TEXAS A&M AGRILIFE RESEARCH & EXTENSION

COTTON ENTOMOLOGY RESEARCH REPORT 2015

TECHNICAL REPORT 16-4

TEXAS A&M AGRILIFE RESEARCH, CRAIG NESSLER, DIRECTOR
THE TEXAS A&M SYSTEM, COLLEGE STATION, TEXAS

COTTON ENTOMOLOGY PROGRAM

RESEARCH ACTIVITY ANNUAL REPORT

2015

SUBMITTED TO:

PLAINS COTTON IMPROVEMENT COMMITTEE
PLAINS COTTON GROWERS, INC.

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Introduction

Plains Cotton Growers, Inc. (PCG) has been a strong supporter of cotton insect research and extension activities in west Texas for many years. Most notably, PCG was instrumental in securing state funds for the Boll Weevil Research Facility at the Lubbock Center, and provided both financial and political support to conduct boll weevil biology and ecology research even before the boll weevil became a significant economic pest of the High Plains region. After the initial entry of the boll weevil into the eastern edge of the High Plains, PCG promoted and along with USDA-APHIS administered the boll weevil diapause suppression program involving a team effort that continued to include Texas A&M University. PCG also supported Texas Cooperative Extension (now Texas A&M AgriLife Extension Service) efforts to annually evaluate the diapause suppression program, conduct applied research trials to develop boll weevil management practices that would enhance the diapause suppression program's efforts and in the 1990s supported an annual survey of High Plains overwintering sites and grid trapping of cotton across the High Plains area. Under the strong and cooperative leadership of PCG, the boll weevil eradication program for the High Plains area progressed much more rapidly than anticipated. Now, the successful boll weevil eradication program has eliminated the boll weevil from this region for over a decade. In 2015, all 11 West Texas zones (Southern Rolling Plains, El Paso/Trans Pecos, St. Lawrence, Permian Basin, Rolling Plains Central, Western High Plains, Southern High Plains/Caprock, Northern Rolling Plains, Northern High Plains, Northwest Plains, and Panhandle) have been declared boll weevil eradicated. The team effort of PCG, Texas A&M AgriLife Research and AgriLife Extension Service over several decades has resulted in a comprehensive understanding of boll weevil ecology and behavior.

With a successful boll weevil eradication program and increased adoption of the Bollgard® technology (now >70%), the cotton insect research and extension program focus has changed considerably during the last 15 years. Our current research/extension focus is on developing ecologically intensive strategies for cotton pest management, including crop phenology, cultivar, non-crop habitat, irrigation, and fertility management towards reducing insect pest pressure. Our research has demonstrated the need for continuing investigation of basic behavior and life patterns of insects while having a strong field-based applied research to bridge the gap between basic, problem-solving science and producer-friendly management recommendations. We have assembled a strong group of people to work as a team to examine multiple disciplines within the broad theme of Cotton IPM. We invest considerable time and manpower resources in investigating the behavior and ecology of major cotton pests of the High Plains with the goal of developing management thresholds based on cotton production technology. Our Program has successfully leveraged research funds based on the funding provided by PCIC to support our Technician position. We are excited about and greatly value our Cotton Entomology research and extension partnerships with multidisciplinary scientists at the Texas A&M AgriLife Center, together with area IPM agents in the region, to continue this partnership as we challenge ourselves to deliver the best cotton insect-pest management recommendations to our Texas High Plains producers.

Texas A&M AgriLife Research & Extension Center at Lubbock

COTTON ENTOMOLOGY PROGRAM

Megha N. Parajulee, Ph.D.

Professor, Faculty Fellow, and Texas A&M Regents Fellow

PROGRAM OVERVIEW: The Cotton Entomology Program at Lubbock combines basic and applied research with strong outreach, industry, and grower partnerships to produce information to enhance the ability of the cotton industry in the Texas High Plains to mitigate cotton yield losses due to insect pests through the use of ecologically intensive integrated pest management. Selected projects of the Program are briefly highlighted in this exhibit.

COTTON FLEAHOPPER POPULATION DYNAMICS AS AFFECTED BY NITROGEN FERTILITY; HALFWAY, TEXAS

A multi-year study investigating the effects of differential nitrogen fertility on cotton fleahopper population dynamics in a typical drip-irrigation Texas High Plains cotton production system has been initiated from the 2014 growing season. Differential nitrogen fertility (0, 50, 100, 150, and 200 lbs N/acre) is being examined for its affect on cotton plant physiological parameters, thereby influencing cotton fleahopper injury potential and plant compensation.

Cotton fleahopper augmentation in multi-plant cages to quantify the response of variable rates of N to FH injury

SEASONAL ABUNDANCE PATTERNS OF BOLLWORM, TOBACCO BUDWORM, AND BEET ARMYWORM MOTHS IN THE TEXAS HIGH PLAINS

A long-term study has been conducted in the Texas High Plains to investigate the year-around weekly moth flight activity patterns of bollworms, tobacco budworms, and beet armyworms. These three species are important cotton pests in the High Plains. The regional adoption of cotton and corn cultivars incorporating *Bt* technology has been instrumental in reducing the current threat of these lepidopteran pests, yet diminishing underground water availability for irrigation is necessitating lower crop inputs, such as transgenic seed costs, for our increasing dryland crop production acreage, increasing the importance of these pests.

Texas Pheromone (TP) and "Bucket" traps used to monitor moths

STATEWIDE SURVEY OF BOLLWORM MOTHS FOR POSSIBLE OLD WORLD BOLLWORM DETECTION IN TEXAS

The objective of this study is to conduct a statewide monitoring of *Helicoverpa armigera* in Texas which will be used to inform growers and consultants and serve as the foundation for the development of management strategies. Plastic bucket traps and pheromone lures will be used to collect moths; moths will be dissected to distinguish Old World and New World bollworm based on genital characteristics.

DEVELOPMENT OF ECONOMIC THRESHOLD AND MANAGEMENT RECOMMENDATIONS FOR *LYGUS* BUG

Texas A&M AgriLife **Cotton Entomology Program** has been providing a unique leadership in *Lygus* research across the United States cottonbelt since 2002. We have quantified the compensation ability of cotton to *Lygus*-induced fruit loss and the recommendation has been made to our producers that pesticide applications prior to 30% pre-flower and 25% early flower fruit shed may not be necessary. We also have developed a late-season insecticide termination guideline for Texas High Plains cotton growers, according to which, insecticide intervention for *Lygus* control may not be warranted when harvestable bolls accumulate ≥350 heat units or the boll is ≥3 cm in diameter after crop cut-out. Current effort concentrates on developing economic threshold-based management recommendations for *Lygus* in Texas High Plains cotton, thereby aiming to minimize economic losses to producers. Continuing studies will examine the effect of *Lygus* on drought-stressed and limited irrigation cotton.

***Lygus* adults and nymphs cause damage to squares, flowers, and bolls**

THRIPS MANAGEMENT IN TEXAS HIGH PLAINS COTTON: INSECTICIDE PRODUCT EVALUATION

Multi-year studies are being conducted at three Texas locations (Hale, Swisher, and Wilbarger counties) to represent cotton fields surrounded by variable vegetation/crop complexes and thrips population pressure in cotton. The study objectives are to: 1) evaluate the foliar insecticide application frequency in managing thrips in seedling cotton, and 2) evaluate the efficacy, residual performance, and economic competitiveness of selected products in thrips management. Insecticides, including seed treatment (thiamethoxam [Cruiser®] and imidacloprid [Aeris®]) and foliar (Orthene®, Bidrin®, and Vydate®) treatments are evaluated for their efficacy and cost effectiveness in managing thrips populations in cotton relative to an untreated control.

Field evaluation of thrips insecticide products

EFFECT OF NITROGEN FERTILIZER ON COTTON FLEAHOPPER DAMAGE POTENTIAL AND CROP RESPONSE TO INJURY

M.N. Parajulee, A. Hakeem, C.K. Dhakal, S.D. Coyle, S.C. Carroll, J.P. Bordovsky

Objective: The objective was to evaluate the effect of nitrogen fertilizer application rates on cotton fleahopper damage potential and cotton's response to fleahopper injury.

Methodology: A high-yielding FiberMax cultivar, FM 9180B2F, was planted at a targeted rate of 54,000 seeds/acre on May 18, 2015. The experiment was a split-plot randomized block design with five nitrogen fertility rate treatments as main plot, two insect augmentation treatments as sub-plots, and five replications. The five main-plot treatments included pre-bloom side-dress applications of augmented nitrogen fertilizer rates of 0, 50, 100, 150, and 200 lbs N/acre using a soil applicator injection rig on July 16. Pre-treatment soil samples (consisting of three soil cores; 0 to 24-inch depth), were collected from each of the 25 experiment plots on June 26. Two 10-ft. sections of uniform cotton were flagged in the middle two rows of each 16-row main-plot that served as two insect treatment sub-plots. The sub-plot treatment included two cotton fleahopper treatments (5 adults per plant vs. no fleahopper as control), contained in multi-plant cages, within designated row sections applied to each of the five nitrogen rates two weeks into cotton squaring (July 21), the most critical phenological stage of cotton for fleahopper management in the Texas High Plains, to simulate an acute infestation of cotton fleahoppers. Crop growth and fruiting patterns were monitored during the crop season.

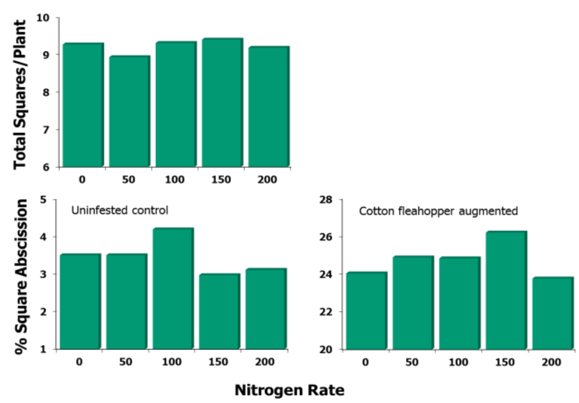

Fig. 1. Per plant square load at the time of cotton fleahopper augmentation (top panel) and percent square abscission (bottom panel) in control versus fleahopper augmented treatments, as influenced by variable rates of nitrogen application, 2015.

Results: Two weeks into squaring, experimental plants had approximately 9 squares per plant. Cotton fleahoppers induced ~25% square abscission across all N treatments (Fig. 1).

All N augmented plots had higher lint yields than on zero N plots, but the crop response to variation in N level was not well defined (Fig. 2). Combined over all N treatments, the acute infestation of fleahoppers rendered the lint yield reduction from 910 lb/acre in the control to 877 lb/acre in fleahopper plots. Lint yield was not significantly affected by ~25% fleahopper-induced square loss at both zero N and 200 lb/acre plots, either via pruning of undesirable fruit load (zero N) or compensation (200 lb N). On the other hand, lint yield was significantly lower in fleahopper augmented 100 lb/acre plots compared to that in uninfested plots, clearly suggesting that the plant response to cotton fleahopper injury is greatly influenced by the availably of nitrogen fertility.

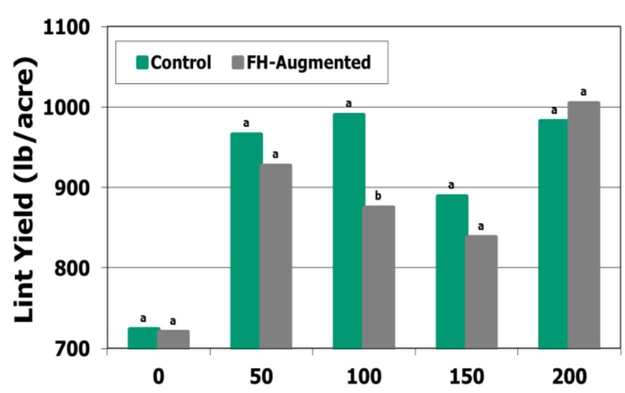

Fig. 2. Effect of nitrogen augmentation rates on lint yield following a single acute infestation of cotton fleahopper versus uninfested control, 2015.

TITLE:

Cotton yield response to cotton fleahopper acute infestations as influenced by irrigation level treatments, Lamesa, TX, 2015.

AUTHORS:

Megha Parajulee – Professor, Faculty Fellow, and Regents Fellow
Abdul Hakeem – Postdoctoral Associate
Sean Coyle – Technician
Chandra Dhakal – Research Assistant
Stanley Carroll – Research Scientist
Wayne Keeling - Professor

MATERIALS AND METHODS:

Plot Size:	4 rows by 300 feet, 3 replications
Planting date:	May 16, 2015
Fertilizer:	120-40-0
Treatments:	
Cultivar:	Deltapine 1454 B2RF FiberMax 2011 GT
Irrigation:	Low: Pre-plant = 0.8 inches; In-season = 3.6 inches High: Pre-plant = 0.8 inches; In-season = 7.1 inches
Cotton fleahopper:	2 insect stages (adults vs nymphs); 3 insect release treatments [control (zero cotton fleahopper), low fleahopper density (2 bugs per plant), high fleahopper density (5 bugs per plant),
Herbicides:	Roundup PowerMax® 1qt/A – January 28 Roundup PowerMax® 1 qt/A + 2,4-D 1 qt/A – April 9 Prowl® 3 pt/A – April 21 Roundup PowerMax® 1 qt/A – June 11 Roundup PowerMax® 1 qt/A + Dual Magnum® 1 pt/A – July 13
Insect release date:	July 2, 2015 (fleahopper susceptible stage)
Plant mapping date:	July 22, 2015 (in-season); October 14, 2015 (pre-harvest)
Harvest date:	October 26, 2015 (hand-harvested)

Cotton fleahopper feeding injury and resulting cotton lint yield were evaluated in two cotton cultivars, as affected by irrigation level, insect stages, and infestation densities. Two seasonal irrigation levels were evaluated, High (7.9”) and Low (4.4”), under a center pivot irrigation system. Laboratory-reared and/or field collected cotton fleahoppers were released onto cotton terminals in 3-ft. (L) x 2-ft. (W) x 3 ft. (H) multi-plant cages (adults; Fig. 1) or in the 3-ft sections of cotton rows on open field (nymphs). Each section contained 7 plants.

Experimental design consisted of two insect stages (adults versus nymphs), three insect release treatments (high, low, and control), two water levels (high versus low), and two cotton cultivars (DP 1454 B2RF and FM 2011 GT), replicated three times and deployed in a randomized complete block design (total 72 plots). Insect release treatments, 1) control (zero fleahopper augmentation), 2) two bugs per plant (low density), and 3) five bugs per plant (high density), were deployed on July 2, 2015 (Fig. 1), and then allowed to feed for one week in order to mimic a natural early-season acute infestation. A single release of cotton fleahoppers was timed to simulate the acute infestation of cotton fleahoppers while cotton was highly vulnerable to the fleahopper injury, which is approximately around the second week of cotton squaring. Plant mapping was conducted before and after cotton fleahopper releases to monitor for altered fruiting patterns. Yield monitoring was achieved via hand-harvesting of each experimental plot on October 26. 2015.

There was no natural infestation of cotton fleahoppers at the experimental farm, so the control plots did not require any insecticidal intervention. Post-release data collection included plant mapping on July 22, a pre-harvest complete plant mapping on October 14, and harvesting on October 26, 2015.

RESULTS AND DISCUSSION:

Averaged across cultivars, irrigation levels, and insect stages, artificial augmentation of cotton fleahoppers caused 7.5 and 12.2% square loss following low and high levels of infestations, respectively, compared to 3.9% square loss in control plots (Fig. 2). This level of square loss in pre-flower cotton is considered a low to moderate level of insect-induced early fruit loss in Texas High Plains cotton. Overall, insect-induced square loss did not vary between the two cultivars, but cotton fleahopper nymphs caused significantly greater square loss compared to the adults. Interestingly, there was a significant cultivar x insect stage interaction in square loss phenomenon; with significantly greater damage by nymphs in DP 1454 B2RF than adults while nymphs and adults caused similar damage to FM 2011 GT (Fig. 2). Fleahopper crop damage, as measured by cotton square loss, did not significantly vary between the two water levels.

Although the crop was at a highly cotton fleahopper susceptible stage, the augmented cotton fleahopper densities of 2 and 5 per plant caused lower levels (7.5 and 12.2%) of fruit abscission than we had anticipated. Nevertheless, lint yield was significantly impacted by the fleahopper augmentation treatment, with significantly lower yields in fleahopper-augmented plots (Fig. 3). Lint yield values were 1415, 1233, and 1149 lb/acre in control, low, and high bug density treatments in high water plots and 1005, 890, 983 lb/acre in low water treatment plots, respectively (Fig. 4). The effect of fleahopper on lint yield was significant under high irrigation plots, but no significant effect of cotton fleahopper was observed in low irrigation plots, indicating plants' from low irrigation plots allowed for lowering of the fruit load via insect-induced fruit loss.

Figure 1. Multi-plant cages deployed in the field to examine the impact of cotton fleahopper densities on cotton yield, Lamesa, TX.

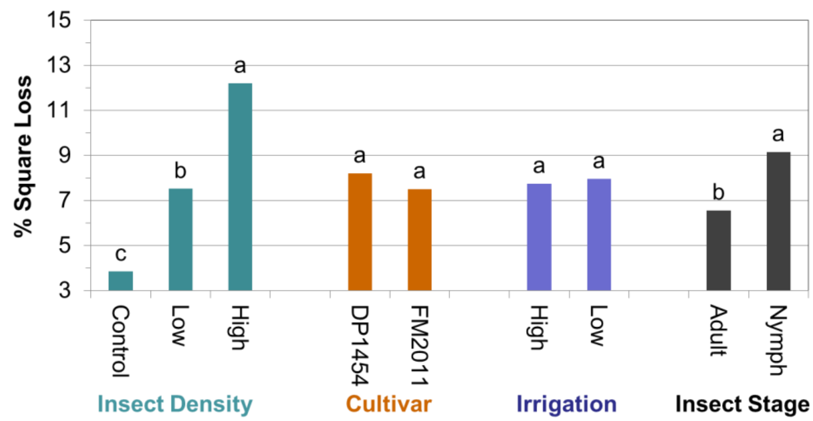

Figure 2. Average percentage square loss following a simulated acute infestation of cotton fleahoppers, achieved by augmenting 2 (low) and 5 (high) bugs per plant during the second week of squaring, under low and high irrigation regimes in two cotton cultivars, Lamesa, Texas, 2015.

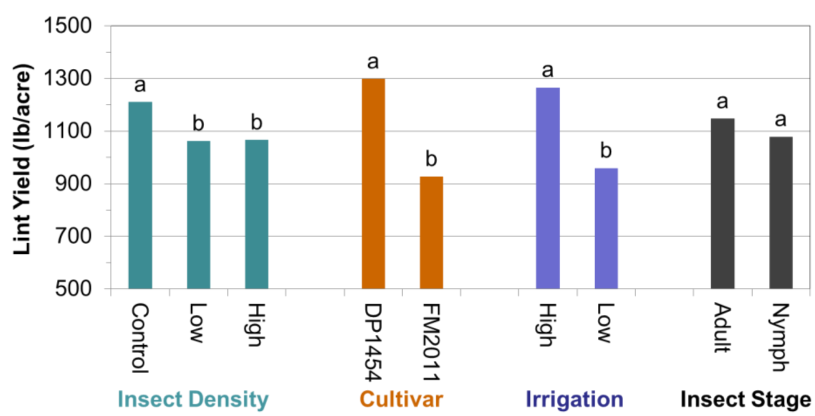

Figure 3. Average lint yield following a simulated infestation of cotton fleahoppers, achieved by augmenting 2 (low) and 5 (high) bugs per plant during the second week of squaring, under low and high irrigation regimes in two cotton cultivars, Lamesa, Texas, 2015.

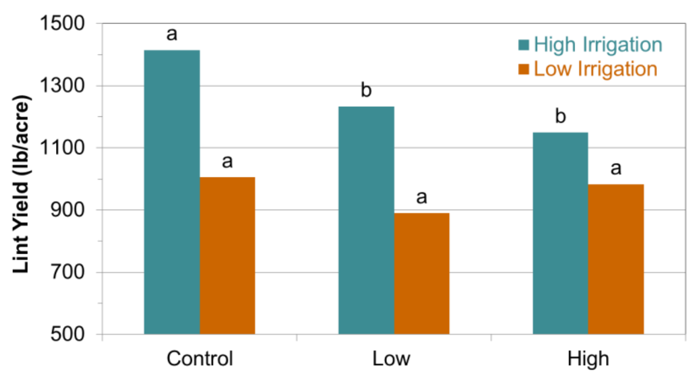

Figure 4. Average lint yield following a simulated acute infestation of cotton fleahoppers under high and low irrigation regimes, Lamesa, Texas, 2015.

ANNUAL REPORT 2015

Cotton Incorporated Core Program

Project Number: 14-457

Evaluation of Cotton Fleahopper Damage Potential and Crop Response to Injury under Variable Nitrogen Fertility Level

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Evaluation of Cotton Fleahopper Damage Potential and Crop Response to Injury under Variable Nitrogen Fertility Level

Project Summary

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), is a significant economic pest of cotton in the Texas High Plains. Injury by cotton fleahoppers to squaring cotton often causes excessive loss of small squares during the early fruiting period of plant development (first 3 weeks of squaring). Both adults and immatures feed on new growth, including small squares. Greater damage is observed on smooth leaf varieties than on hirsute varieties, which may extend the susceptible period into early bloom, especially under a high-input production regime. Cotton is affected by cotton fleahopper injury from about the fifth true-leaf through first week after initiation of flowering. Squares up to pinhead size are most susceptible to damage, and yield loss is most likely from feeding during the first three weeks of fruiting. Cotton fleahopper damage also delays crop maturity and thus increases the vulnerability of cotton to late season pests such as heliothine caterpillars and *Lygus* bugs. The objective of this study was to evaluate the cotton crop growth parameters and lint yield following cotton fleahopper acute infestations under a range of nitrogen fertility rates. The five main-plot treatments included pre-bloom side-dress applications of augmented nitrogen fertilizer rates of 0, 50, 100, 150, and 200 lbs N/acre using a soil applicator injection rig on 23 July 2014 and 16 July 2015. The sub-plot treatment included two cotton fleahopper augmentation treatments [5 cotton fleahopper nymphs (2014) or adults (2015) per plant versus no fleahopper augmentation as control] applied to each of the five nitrogen fertility rates two weeks into cotton squaring, the most critical phenological stage of cotton for cotton fleahopper management in the Texas High Plains. Cotton fleahopper infestation treatments caused 14-27% and 24-26% square loss in 2014 and 2015, respectively. Cotton fleahopper induced fruit loss resulted in significant crop maturity delay in 2014, as measured by number of unopened bolls (7.7% non-harvestable bolls in the infested plots versus 1.8% in control plots) at harvest. There was no maturity delay penalty in 2015 due to an unseasonably warmer fall. As expected, lint yield varied with N level regardless of the cotton fleahopper infestation in both years. In uninfested control plots, lint yield displayed a characteristic staircase effect of nitrogen rate, with lowest lint yield (862 lb/acre) in zero N and highest lint yield (1,081 lb/acre) in 200 N treatments, with numerical increase in lint yield for each incremental nitrogen application of 50 lb/acre. In 2015, all N augmented plots had higher lint yield than on zero N plots, but the N density response was not well defined. Combined over all N treatments, the acute infestation of cotton fleahoppers rendered the lint yield reduction from 975 and 910 lb/acre in the uninfested control to 846 and 877 lb/acre in fleahopper augmented treatments in 2014 and 2015, respectively. In both years, cotton lint yield was not significantly affected by ~25% fleahopper-induced square loss three weeks into squaring at both zero N and 200 lb/acre plots, either via pruning of undesirable fruit load (zero N) or compensation (200 lb N), whereas lint yield was significantly lower in fleahopper augmented 100 lb/acre plots compared to that in uninfested plots, clearly suggesting that the plant response to cotton fleahopper injury is greatly influenced by nitrogen fertility.

Introduction

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), is a significant economic pest of cotton in the Texas High Plains. Injury by cotton fleahoppers to squaring cotton often causes excessive loss of small squares during the early fruiting period of plant development (first 3 weeks of squaring). Both adults and immatures feed on new growth, including small squares. Greater damage is observed on smooth leaf varieties than on hirsute varieties (Knutson et al. 2013), which may extend the susceptible period into early bloom, especially under a high-input production regime. Cotton is affected by cotton fleahopper injury from about the fifth true-leaf through first week after initiation of flowering. Squares up to pinhead size are most susceptible to damage, and yield loss is most likely from feeding during the first three weeks of fruiting (Reinhard 1926). Cotton fleahopper damage also delays crop maturity and thus increases the vulnerability of cotton to late season pests such as heliothine caterpillars and *Lygus* bugs, particularly when natural enemies are destroyed by insecticides directed against cotton fleahoppers (Chen et al. 2007).

Predominantly, cotton fleahoppers feed upon pinhead-sized or smaller squares, which results in abortion of these young fruits, thereby impacting yields. While cotton fleahopper feeding preferences serve as a baseline for their management in cotton fields, a detailed understanding of cotton plant responses to fleahopper damage remains unachieved (Parajulee et al. 2006, Chen et al. 2007). Cotton plant growth is sensitive to numerous environmental and management input factors, particularly irrigation and nitrogen fertility. Cotton growth responses to various input factors are well-documented and growth models have been developed. However, the specific cotton plant responses to cotton fleahopper injury under a range of nitrogen fertility remain uninvestigated. This study was designed to evaluate the cotton crop growth parameters and lint yield following cotton fleahopper acute infestations under a range of nitrogen fertility rates.

Materials and Methods

This study was conducted at the Texas A&M AgriLife Research farm near Plainview, Texas. A 5-acre subsurface drip irrigation system has been in place for 14 years and nitrogen fertility treatments have been applied in a randomized block design with five replications since 2002 (Fig. 1). The present study utilized the same experimental set up as for the last 13 years. Pre-plant land preparations on the field of 30-in row-spacings included an application and incorporation of Treflan® (trifluralin) @ 2 pints/acre on 19 February 2014 and 12 January, 2015. The field did not receive pre-plant fertility applications.

The 2014 study was planted with FiberMax 9063 B2R at a targeted rate of 54,000 seeds/acre on 16 June and post-emergence herbicide treatments were applied on 27 June (Crop Smart® @ 32 oz/acre; Warrant® @ 3 pints/acre) and 7 July (Crop Smart® @ 40 oz/acre). The 2015 test was planted to Fibermax 9180 B2F at a targeted rate of 60,000 seeds/acre followed by an 'over-the-top' Caparol® 4L (prometryn; 3 pints/acre) application immediately after planting on 18 May, with post-emergence herbicides applications on 30 June (RoundUp® @ 32 oz/acre) and 29 July (Warrant® 3 pt/acre) for weed management.

Experimental plots were 16 rows wide x 120 ft long and 5 ft alleys separated the plots. The experiment was a split-plot randomized block design with five nitrogen fertility rate treatments as main plot, two insect augmentation treatments as sub-plots, and five replications. The five main-plot treatments included pre-bloom side-dress applications of augmented nitrogen fertilizer rates of 0, 50, 100, 150, and 200 lbs N/acre using a soil applicator injection rig on 23 July 2014 and 26 June 2015. The individual plots have been receiving the same nitrogen augmentation rates for the past 14 years. The pre-treatment residual nitrogen soil samples were pulled on 10 July 2014 and 26 June 2015 from each of the 25 experimental plots. The soil samples were quickly placed into an unused greenhouse to quickly remove the soil moisture. These dried samples were processed through a soil grinder prior to shipment to Ward Laboratories (Kearney, NE) for residual nitrogen analyses. The five fertility treatment applications were applied by side-dressing the 25 experimental plots with the appropriate nitrogen levels on 23 July 2014 and 16 July 2015. Two 10-ft. sections of uniform cotton were flagged in the middle two rows of each 16-row main-plot that served as two insect treatment sub-plots. The sub-plot treatments included two cotton fleahopper augmentation treatments (5 cotton fleahopper nymphs per plant uncaged [2014] or 5 cotton fleahopper adults per plant in multi-plant cages [2015] versus no fleahopper augmentation as control) applied to squaring cotton within these designated row sections to simulate an acute infestation of cotton fleahoppers. This early squaring period is the most critical phenological stage of cotton for cotton fleahopper management in the Texas High Plains (Parajulee et al. 2006).

Woolly croton was harvested from rangeland sites near College Station, Texas, in early February and then placed into cold storage. Forty 1-gallon sheet metal cans (ends of cylinder-type cans covered with window screen), each containing 4 ounces of dry croton twigs per can, were initiated to generate the required number of cotton fleahoppers for the experiment (Hakeem and Parajulee 2015). Conditions conducive to cotton fleahopper emergence were simulated in a laboratory environment in order to induce hatching of overwintered eggs embedded in the croton stems, and emerged cotton fleahoppers were subsequently reared on fresh green beans. Field collected cotton fleahopper adults augmented the laboratory colony in 2015. The single release of cotton fleahoppers (nymphs in 2014 and adults in 2015) mentioned above was timed to simulate the acute heavy infestation of cotton fleahoppers (4-5 days of feeding) while cotton was highly vulnerable to the fleahopper injury. It was planned so that this arrangement would ensure significantly high levels of fleahopper-induced square damage on treatment plots to quantify the variation in damage potential as influenced by soil applied N. The release was accomplished on 30 July 2014 and 21 July 2015 by aspirating third-instar fleahopper nymphs or adults from the laboratory reared and/or adapted colonies, transferring them into 0.75” X 1.5” plastic vials, then cautiously depositing them onto the terminals of plants in each treatment plot at the rate of 5 cotton fleahoppers per plant; the control plots received no fleahoppers and were kept fleahopper-free during the entire study period. Natural infestations of cotton fleahoppers did not occur at our site due to the severe crop delay in 2014 and frequent rain showers in 2015. Therefore, the control sections within each of the 25 plots in 2014 did not receive supplemental insecticidal interventions until an Orthene® 97UP insecticide application was applied on 7 August 2014 to all experimental units (both fleahopper release sections and control sections within each of the 25 main-plots) to ensure complete removal of all cotton fleahoppers following their release and feeding period. For 2015 study, a 7-plant unit was caged within the marked cotton row sections and adult cotton fleahoppers were released in each cage, but the caging of adult treatment section with large cages for <7 days was expected to hinder the plant growth minimally. Seven days after cotton fleahopper augmentation, the entire test was sprayed with Orthene 97UP @ 12 oz acre on 28 July 2015. In both years, the entire test was kept insect-free for the remainder of the study to isolate the effect of cotton fleahopper injury only. All control and fleahopper-augmented sections were monitored for fleahopper-induced fruit loss on 14 August 2014 and 6 August 2015.

Additional data collected included monitoring of plant height, leaf chlorophyll content, leaf nitrogen content, and squaring patterns in all 50 experimental units (5 N rates x 2 insect treatments x 5 replications), starting from the first week of squaring (pre-release data) and approximately weekly thereafter well into the fall crop developmental period. The dates in which ten 5th main stem leaves (from the plant top) were collected for chlorophyll readings, leaf area measurements, leaf dry weights, and end-of-study laboratory leaf nitrogen analysis in 2014 included 25 July; 5, 22, and 28 August; 5 and 26 September; and 2 and 8 October 2014; the 2015 samples dates for these parameters were 30 July; 6, 13, 20, and 27 August; and 4 and 11 September 2015. In-season plant mapping and plant height data from five randomly selected plants per plot were collected on 26 August 2014 and 30 July 2015. Five randomly selected plants in each of the 25 experimental plots (125 total plants) were dug-up and returned to the laboratory for measurement of detailed individual plant biomass of the following: 1) root, 2) shoot, 3) leaves, and 4) fruits. Later on 26 September 2014 and 20 August 2015, 15 randomly selected bolls were collected from the 5th mainstem node from the top of the plants and then the 375 total bolls (15 bolls per plot X 25 plots) were placed into an ice chest and returned to the laboratory to measure boll parameters including: 1) boll diameter, 2) boll fresh weight, 3) boll carpel wall puncture pressure, and 4) boll dry weight following placement into a drying oven.

The timing of crop 'cut-out' within individual plots was estimated by counting the Nodes Above White Flower (NAWF) on a series of randomly selected plants per plot on 28 August; and 5 and 19 September 2014 and 10, 13, 20, and 27 August; and 4 and 11 September 2015. The 2014 test was prepared for harvest by first spraying a boll opener (Boll Buster® 1 quart per acre) and a defoliant [ET® (pyraflufen) 1.25 oz per acre] in a tank mix on 23 October, followed by an application of a desiccant (Helmquat® 3SL 1 quart per acre) to finish terminating the cotton plants on 3 November 2014. The 2015 test was terminated by spraying a boll opener (Boll Buster® 1 quart per acre) and a defoliant [ET® (pyraflufen) 1.25 oz per acre] in a tank mix on 14 October, followed by an application of a desiccant (Helmquat® 3SL 1 quart per acre) to terminate the plants on 29 November 2015. Final plant mapping and harvesting of test sections were performed on 20 November 2014 and 2 November 2015 and the ginned lint samples were sent to Cotton Incorporated for fiber quality analysis.

0	50	200	50	200
100	100	0	100	50
200	150	50	150	0
50	200	100	200	100
150	0	150	0	150

Figure 1. Helms Farm nitrogen study experimental plot layout following a five-treatment x five-replication randomized block design. Each of the 25 plots received one of the five nitrogen augmentation treatments including 0, 50, 100, 150, or 200 lbs N/acre, Hale County, TX.

Results and Discussion

Influence of N fertility level on cotton plant growth parameters. Soil residual N levels were much higher in 2014 compared to that in 2015 (Fig. 2). The unusual heavy rainfall throughout spring of 2015 likely leached excess residual nitrogen build-up from prior years of drought conditions, resulting in much lower residual N in 2015. Residual N levels generally increased with increased level of applied N. In 2014, residual N levels were significantly higher in plots that received the two highest application rates of N fertilizer versus plots receiving 50 lb/acre N applications or no N augmentation; plots that received 100 lb N/acre had an intermediate level of residual nitrogen (Fig. 2). The two highest N augmentation plots (150 and 200 lb/acre) resulted in three-times higher amount of soil residual N compared to that in zero and 50 lb/acre plots. In 2015, plots receiving 150 and 200 lb/acre N had accumulated significantly higher residual N compared to that in zero and 50 lb/acre N plots. These experimental plots had been receiving same assigned levels of applied N for the previous 13 years and the relationship between applied N rates and resulting residual N has generally followed this trend for all previous years.

Variation in residual N did not show significant variable effect on early cotton growth parameters, such as plant height, leaf area, and chlorophyll content. However, the effect of N application rate was more pronounced as the season progressed, especially in a drier year such as 2014 (Fig. 3). However, in a wet year such as 2015, the effect of N application rate did not vary temporally within the season (Fig. 4). In 2014, the effect of N application rate was less pronounced in leaf surface area compared to that for chlorophyll concentration and leaf N content of the fifth mainstem node leaf. Measured leaf chlorophyll content varied with nitrogen application level, and leaf chlorophyll contents from cotton in those plots which received 0 lb N/acre were significantly lower than all others (Figs. 3-4). Chlorophyll concentration in zero N plots was 5 or more units lower than that for 50 lb N/acre plots throughout the growing season, while the concentration further declined as the season progressed, especially in 2014. In 2015, all N augmented cotton plots exhibited relatively consistent leaf parameters but significantly varied to that in zero N plots (Fig. 4). It is noteworthy that the leaf chlorophyll content in zero N treatment plots declined precipitously beginning in late August, when plants began allocating much of their resources to boll maturation, whereas this phenomenon did not occur in plots that received ≥50 lb N/acre. In 2014, percentage leaf nitrogen declined as the season progressed, especially when plants began diverting their energy to fruit maturation (mid- to late August). However, the leaf nitrogen content in zero N plots began to decline soon after cotton began flowering, but it declined much more rapidly in zero N plots than for N augmented plots when plants began allocating much of their resources to boll maturation (Fig. 3). In 2015, percentage leaf nitrogen did not vary significantly as season progressed, but the leaf nitrogen content in zero N plots remained consistently lower than that for N augmented plots (Fig. 4).

Plant parameter values such as plant height, leaf area (leaf size), leaf chlorophyll concentration, and percentage leaf nitrogen were much lower in zero N plots compared to that in all N augmented plots by the time crop attained full maturity (Figs. 5-8), indicating a high degree of physiological stress on plants receiving zero pounds of augmented nitrogen. Lower rates of N augmentation resulted in lower plant parameter values compared to that for high rates of N augmentation.

Variable rates of N augmentation affecting plant height, leaf size, leaf chlorophyll, and leaf nitrogen content correspondingly impacted leaf dry weight and boll dry weight at full crop maturity. Fifth mainstem leaf dry weight was significantly lower at zero N plots (Figs. 8-9). Leaf dry-weight values were similar in all N augmented plots although the two lower N augmented treatments (50 and 100 lb/acre) had numerically lower leaf dry weight compared to that for two highest N rates.

Nitrogen fertility level also influenced boll maturity. Plants in zero N plots advanced to reproductive phase earlier and bolls formed and matured significantly earlier than in N augmented plots. As a result, dry weight of fifth mainstem node bolls was significantly greater in zero N plots compared to that for N augmented plots (Fig. 9). Laboratory measurement of boll exocarp penetrability in 2014 showed that the fifth mainstem node bolls from zero N augmented plots required significantly greater pressure to puncture the exocarp versus that required to do so for bolls from N augmented plots; however, heavy and frequent rain events in 2015 eliminated the moisture stress in zero N plots during early boll development phase, resulting in no significant penetrability differences in bolls across all N treatments (Fig. 10).

Variation in soil residual N levels, coupled with variable N application, resulted in phenotypic expression of nitrogen deficiency in cotton across treatment plots, more pronouncedly between zero N plots and N augmented plots, which were reflected on temporal chlorophyll contents of the fifth leaf (Fig. 3). However, such phenotypic expression of N deficiency in zero or low N level treatments was not observed in 2015.

N fertility level and cotton fleahopper infestation. Cotton plants were two weeks into squaring when an acute infestation of 5 cotton fleahoppers per plant was deployed. Pre-release monitoring of squaring profiles showed that plants had ~6 (2014) to ~9 (2015) squares per plant across all N treatments. Total square density did not vary with N treatments prior to cotton fleahopper infestation (Figs. 11-12). This density (5 cotton fleahoppers per plant) is considered equivalent of 1 cotton fleahopper per plant, with 20% field survivorship and visual observation retrieval of released nymphs or adults. The density is also equivalent to 3-4 times current cotton fleahopper threshold (25-30 cotton fleahoppers per 100 plants) for the Texas High Plains.

One week of cotton fleahopper infestation resulted in significant square abscission in cotton fleahopper augmented plots, but negligible square abscission (2-4% or less) was observed in uninfested control plots (Figs. 11-12). While total square density did not vary across N treatments, cotton fleahopper-induced square abscission levels varied significantly with N application rates in 2014, but it did not vary across N treatments in 2015. In general, higher N rate favored lesser impact of cotton fleahopper injury. In 2014, square abscission rate was numerically highest at zero N plots, followed numerically by 50 and 100 lb N/acre plots, yet all values were statistically similar. However, abscission rates were reduced to 19 and 14% in 150 and 200 N treatments, respectively (Fig. 11). In 2015, square abscission rates were similar at ~25% across all N treatments (Fig. 12). No biological or physiological reasons are speculated for reduced square abscission observed in the two highest N rate plots in 2014.

In 2014, cotton fleahopper infestation caused noticeable crop maturity delay, as measured by number of unopened bolls (non-harvestable bolls) present at harvest. Averaged across all N treatments, percentage unopened bolls were 7.7% in cotton fleahopper augmented plots compared with 1.8% unopened bolls in uninfested (control) plots; N augmentation levels did not significantly influence the percentage boll opening at the time of harvest (Fig. 13). Nevertheless, because the level of square abscission was not excessive (14-27%) for pre-flower cotton (75% fruit set is considered a lower limit for Texas High Plains cotton into the third week of squaring), the crop did not suffer a major crop maturity delay due to cotton fleahopper infestation. The 2015 crop season was characterized by frequent rain events throughout the spring and early summer months, followed by a relatively warmer and extended fall, which allowed for full crop maturity across all N application regimes.

As expected, lint yield varied with N level regardless of the cotton fleahopper infestation (Figs. 14-16). In uninfested control plots in 2014, lint yield displayed a characteristic staircase effect of nitrogen application rate, with lowest lint yield (862 lb/acre) in zero N and highest lint yield (1,081 lb/acre) in 200 N treatments, with numerical increase in lint yield for each incremental nitrogen application of 50 lb/acre. In 2015, all N augmented plots had higher lint yield than on zero N plots, but the crop response to variation in N density was not well defined. Combined over all N treatments, the acute infestation of cotton fleahoppers rendered the lint yield reduction from 975 lb/acre and 910 lb/acre in the uninfested control to 846 lb/acre and 877 lb/acre in fleahopper augmented treatments in 2014 and 2015, respectively. In both years, cotton lint yield was not significantly affected by ~25% fleahopper-induced square loss three weeks into squaring at both zero N and 200 lb/acre plots, either via pruning of undesirable fruit load (zero N) or compensation (200 lb N). On the other hand, lint yield was significantly lower in fleahopper augmented 100 lb/acre plots compared to that in uninfested plots, clearly suggesting that the plant response to cotton fleahopper injury is greatly influenced by the availably of nitrogen fertility.

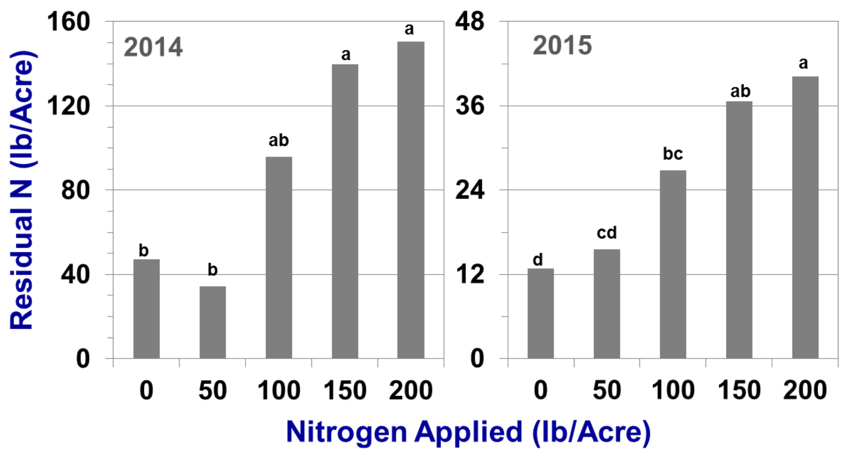

Figure 2. Effect of prior years' N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulation in the soil for the current crop year, 2014-2015.

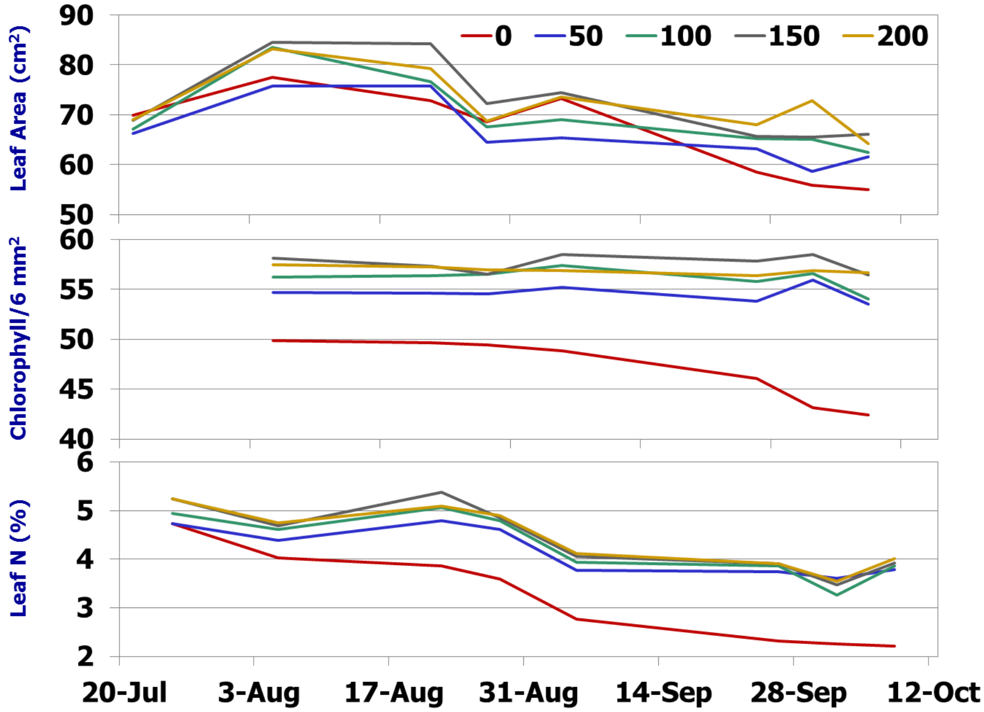

Figure 3. Temporal dynamics of leaf growth (leaf area), chlorophyll concentration, and percentage leaf nitrogen content measured on fifth mainstem leaf as influenced by the variable rates of augmented nitrogen (lb N/acre), 2014.

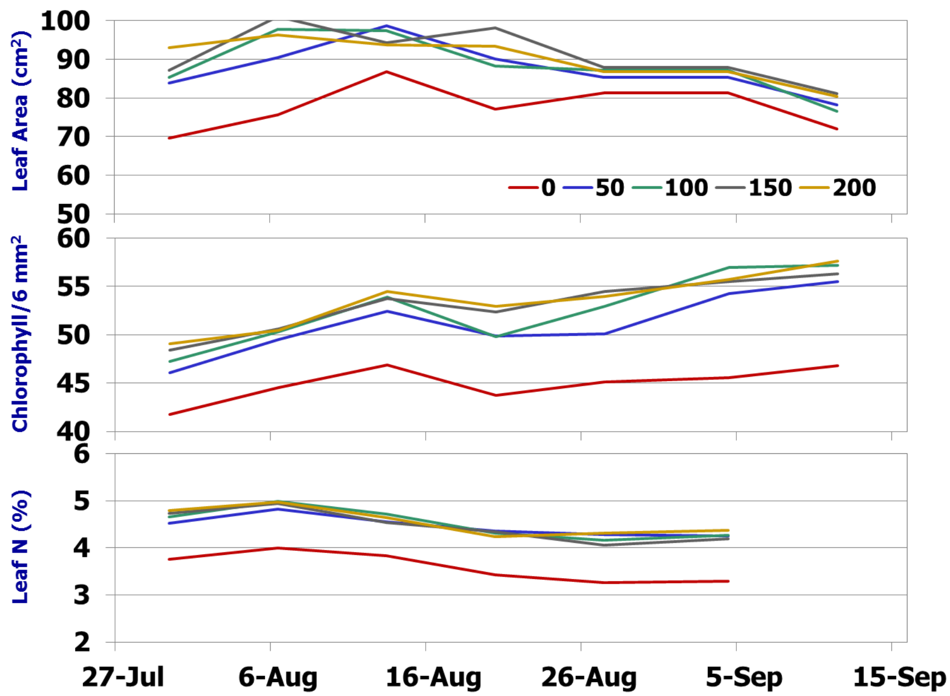

Figure 4. Temporal dynamics of leaf growth (leaf area), chlorophyll concentration, and percentage leaf nitrogen content measured on fifth mainstem leaf as influenced by the variable rates of augmented nitrogen (lb N/acre), 2015.

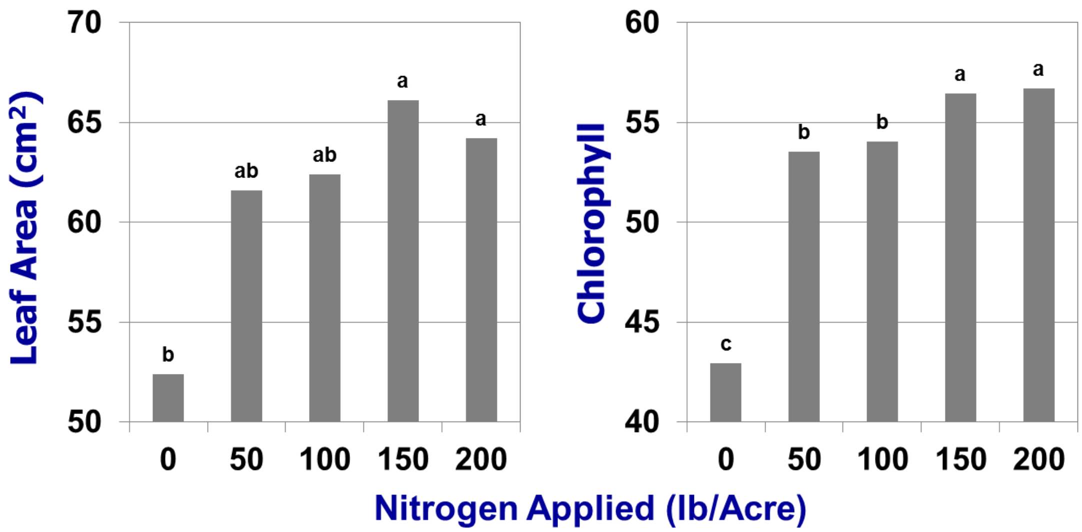

Figure 5. Average leaf surface area (left) and chlorophyll concentration or SPAD values (right) of the fifth mainstem node leaf on a full-canopy crop as affected by N treatments, August 26, 2014.

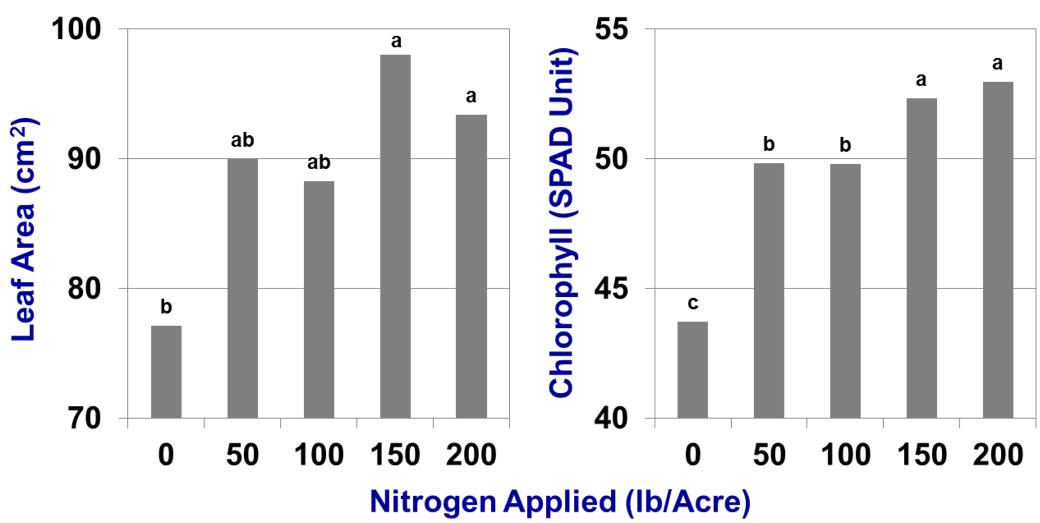

Figure 6. Average leaf surface area (left) and chlorophyll concentration or SPAD values (right) of the fifth mainstem node leaf on a mid-season crop as affected by N treatments, August 20, 2015.

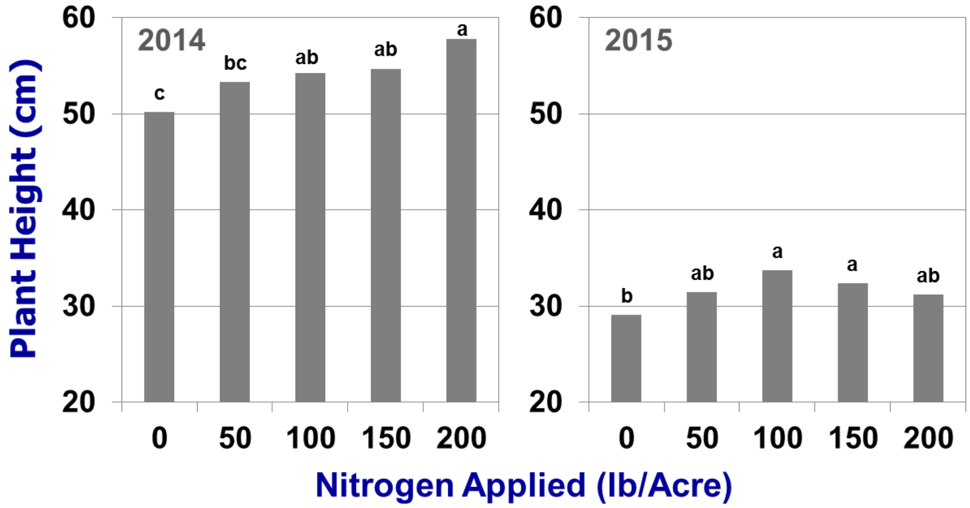

Figure 7. Effect of variable nitrogen treatments on cotton plant height at full crop canopy growth (August 26, 2014) and mid-season (July 30, 2015).

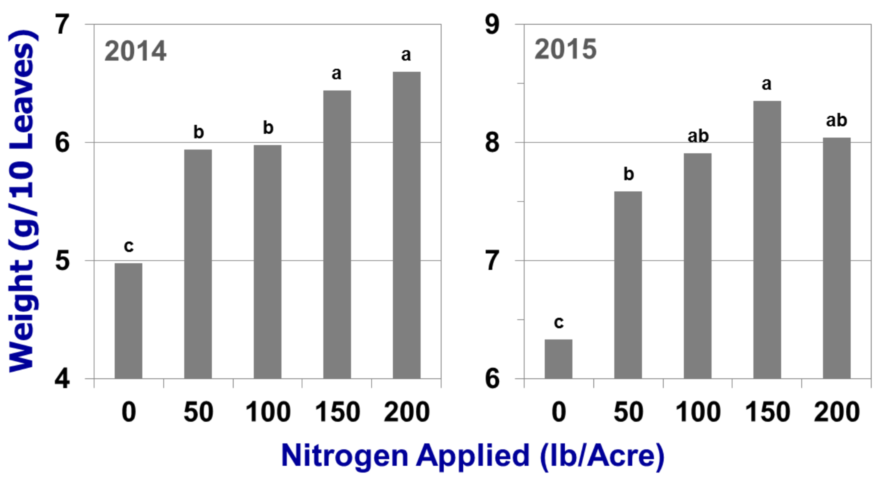

Figure 8. Effect of variable nitrogen on fifth mainstem leaf dry weight, averaged over 6 sample weeks during the cotton growing season, Hale Co., Texas, 2014-2015.

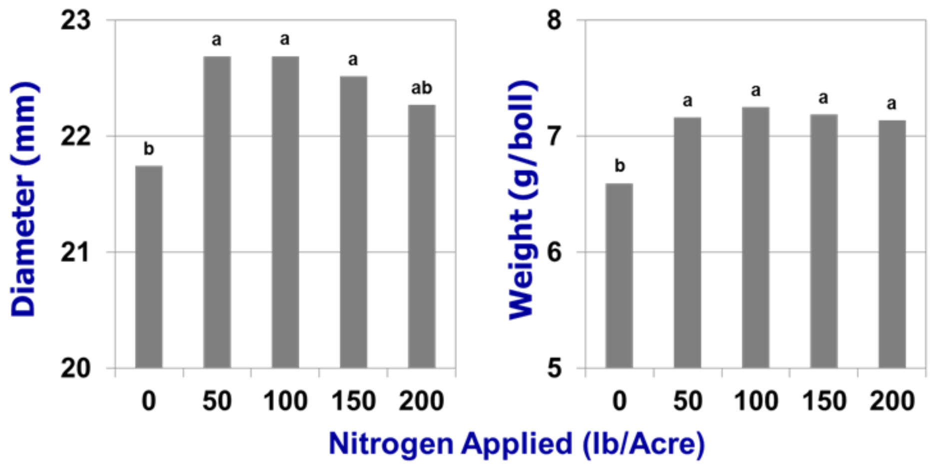

Figure 9. Effect of variable nitrogen on fifth mainstem leaf dry weight and fifth mainstem node boll dry-weight at full crop maturity, September 26, 2014.

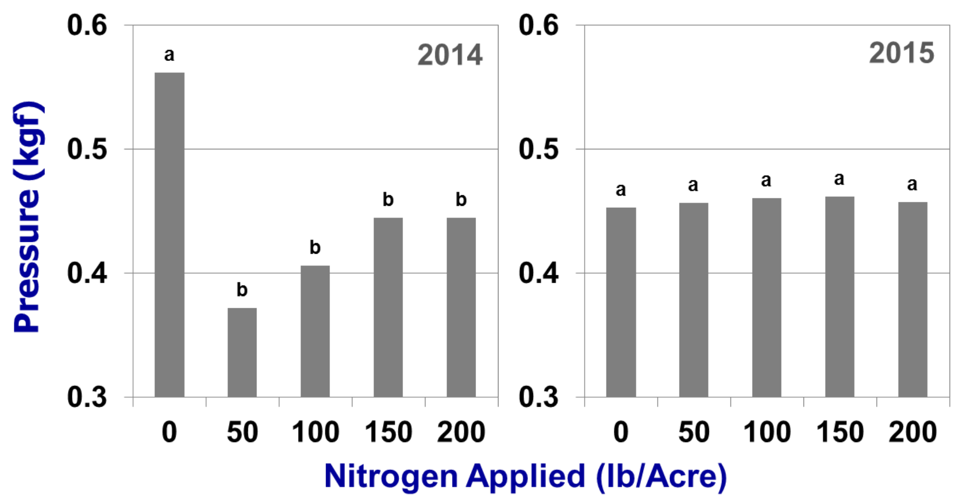

Figure 10. Effect of variable nitrogen on boll maturity as measured by the pressure required to puncture the carpel wall of the fifth mainstem node position bolls, September 26 (2014) and August 20 (2015).

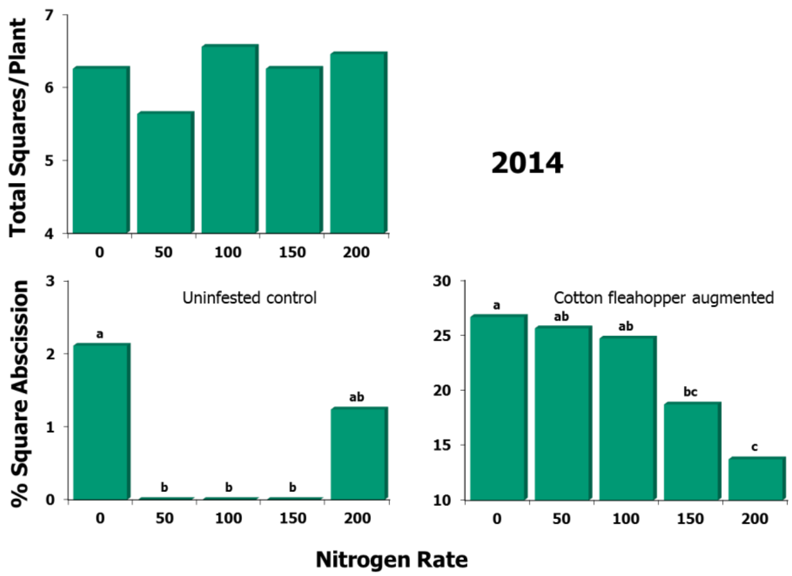

Figure 11. Total square density (number of squares set per plant) at the time of cotton fleahopper augmentation (top panel) and percentage square abscission (bottom panel) in control versus cotton fleahopper augmented treatments, as influenced by augmented variable rates of nitrogen application (0, 50, 100, 150, and 200 lb per acre), 2014, Hale County, TX.

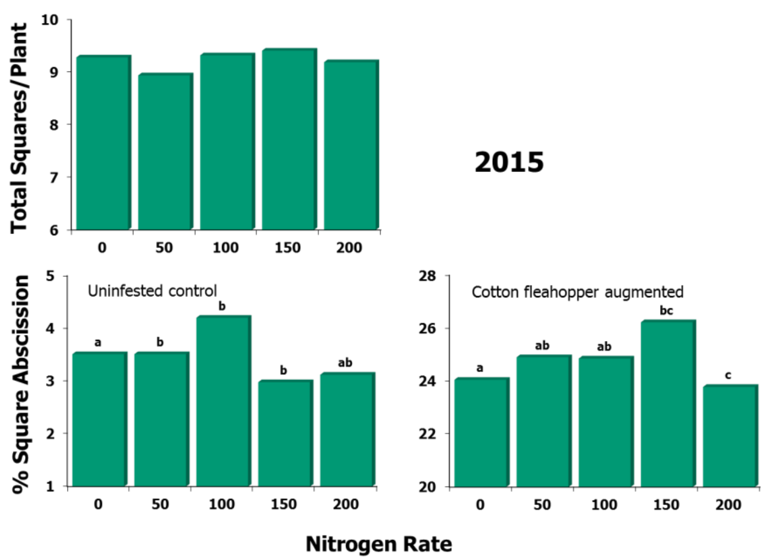

Figure 12. Total square density (number of squares set per plant) at the time of cotton fleahopper augmentation (top panel) and percentage square abscission (bottom panel) in control versus cotton fleahopper augmented treatments, as influenced by augmented variable rates of nitrogen application (0, 50, 100, 150, and 200 lb per acre), 2015, Hale County, TX.

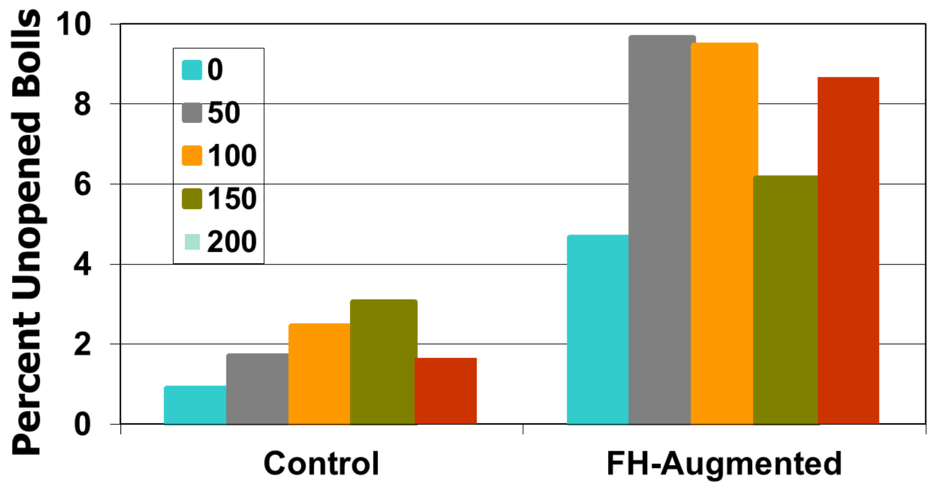

Figure 13. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on cotton maturity as measured by number of unopened (non-harvestable) bolls at harvest, November 20, 2014, Hale County, TX.

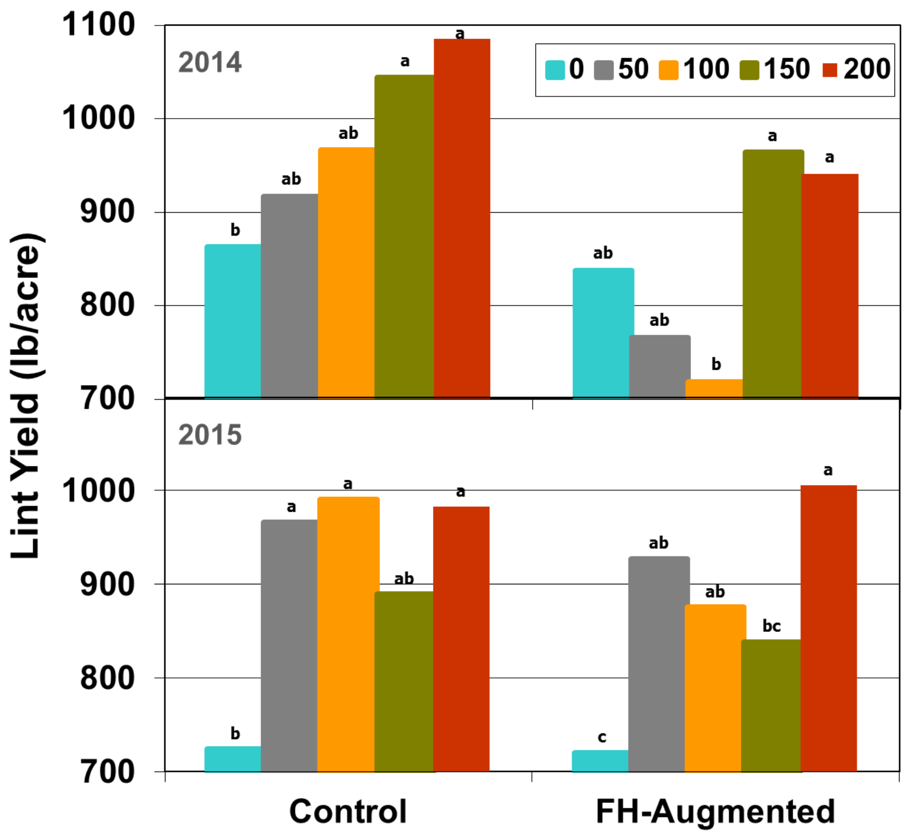

Figure 14. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on lint yield following a single acute infestation of cotton fleahopper versus uninfested control, 2014-2015, Hale County, TX.

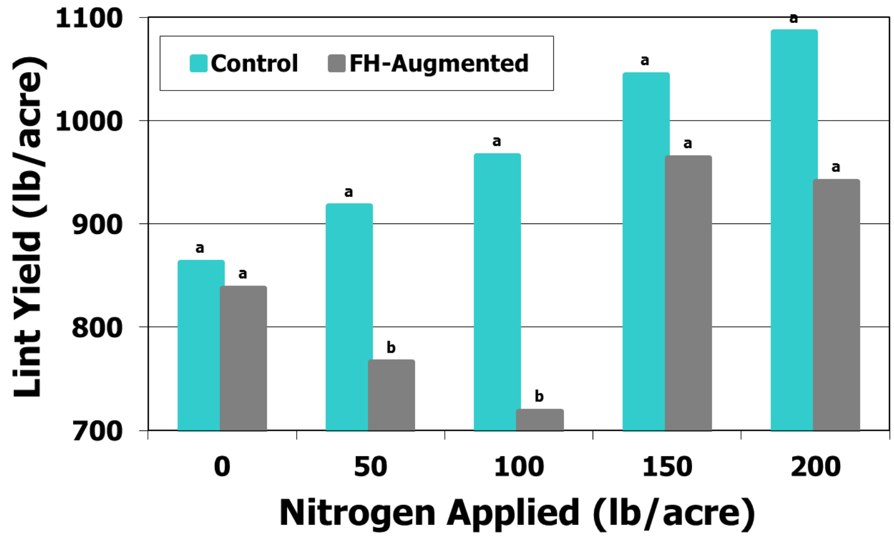

Figure 15. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on cotton lint yield following a single acute infestation of cotton fleahopper versus uninfested control, 2014, Hale County, TX.

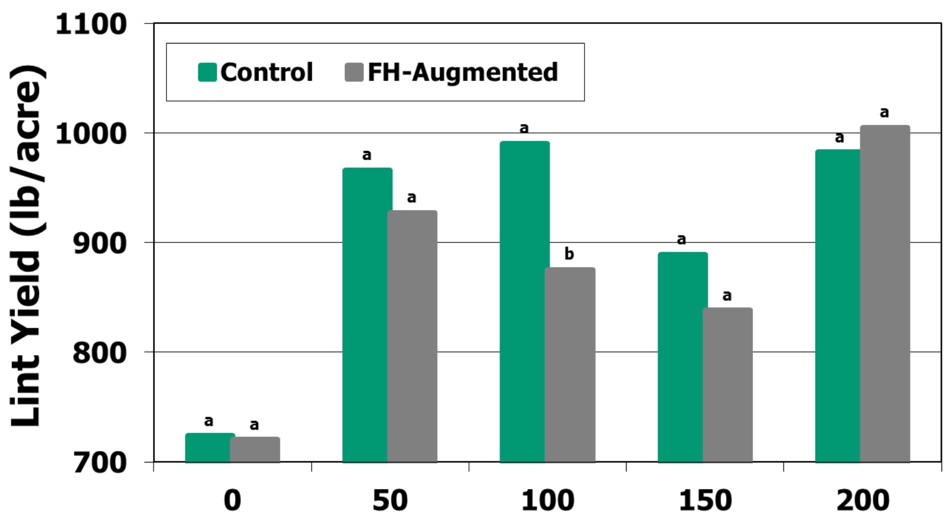

Figure 16. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on cotton lint yield following a single acute infestation of cotton fleahopper versus uninfested control, 2015, Hale County, TX.

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PROJECT FINAL REPORT
2012-2015

Cotton Incorporated/Texas State Support Program

Project Number: 12-112TX

Development of Economic Threshold and Management Recommendations for *Lygus* in Texas High Plains Cotton

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Development of Economic Threshold and Management Recommendations for *Lygus* in Texas High Plains Cotton

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PROJECT SUMMARY

Western tarnished plant bug, *Lygus hesperus*, is the primary *Lygus* species inhabiting cotton and several other crop hosts in the Texas High Plains. In Texas High Plains cotton, *Lygus* is generally more pestiferous in the boll development stage than in early squaring stage. Our recent study on boll damage assessment based on heat unit-delineated maturity provided a boll-safe cutoff value of 350 heat units (~2-3 weeks from flowering), although *Lygus* adults and nymphs both cause external lesions on bolls throughout boll development and may give farmers a false impression of *Lygus* damage. A four-year State Support funded project revealed that late-instar nymphs caused significantly more damage to maturing bolls than adults, and inflicted 23, 29, and 15% more loss in lint yield, seed weight, and seed counts per boll, respectively, versus adults. Nevertheless, no economic threshold for *Lygus* boll management has been developed for Texas cotton. The major goal of this project was to develop economic threshold-based management recommendations for *Lygus* in Texas High Plains cotton, thereby aiming to minimize economic losses to producers. Specific objectives were to: 1) determine the maximum potential for *Lygus* to inflict damage to cotton bolls at various boll maturity levels (ages), 2) characterize the cotton boll preference behavior of *Lygus*, and 3) establish the *Lygus* economic threshold for Texas cotton. Boll damage potential of *Lygus hesperus* was determined in a no-choice cup-cage study. Ten cohorts of cup-caged single bolls (1-20 days old) were each exposed to a *Lygus* adult for 48 hours and the boll damages were quantified. After bolls reached about two weeks of age, *Lygus* caused very little seed damage, which as expected, also did not result in significant lint yield loss. Cotton bolls were safe from *Lygus* damage when they reached >28 mm diameter or their carpel wall hardness was 0.7 lb per square foot or greater. Cotton boll feeding preferences of *Lygus hesperus*, within-plant boll distribution profile, and *Lygus* damage to cotton bolls at various *Lygus* densities were determined in a whole-plant and multi-plant cage field studies. Caged cotton plants were exposed to five levels of *Lygus* (0, 1, 2, 4, and 6 adults per plant) for one week when plants were at two selected boll development stages (350 and 550 HU after first flower). When the crop matured from 350 HU to 550 HU after first flower, the percentage of bolls vulnerable to *Lygus* feeding damage was reduced from 50% to 30%. Internal warts were mostly limited to the bolls measuring <35 mm in diameter. In this open-choice boll feeding situation, *Lygus* preferred to feed on bolls that were 10-30 mm in diameter. Averaged over four years, on a 1,200 lb/acre crop, artificial augmentation of 1, 2, 4, and 6 *Lygus* per plant at 350 HU after first flower reduced the cotton lint yield by 199, 271, 407, and 433 lb/acre, respectively, whereas the yield reduction values for the same *Lygus* densities were 52, 142, 229, and 269 lb/acre during the late season (550 HU from first flower). Thus, the *Lygus* yield reduction potential significantly decreased when cotton matured from 350 HU to 550 HU, with 71 lb/acre lint reduction per *Lygus* per plant infestation in late season compared with 127 lb/acre lint reduction per *Lygus* per plant in mid-season. Based on this study, *Lygus* management options for Texas High Plains cotton after the initiation of first flower are recommended as follows: careful monitoring of cotton crop and adjacent habitats for *Lygus* abundance and movement behavior and avoidance of insecticide treatment for *Lygus* in cotton prior to 200 HU from first flower, 2-4 *Lygus* per 6 row-ft for mid-season cotton, and 4-6 *Lygus* per 6 row-ft for late season cotton.

Introduction

Cotton, *Gossypium hirsutum* L., is a major cash crop in the U.S. and worldwide. The U.S. is the world's third largest cotton producer and the U.S. cotton industry is valued at more than 25 billion dollars per year. In Texas, approximately six million acres of cotton have been planted annually in recent years, and Texas is the largest cotton producing state (Williams 2013). *Lygus hesperus* is an important economic pest of cotton in some regions of the United States and it is an emerging pest of Texas High Plains cotton. In 2012, a 2.04% reduction in U.S. cotton yields was attributable to arthropod pests – 0.7% due to *Lygus* species, which was ranked top among other yield-reducing pests (Williams 2013) and also cost more per infested acre because multiple applications were often required. In Texas, over 2 million acres of cotton were infested by *Lygus* in 2012 (Williams 2013). *Lygus* can cause severe cotton square loss, anther damage, and seed damage depending upon the crop growth stage the infestation occurs. Both adult and nymphal stages of *Lygus* can inflict damage to cotton fruiting structures. *Lygus* late-instar nymphs are capable of inflicting greater internal damage to maturing bolls than are adults, and this was especially true for 1-2 week old (150-250 HU) bolls (Jubb and Carruth 1971, Parajulee *et al.* 2011). In the Texas High Plains region, *Lygus* generally infest cotton fields during the latter part of the cropping season, thus causing mostly damage to the cotton bolls. Following the introduction of *Bt*-technology (Bollgard® cotton), outbreaks of lepidopteran pests have been drastically reduced, and in recent years, secondary piercing-sucking pests such as *Lygus* are of increasing concern to Texas High Plains producers (Parajulee *et al.* 2008).

Cotton boll profiles change as crop matures, and as a result, the number of *Lygus* susceptible and/or tolerant bolls to *Lygus* damage also change. As boll maturity profiles change, *Lygus* boll selection and feeding behavior may also change which can result in different levels of crop injury and yield loss. There is a strong relationship between boll maturity and *Lygus* feeding damage, thus understanding the boll maturation profile and characterizing *Lygus* damage risk dynamics is very important. Because reliable *Lygus*-resistant or tolerant cotton cultivars are unavailable, cotton producers primarily rely on pesticides for *Lygus* management. Current pesticide application decisions are based on field scouting, whereby spray applications are typically warranted when *Lygus* populations exceed locally established economic threshold (ET) levels.

Oosterhuis and Kim (2004) reported that cotton bolls that accumulated 350-450 heat units were safe from piercing-sucking insects. It is expected that *Lygus hesperus* may also be unable to damage cotton bolls once a certain boll maturity level has been reached, after which pesticide applications would not be necessary. However, the actual boll damage potential of *Lygus hesperus* is largely unknown. One important question in this study was: At what point do maturing bolls or the entire crop become "safe" from *Lygus* feeding damage, and, consequently, when does insecticide use become unnecessary? Given the availability of tools to identify when the bolls are safe, timing of insecticide use termination may be refined to minimize unnecessary economic and ecological costs.

The objectives of our field experiments were to: 1) determine the maximum potential for *Lygus* to inflict damage to cotton bolls at various boll maturity levels (ages), 2) determine the cotton boll maturity profile during two boll development stages (at 350 and 550 HU After First

Flowering [AFF]), 3) determine the boll feeding preference of *Lygus hesperus* adults as affected by the change in boll maturity profile as the crop matures from 350 HU to 550 HU AFF, and 4) quantify the yield loss caused by five different levels of *Lygus* infestations (0, 1, 2, 4, and 6 *Lygus* adults per plant). The overall goal was to better understand the boll feeding biology and behavior of *Lygus hesperus* in order to further develop a dynamic economic threshold for improved *Lygus* management in Texas High Plains cotton.

Materials and Methods

Estimating *Lygus* Boll Damage Potential

A field study to quantify adult *Lygus hesperus* cotton boll damage potential was conducted at the Texas A&M AgriLife Research and Extension Center farm located near Lubbock, Texas. Cotton cultivar ST5458B2RF was planted on May 18 (2012), May 22 (2013), and May 15 (2014), and ST4946GLB2 on June 3 (2015) in a drip-irrigated field with 40-inch row spacing. The targeted seeding rate was 56,000 seeds per acre. On June 2, the 2012 study was treated with Orthene® 97S for thrips at a rate of 3.0 oz per acre and with Cornerstone Plus® herbicide (41% glyphosate) at 32 oz per acre for weed management, whereas the 2013 and 2014 study plots did not receive insecticide interventions for thrips control and weeds were removed via hand-hoeing. In 2015, RoundUp® @ 32 oz/acre and Warrant® 3 pt/acre were applied on June 30 and July 29, respectively, for weed management.

2012 Study. The experimental design was a split-plot randomized block with three replications. Ten cotton boll age cohorts (1 to 20 days from flowering at 1-day increment) served as the main plot and two *Lygus* infestation levels (I: one adult *Lygus* feeding for 48 hours, and II: control or zero bugs) served as subplots. Thus, there were 30 main plots (3 blocks x 10 boll age cohorts), each of which consisted of 100 ft long cotton rows. In each main plot, 20 randomly selected white flowers were individually cup-caged using modified polystyrene foam and cloth-net "cup cages" (Fig. 1). Thus, a total of 600 white flowers were cup-caged (30 main plots x 20 flowers per main plot). Two treatment levels (control and single *Lygus* bug infestation) were applied in each main plot. Each plot contained 20 cup-caged bolls of which 5 bolls were used as controls, and the remaining 15 bolls were exposed to *Lygus* feeding. Cotton bolls in the Texas High Plains region typically accumulate 14-30 HU per day in August; thus, in ten days following cup-caging the fruit, on August 20, the August 1st cup-caged bolls had received about 450 HU, whereas the August 10th cup-caged bolls had accumulated approximately 200 HU. Once the cotton bolls received 200-450 HU, individual *Lygus* adults were released in the appropriate cages and allowed to feed for 48 hours. *Lygus* adults were initially reared on artificial diet, but were "trained" on fresh green beans and cotton squares for a week prior to using them for the boll feeding experiment. Prior to release into the cup-cages, the *Lygus* adults were starved for 4-5 h. Five *Lygus* infested bolls from each plot were used for boll size, weight, carpel wall hardness and *Lygus* damage assessment (internal and external *Lygus* damage lesions), while the remaining ten *Lygus* infested bolls were kept for yield assessments. Both control bolls and the bolls kept for yield assessment were harvested during the first week of November, 2012.

2013 Study. The study was deployed in a split-plot randomized block design with three replications (blocks) to quantify the effect of *Lygus* density and infestation timing on cotton yield and quality. The study consisted of two *Lygus* infestation levels (one adult *Lygus* feeding for 48 hours versus zero bugs) as main plot factors and ten cotton boll age cohorts (every-other-day caging of bolls from Day 1 to Day 20) as subplot factors. Thus, there were 60 experimental units. Each experimental unit had eight individually caged bolls as subsamples, thus, this study comprised of a total of **480 individually caged cotton bolls** (three blocks x two *Lygus* infestation levels x ten boll age cohorts x eight subsamples).

Cotton field was divided into three blocks. Each block consisted of 10 cotton rows, representing 10 boll age cohorts. Every two days for a period of 20 consecutive days (July 29 to August 18), one cotton row (a main plot) was randomly selected and twenty randomly selected new, white flowers were individually tagged, yielding 10 cotton boll age cohorts. On Day 21 (August 19), all 480 bolls were caged using modified polystyrene foam and cloth-net "cup cages" and individual *Lygus* adults were released in the appropriate cages and allowed to feed for 48 hours. Control cages received zero insect augmentation. After 48 hours, released *Lygus* bugs were killed in all cages and 50% of the infested bolls from each boll age cohort were retrieved and processed in the laboratory to evaluate internal and external *Lygus* damage lesions, boll weight, diameter, and boll hardness. The remaining 50% of the infested bolls were kept for harvest to determine yield and lint quality.

2014 Study. The study was deployed in a split-plot randomized block design with three replications (blocks) to quantify the damage potential of *Lygus* adults and late-instar nymphs with respect to cotton boll development stage. The study consisted of three *Lygus* infestation levels (one adult *Lygus* feeding for 48 hours, one late-instar nymph feeding for 48 hours, and zero bugs per boll) as main plot factors and ten cotton boll age cohorts (every-other-day caging of white flowers, also referred to as 1-day old bolls, from Day 1 to Day 20) as subplot factors. Thus, there were 90 experimental units. Each experimental unit had four individually caged bolls as subsamples, thus, this study comprised of a total of 360 **individually caged cotton bolls** (three blocks x three *Lygus* infestation levels x ten boll age cohorts x four subsamples).

Cotton field was divided into three blocks. Each block consisted of 10 cotton rows, representing 10 boll age cohorts. Every two days for a period of 20 consecutive days (4 August to 22 August), one cotton row (a main plot) was randomly selected and fifty randomly selected new, white flowers were individually tagged, yielding 10 cotton boll age cohorts. On Day 20 (23 August), all 360 bolls were caged using modified polystyrene foam and cloth-net "cup cages" and individual *Lygus* adults or nymphs were released in the appropriate cages and allowed to feed for 48 hours. Control cages received zero insect augmentation. After 48 hours, released *Lygus* bugs were killed in all cages and 25% of the infested bolls from each boll age cohort were retrieved and processed in the laboratory to evaluate internal and external *Lygus* damage lesions, boll weight, diameter, and boll hardness. The remaining 75% of the infested bolls were kept for harvest to determine yield and lint quality. These individual bolls were harvest on October 22.

2015 Study. A split-plot randomized block field study was deployed with 10-15 replications. The study was an extensive undertaking in which ten cotton boll age cohorts (tagging of bolls every-other-day from Day 1 to Day 20) served as main plot and three *Lygus* infestation levels (I: one *Lygus* nymph feeding for 72 hours, II: one *Lygus* adult feeding for 72 hours, and III: control or zero bugs) within each boll age served as subplots. Individually tagged (and caged on Day 21) bolls served as replicate, thus, this study comprised of a total of **750 individually caged cotton bolls** (25 replications x three *Lygus* infestation levels x ten boll age cohorts). To ensure the retention of 750 caged bolls after 20 days of tagging, we tagged 1000 bolls (100 bolls per day). Every two days for a period of 20 consecutive days (July 27, 29, 31, August 2, 4, 6, 8, 10, 12, and 14), one cotton row was consecutively selected and 100 randomly selected new, white flowers were individually tagged, yielding 10 cotton boll age cohorts.

Cotton bolls in the Texas High Plains accumulate an average of 20 HU per day, thus optimally; the oldest cup-caged boll will (at 20 days) have accumulated 400 HU. On Day 21 (August 15), all tagged bolls were caged using modified polystyrene foam and cloth-net cup cages. Individual *Lygus* adults (n=25 bolls per boll-age cohort) and nymphs (n=12 bolls per boll-age cohort) were released in the appropriate cages and allowed to feed for 72 hours. Control cages (n=13 bolls per boll-age cohort) received zero insect augmentation. After 72 hours, released *Lygus* bugs were killed in all cages on August 19 and 3 (control), 4 (nymph-augmented), and 6 (adult-augmented) bolls from each boll age cohort were retrieved and processed in the laboratory to evaluate internal and external *Lygus* damage lesions. The remaining bolls were kept for harvest to determine yield and lint quality. These single bolls were harvested on October 9.

Figure 1. Deployment of cup-cages to enclose age-specific bolls for *Lygus* damage potential study, Lubbock, TX, 2012-2015.

Determination of Boll Maturation Profile, Feeding Preference and Economic Threshold

A field study was conducted to quantify the effect of *Lygus* density and infestation timing on cotton yield and fiber quality. Cotton planting and field management operations were the same as described for *Estimating Lygus Boll Damage Potential* section above.

For 2012-2014 studies, the experiment was laid out in a split-plot randomized block design with three replications, two main plot factors (two cotton boll developmental stages [early boll development and late boll development]), and four subplot factors (four levels of *Lygus* infestation [control or zero bugs, one bug/plant, two bugs/plant, and four or six bugs/plant]). There were a total of 24 experimental units. Each experimental unit had 8 cotton plants as subsamples (3 used for damage assessment and 5 for yield and quality assessment). A total of 192 whole-plant sleeve-caged cotton plants (three blocks x two cotton boll stages x four *Lygus* densities x eight subsamples) were used for this study (Fig. 2).

The cotton field study site was closely monitored and kept virtually arthropod pest-free until cages were deployed on July 24, July 29, and July 28 in 2012, 2013, and 2014, respectively. When the cotton plants reached the target maturity level (350 HU >60 °F after first flower on August 7, August 13, and August 17 in 2012, 2013, and 2014, respectively, and 550 HU >60 °F after first flower on August 21, August 29, and August 27 in 2012, 2013, and 2014, respectively), field-collected *Lygus* were released into the whole-plant sleeve-cages at the rates of 0, 1, 2, and 4 *Lygus*/plant in 2012 and 2013; the infestation densities were changed to 0, 2, 4, and 6 *Lygus*/plant in 2014 to increase the damage intensity. *Lygus* adults were collected from nearby alfalfa field or from adjacent counties and then acclimatized in the laboratory for 48 hours before using them for the boll feeding experiment. Cotton plants were exposed to the *Lygus* adults for ~7 days, after which time, the insects were killed via a pesticide application. Three randomly selected cotton plants from each plot were clipped near the soil surface and brought to the laboratory on August 13, August 19, and August 27 for the 350 HU and August 29, September 2, and September 5 for the 550 HU plots in 2012, 2013, and 2014, respectively.

In 2015, the study layout was similar to that for previous years, but we evaluated three main plot factors (three cotton boll developmental stages [early, mid, and late boll development]), and five subplot factors (five levels of *Lygus* infestation [control or zero bugs, one bug/plant, two bugs/plant, four bugs/plant, and six bugs/plant]), replicated four times. Three boll development stages were characterized as bolls having accumulated 200 HU, 350 HU, and 550 HU, representing "early", "mid", and "late" *Lygus* boll infestations, respectively. Forty-five plot sections (3-ft row section with >50% of the plants with the first white flower on the plant) were marked on 27 July and the daily heat unit accumulation (>60 ^{o}F) was calculated to prepare for treatment deployment. Plant stand inside each 3-ft section was thinned to maintain 6 plants per section (1 was used for damage assessment and 5 for yield and quality assessment) on the day of cage deployment. On 5 August, heat unit accumulations reached ca. 200 HU. The experimental cages were deployed on pre-selected 6-plant sections and the density treatments were applied on this date. *Lygus* adults and nymphs were collected from a nearby alfalfa field and released @ 0, 1, 2, 4, or 6 *Lygus* per cage. Separate studies were conducted for *Lygus* adults and nymphs. Cages were removed on August 15, one plant per cage was removed for damage assessment, and the remaining plants were sprayed with an insecticide to kill augmented *Lygus*. *Lygus* feeding on mid- and late boll development stages were evaluated by releasing the same *Lygus* density treatments as described for early boll development stage. *Lygus* density treatments were deployed on August 9 (350 HU) and 18 (550 HU) and terminated on August 19 and 28 for mid and late boll development stages, respectively.

The cotton crop was defoliated by spraying FOLEX® 6EC (12 oz per acre) and a boll opener (Ethephon® 6; 32 oz per acre) in a tank mix in 2012-2014, but the 2015 crop was terminated via natural freezing. After the crop was ready to harvest, the remaining 5 caged plants from each plot, which had been maintained pest-free, were harvested manually to evaluate the lint yields and fiber quality. Harvested single-plant samples (2012-2014) or 5-plant sample per cage (2015) were ginned individually via table-top gin and samples were analyzed for fiber quality (HVI) parameters at Cotton Incorporated. ANOVA, GLM model via SAS (2010) was used to evaluate the treatment effects (α=0.1) and treatment means were compared by LSMEAN procedure.

Figure 2. Field deployment of single-plant cages (2012-2014) and multi-plant cages (2015) for *Lygus* threshold study, Lubbock.

Results and Discussion

Boll Development vs. *Lygus* Damage Potential

The Lubbock area cotton crop during the August 1-20 period in 2012 received ≈24 HU per day and bolls developed rapidly. The diameter of the cotton bolls grew at an average rate of 1.2 mm per day and gained an average of 1.4 grams of weight per day. As the bolls matured and became larger, the carpel walls became harder as evidenced by the pressure required to puncture the carpel wall, increasing at a rate of 0.018 lb per square foot per day (Fig. 3). The 2013 and 2014 boll development patterns were similar to that for 2012. When forced to feed on a single boll, each *Lygus* adult inflicted, averaged across all boll age cohorts, 10-28 external lesions per boll in 48 hours. Numerous external lesions were found in all bolls, irrespective of their age. It indicates that in a "no-choice" feeding situation *Lygus* can cause external feeding injury to all bolls, but the actual number of damaged seeds was significantly reduced as bolls became older, larger and tougher to puncture. When bolls reached an age of 16 days (2012) or 13 days (2013), *Lygus* caused very little seed damage (<2 seeds per boll) that did not result in significant lint yield reductions (Figs. 4-5). We were unable to derive this relationship for 2014 data due to field management failure prior to harvest. When cotton bolls received >350 HU after first flower, they were safe from *Lygus*-induced fiber yield loss. Cotton bolls were observed to be safe from *Lygus* damage when the bolls: 1) exceeded >28 mm in diameter, 2) weighed >14 g, or 3) carpel wall puncture force exceeded 0.7 lb per square foot (Figs. 3-5).

Boll damage potential significantly increased as bolls mature from Day 1 to Day 7, demonstrating that the 1-wk old bolls are the most sensitive to *Lygus* injury. The damage potential begins to decrease after 7 days, but bolls are still susceptible to *Lygus* injury for about another 5-6 days. Considering year-to-year variations, it appears that the maturing bolls are no longer susceptible to *Lygus* injury two weeks after white flower (Figs. 4 and 5).

In a multi-plant cage study (2015), external feeding mark (sunken lesions on the external surface of the boll) numbers were considerably higher in early season bolls compared to that in late season. Number of external lesions per boll increased with increased *Lygus* density, which is especially pronounced during the early season period (Fig. 6). Four *Lygus* per plant caused significantly higher external lesions compared to the control and the 1 and 2 *Lygus* per plant treatments; however, increasing the density to six *Lygus* per plant did not increase the external feeding injury marks (Fig. 6). Our previous study suggested that the survivorship of the field-collected and cage-released *Lygus* adults in the Texas High Plains is about 20-25%. Therefore, our highest actual density was set around 1-1.5 bugs per plant. Internal injury followed the similar trend as for external lesions, with an increased number of internal injury warts as *Lygus* densities increased. Regardless of the crop's phenological stages, *Lygus* caused internal injuries to the bolls compared to that in control cages. However, a density-dependent relationship between *Lygus* density and internal boll damage was more evident in late season (Fig. 7).

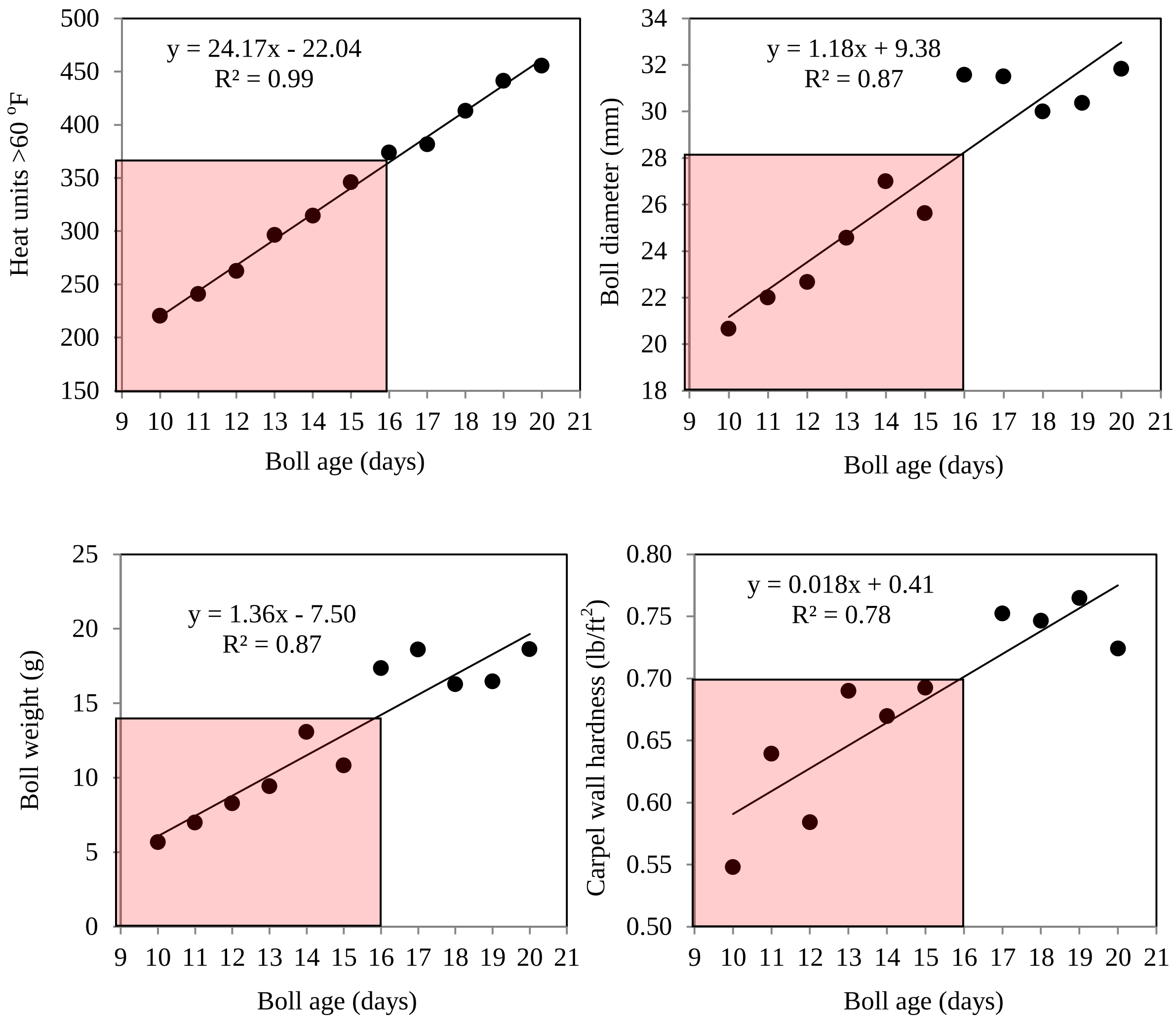

Figure 3. Cotton boll age relationships as associated to heat unit accumulations, boll size, boll weight, and carpel wall hardness, Lubbock, Texas, 2012.

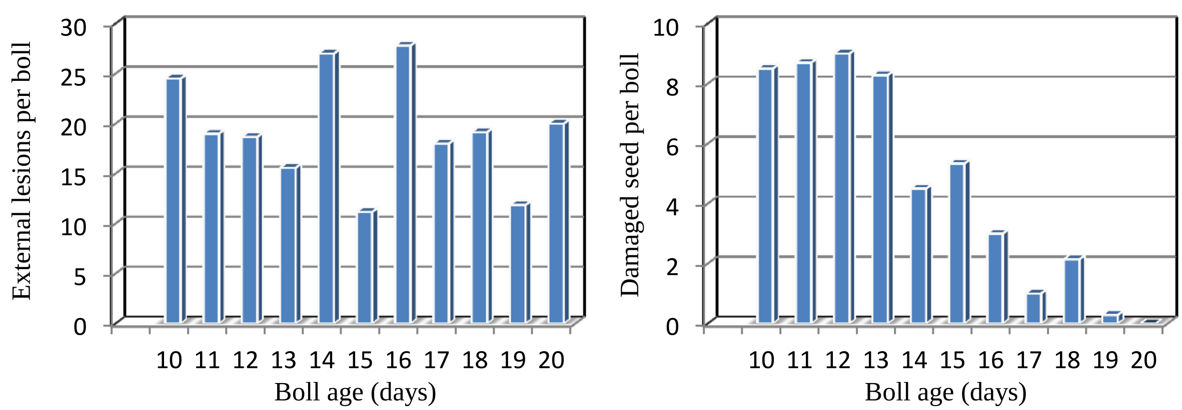

Figure 4. Cotton boll injury (external lesions and damaged seeds) at various boll ages following a 48-h feeding of a single *Lygus* adult, Lubbock, TX, 2012.

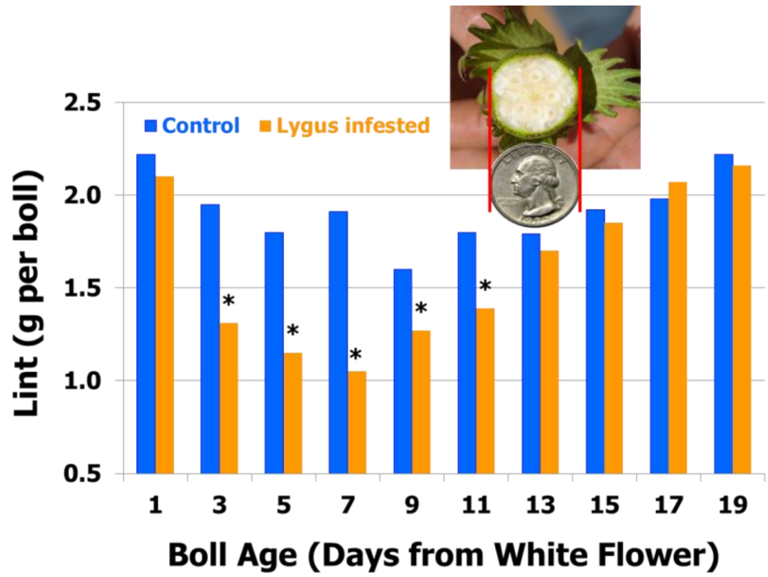

Figure 5. Single-boll lint yield (grams per boll) following 48 hours of feeding by a single *Lygus* adult versus uninfested boll at boll ages ranging from Day 1 to Day 19, Lubbock, TX, 2013. * indicates that the *Lygus*-infested bolls resulted in significantly lower yield than non-infested bolls.

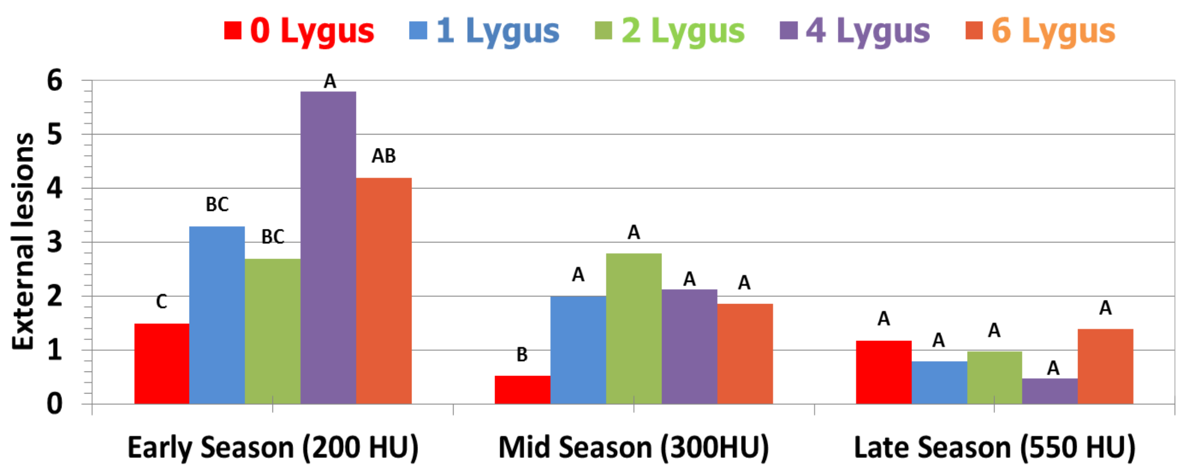

Figure 6. Cotton boll external injury (external lesions) at two phenological stages of cotton following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages, Lubbock, TX, 2015.

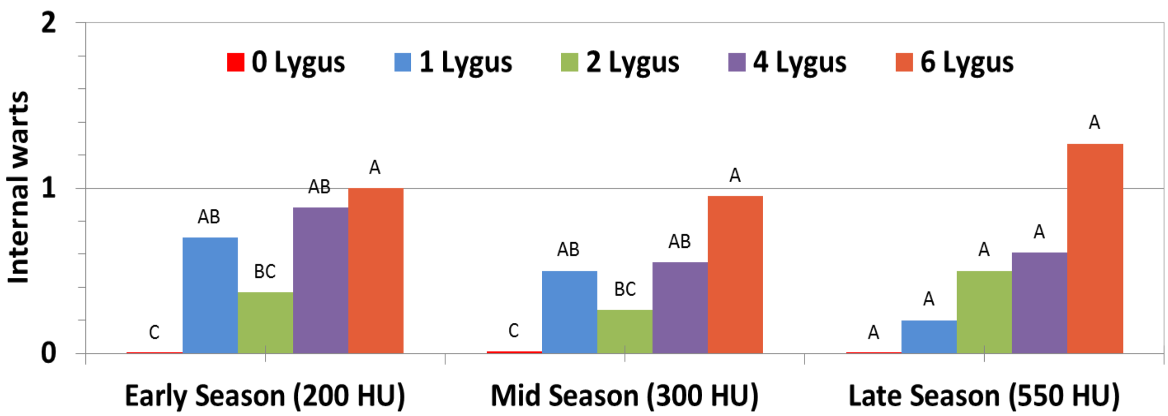

Figure 7. Cotton boll internal injury (internal warts) at two phenological stages of cotton following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages, Lubbock, TX, 2015.

Fruiting Profile

At 350 HU after first flower, an average of 57% fruit retention was observed, but fruit retention was decreased to 37% when cotton reached 550 HU after first flower. Cotton plants at 350 HU were observed to have 84% bolls, 14% squares and 2% flowers, while at 550 HU, the cotton plants had 99% bolls, 1% squares, and no flowers. Although there were a higher percentage of cotton bolls on 550 HU plants, the actual number of bolls per plant decreased from an average of 8.8 bolls per plant at 350 HU to 6.3 bolls at 550 HU. Approximately 28.4% of the bolls were naturally aborted from the plants as they matured from the 350 HU to 550 HU stage (Fig. 8).

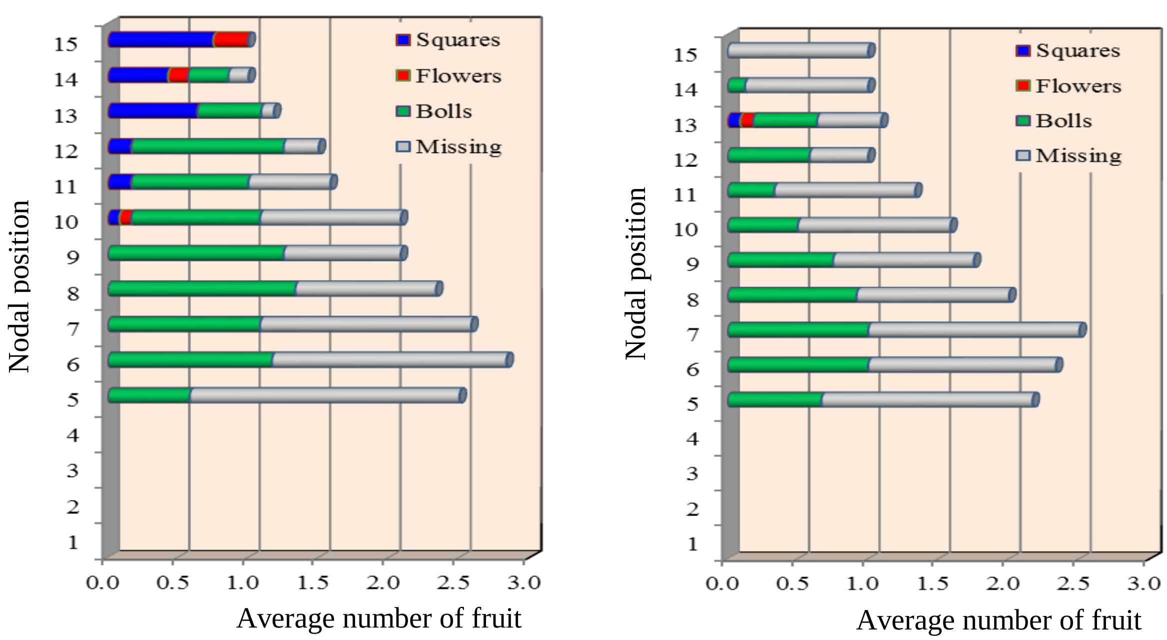

Figure 8. Fruiting profile at 350 (left) and 550 (right) HU after first flower, Lubbock, TX, 2012.

Most of the bolls were from first fruiting positions of the sympodial branches. At 350 HU, 66%, 24%, 8%, and 2% bolls were from the first, second, third and fourth sympodial branch fruiting positions, respectively; while at 550 HU, 81%, 16%, 3%, and 0% bolls were from the first, second, third and fourth sympodial branch fruiting positions, respectively (Fig. 9). When the cotton plants matured from 350 HU to 550 HU, they dropped all of the 4th fruiting position and most of the 3rd fruiting position bolls. Since 97% of the bolls were on first and second fruiting positions on the cotton plants at the 550 HU stage, our sampling and crop protection efforts should be focused on protecting primarily the first and second position bolls at this stage. However, fruiting profiles may vary with cotton cultivar, cotton growing region, and crop management practices and input use patterns.

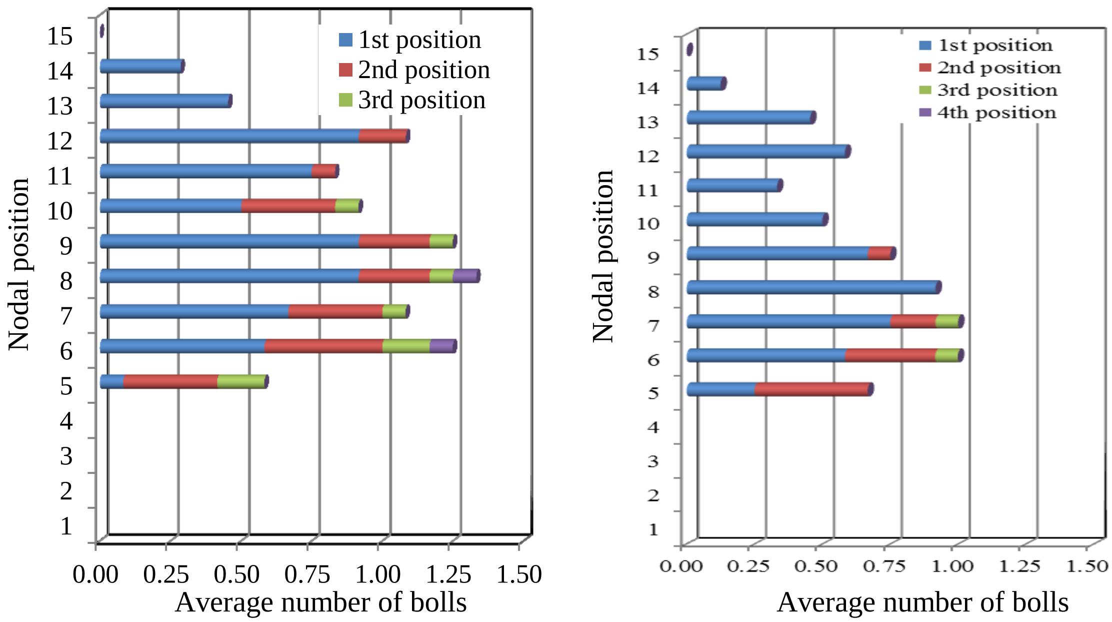

Figure 9. Boll distribution on sympodial branches at 350 (left) and 550 (right) HU after first flower, Lubbock, TX, 2012.

Boll Maturation Profile

Thirty-two cotton plants were harvested (16 plants each from 350 HU and 550 HU plots) from which 643 bolls were retrieved. Boll diameter was measured using a Vernier caliper and bolls were categorized into 6 boll size groups (5-10, 11-15, 16-20, 21-25, 26-30 and 31-35 mm). Our past research indicates >25 mm diameter sized cotton bolls are safe from *Lygus* damage. Plants at 350 HU had 47% of the bolls safe from *Lygus* damage (larger than 25 mm diameter), whereas after 2 additional weeks, cotton in the same field had 70% of the bolls safe from *Lygus* damage. When the cotton crop matured from 350 to 550 HU, the proportion of bolls vulnerable to *Lygus* feeding damage was reduced from 53% to 30%. Therefore, it is likely that with a similar level of *Lygus* infestation, *Lygus* may cause a greater amount of cotton yield loss when infesting a mid-season crop (350 HU) compared to that for a late season infestation (550 HU).

For our 2012 cotton crop, within-plant cotton boll maturation profile shows that bolls distributed from the 5th to 14th nodes (Fig. 8). At the 350 HU stage, the top 4 bolls (from 10-13th node) were <25 mm diameter size and were vulnerable to *Lygus* damage if bugs were present (data not shown). When the cotton reached 550 HU, only the top 3 bolls (nodes 11-13) were <25 mm diameter size and therefore vulnerable to *Lygus* damage, if present. Bolls from the 5th to 9th nodes were larger and less vulnerable to *Lygus* feeding damage.

There was a very strong positive relationship between boll size (diameter) and the hardness of the boll carpel wall. As we move from the top to bottom nodes of a cotton plant, as expected, we found larger bolls with harder carpel walls (Fig. 10). The vertical boll profile suggests that cotton growers or crop consultants need to focus their *Lygus* damage evaluations primarily during the 350-550 HU, and mostly on the top 3-4 bolls, since they are the most vulnerable to *Lygus* feeding injury. The 2013 data also showed similar trends in terms of within-plant boll maturation distribution.

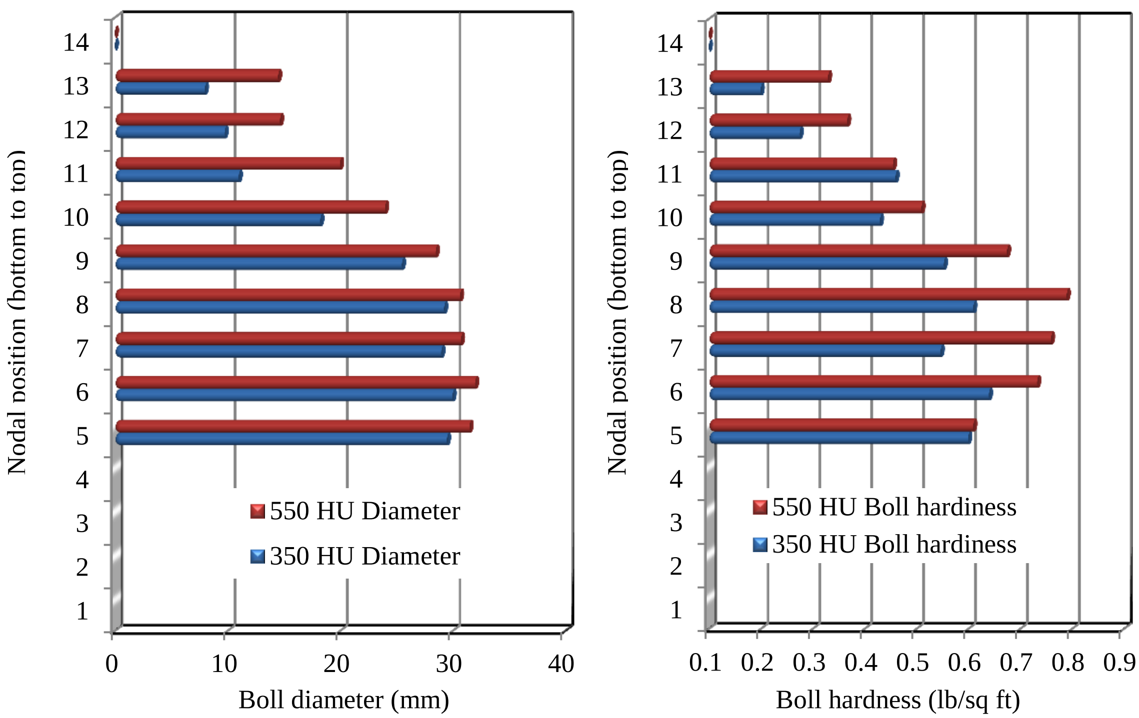

Figure 10. First position boll size profiles of 350 and 550 HU cotton after first flower, Lubbock, TX, 2012.

Lygus Boll Feeding Preference and Boll Damage

In the whole-plant caging study, *Lygus* external feeding lesions were found in bolls of all sizes, indicating *Lygus* attempted to feed on cotton bolls irrespective of boll size. Nevertheless, successful punctures and the resulting internal warts were limited to the bolls <35 mm in diameter. A significantly higher proportion of bolls had internal warts (>20% of bolls) for <30 mm bolls, indicating that in an open-choice situation, *Lygus* preferred to feed on bolls that were <30 mm in diameter (Fig. 11). Cotton plants at the 350 HU had 90% of the bolls measuring <30 mm in diameter, whereas plants at the 550 HU had 78% of the bolls at <30 mm diameter (Fig.

11). The no-choice cup-cage study showed bolls that are >25 mm diameter were safe from *Lygus* damage, whereas in the open-choice whole-plant caging study, *Lygus* preferred to feed on bolls up to 30 mm in diameter. This slight discrepancy might be due to differences in cotton boll development inside cup-cages versus whole-plant cages, or due to differences in *Lygus* behavior in the presence of different boll size options and containments. Evaluation of internal lesions and internal warts suggests there is not a significant relationship between external *Lygus* feeding lesions and actual seed damage due to *Lygus* feeding (Fig. 12), but there were strong relationships between the number of internal warts and number of *Lygus* damaged seed. It clearly indicates that estimating *Lygus* damage by using external lesions can be misleading; therefore, it is best to use the number of internal warts to estimate the degree of *Lygus* crop damage.

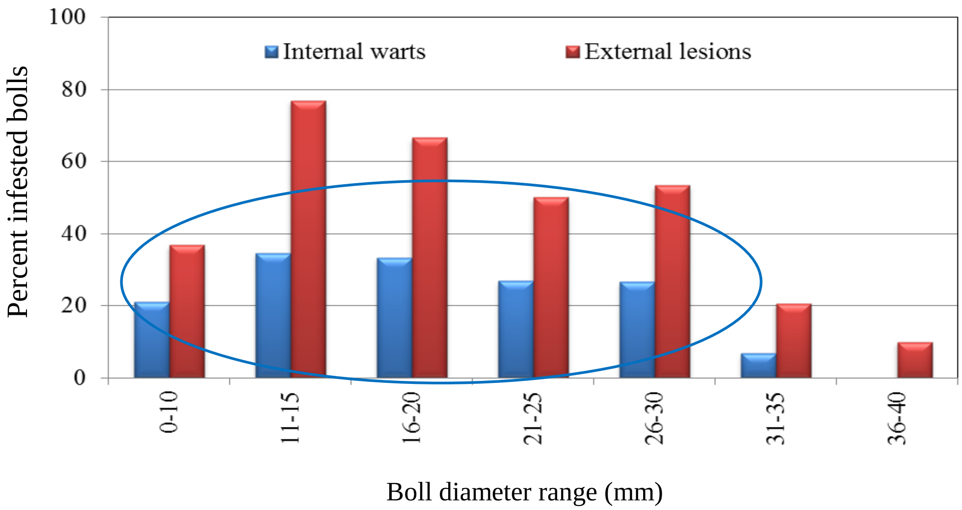

Figure 11. Boll feeding preference of *Lygus* in whole-plant cages based upon the proportion of external and internal boll damage. Lubbock County, TX, 2012.

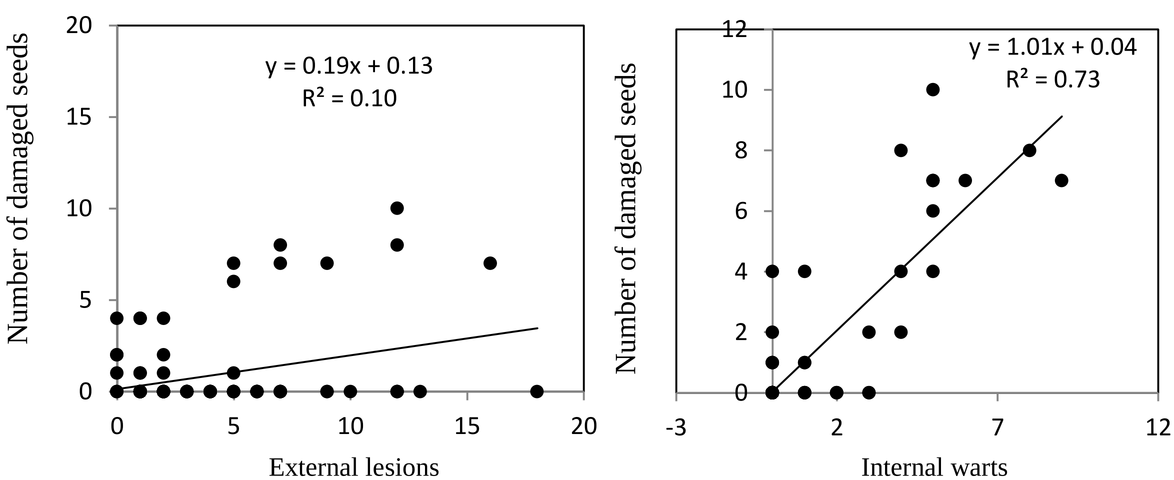

Figure 12. Relationships between the number of damaged seeds per boll and the number of external lesions or internal warts, Lubbock, TX, 2012.

Yield Loss

In general, as expected, *Lygus* augmentation reduced the lint yield compared to that in uninfested control cages (Figs. 13-16). However, the damaging effect of *Lygus* was more pronounced during mid-season (350 HU from first flower) compared to that in late season (550 HU from first flower) for all four years of the study.

In 2012, artificial augmentation of 2-4 *Lygus* bugs per plant at 350 HU after first flower significantly reduced the cotton lint yield, but the same level of *Lygus* infestation at 550 HU did not result in significant lint yield reduction compared with that in uninfested control plants (Fig. 13). Augmentation of 1, 2, and 4 *Lygus* bugs per plant at 350 HU after first flower reduced the cotton lint yield by 116, 425, and 580 lb/acre, respectively, whereas the yield reductions for the same *Lygus* densities were 125, 149, and 185 lb/acre during the late season (550 HU from first flower).

These data suggest that the maturing bolls are much more tolerant to *Lygus* injury when the plant attains 550 HU from first flower. It is also possible that *Lygus* bugs may choose to feed on superfluous bolls or squares and the yield contributing fruits may not be significantly impacted by such late infestations. Because potential yield loss risks due to certain *Lygus* density infestations vary with boll maturation profile, the *Lygus* management economic threshold should be optimized for a dynamic ET to accommodate for within-plant fruit maturity profiles.

In 2013, cotton lint yields in mid-season plots (cages) were much lower than in 2012, but the augmentation of 1, 2, and 4 *Lygus* bugs per plant reduced the cotton lint yield by 157, 106, and 281 lb/acre, respectively (Fig. 14). While these lint yield reduction values were not statistically significant, owing to greater variation in data, the trend was convincingly supportive of a clear influence of *Lygus* augmentation on yield reduction and the data trend was similar to that in 2012. Overall, lint yield was higher in late-season test plants compared to that in mid-season test plants, but the augmentation of 1 *Lygus* per plant did not result in significant yield reduction, whereas 2 and 4 *Lygus* per plant reduced 143 and 159 lb/acre, respectively (Fig. 14).

In 2014, augmentation of 2, 4, and 6 *Lygus* per plant at 350 HU after first flower reduced the cotton lint yield by 407, 406, and 516 lb/acre, respectively, whereas the yield reductions for the same *Lygus* densities were 282, 295, and 415 lb/acre during the late season (550 HU from first flower) (Fig. 15). Overall yield in 2014 was higher than in 2012 and 2013, but the damage inflicted by 2 and 4 *Lygus* per plant on mid-season cotton was comparable to that for 2012, whereas the damage inflicted in late season cotton was higher in 2014 compared to that in 2012 or 2013.

In 2015, overall, lint decreased for each successive phenological stages of cotton due to an artifact of experimental logistics (Fig. 16). Each cage contained about 12 plants and plants were thinned to 6 per cages at the time of insect release. As a result, the early season test had significantly more time to compensate for thinned plant densities compared to when we thinned the densities in successive phenological stages. Within each phenological stage, higher *Lygus* densities significantly reduced the lint yield compared to that in control cages. Early season crop compensated for boll injury and the yield in 0, 1, and 2 *Lygus*-augmented cages were similar. During mid-season, *Lygus* infestations reduced yield significantly for all densities, indicating the greater vulnerability of the mid-season crop to *Lygus* injury in the Texas High Plains. In late season, low densities of *Lygus* (1 or 2 *Lygus*/plant) overcompensated the yield as *Lygus* likely fed on young, non-harvestable fruits which provided an opportunity for harvestable bolls to mature (Fig. 16). The seed yield followed the same pattern as observed for lint yield (Fig. 17).

Lygus-induced lint yield reduction for a given *Lygus* density was lower for late season compared to that for mid-season infestations in all four years of the study (Figs. 13-16). Averaged over four years, on a 1,200 lb/acre crop, artificial augmentation of 1, 2, 4, and 6 *Lygus* per plant at 350 HU after first flower reduced the cotton lint yield by 199, 271, 407, and 433 lb/acre, respectively, whereas the yield reduction values for the same *Lygus* densities were 52, 142, 229, and 269 lb/acre during the late season (550 HU from first flower). Thus, the *Lygus* yield reduction potential significantly decreased when cotton matured from 350 HU to 550 HU.

These data clearly suggest that the maturing bolls are more tolerant to *Lygus* injury when the plant attains 550 HU from first flower. It is also possible that *Lygus* bugs may choose to feed on superfluous bolls or squares and the yield contributing fruits may not be significantly impacted by such late infestations. Because potential yield loss risks due to certain *Lygus* density infestations vary with boll maturation profile, the *Lygus* management economic threshold should be optimized for a dynamic ET to accommodate for within-plant fruit maturity profiles. Regression analysis of the four-year data suggests that *Lygus* adults could inflict maximum lint losses of 127 and 71 lb/acre, respectively, for mid- versus late season infestations of per unit (1 adult) *Lygus* per plant (Fig. 18). Based on this study, *Lygus* management options for Texas High Plains cotton after the initiation of first flower are recommended as follows: 1) careful monitoring of cotton crop and adjacent habitats for *Lygus* abundance and movement behavior and avoidance of insecticide treatment for *Lygus* in cotton prior to 200 HU from first flower, 2) 2-4 *Lygus* per 6 row-ft for mid-season cotton, and 3) 4-6 *Lygus* per 6 row-ft for late season cotton.

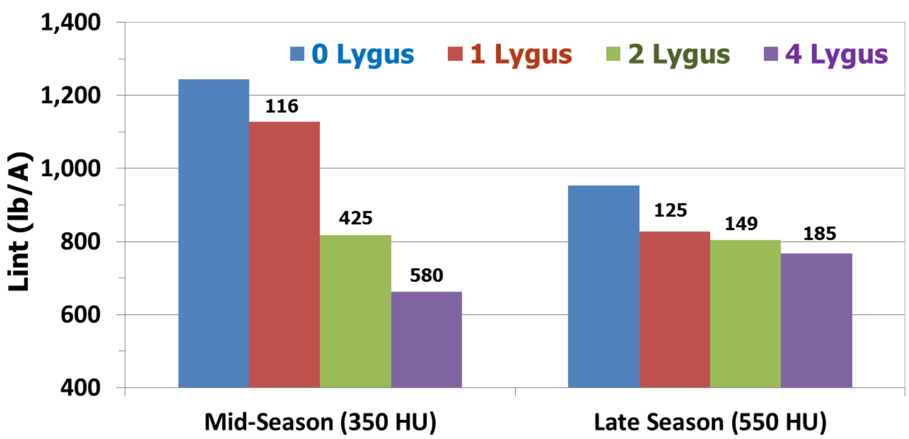

Figure 13. Influence of varying levels of *Lygus* infestations on lint yields at two crop phenological stages, as measured by heat-unit accumulation beyond first white flower, Lubbock County, TX, 2012.

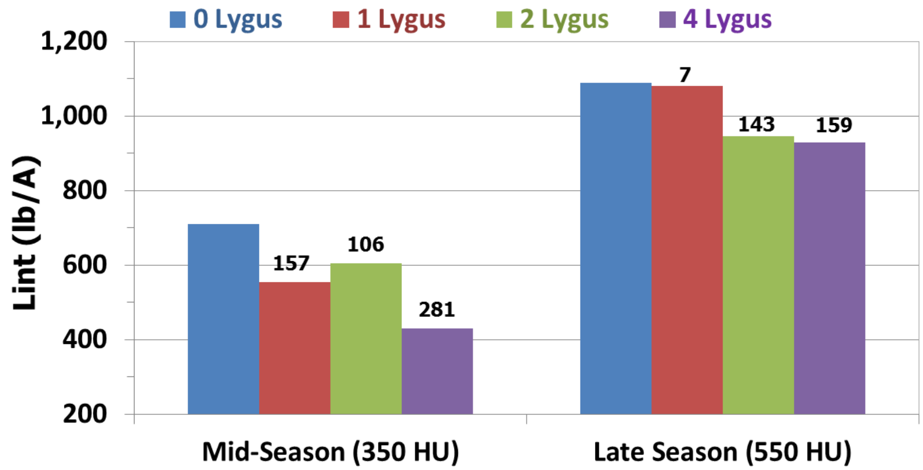

Figure 14. Influence of varying levels of *Lygus* infestations on lint yields at two crop phenological stages, as measured by heat-unit accumulation beyond first white flower, Lubbock County, TX, 2013.

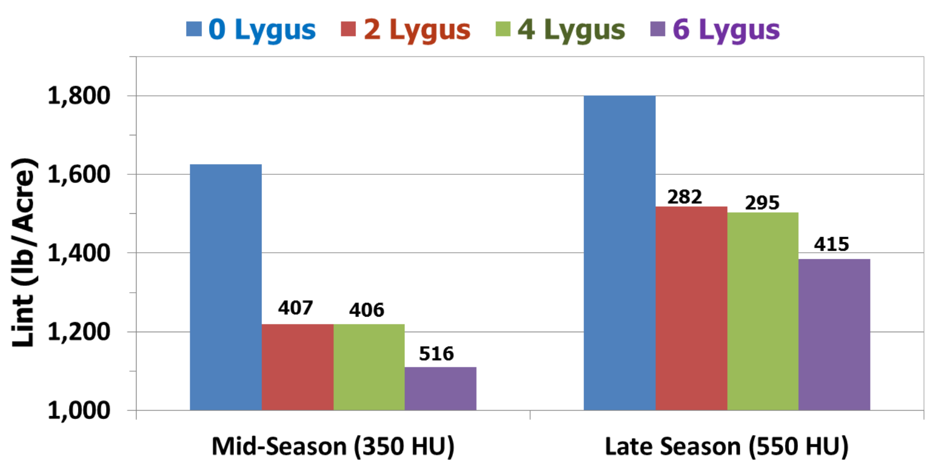

Figure 15. Influence of varying levels of *Lygus* infestations on lint yields at two crop phenological stages, as measured by heat-unit accumulation beyond first white flower, Lubbock County, TX, 2014.

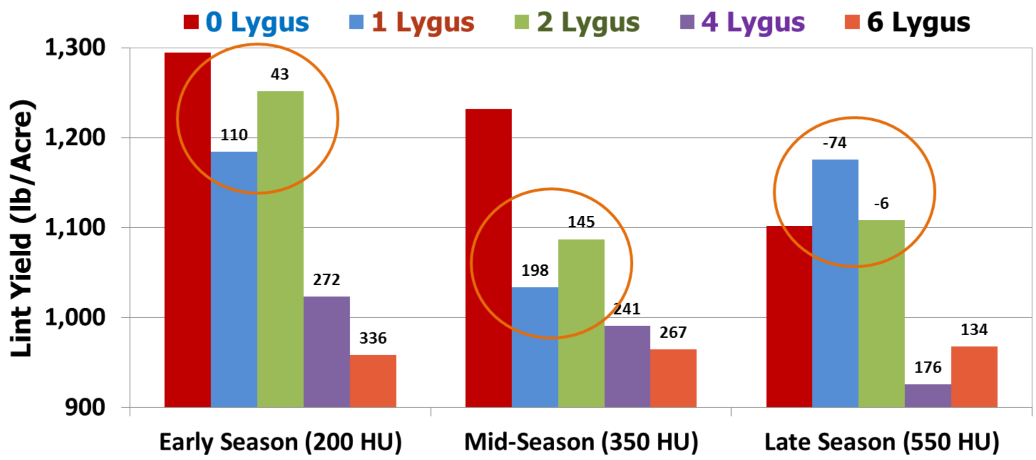

Figure 16. Cotton lint yield following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages at three cotton phenological stages, Lubbock, TX, 2015.

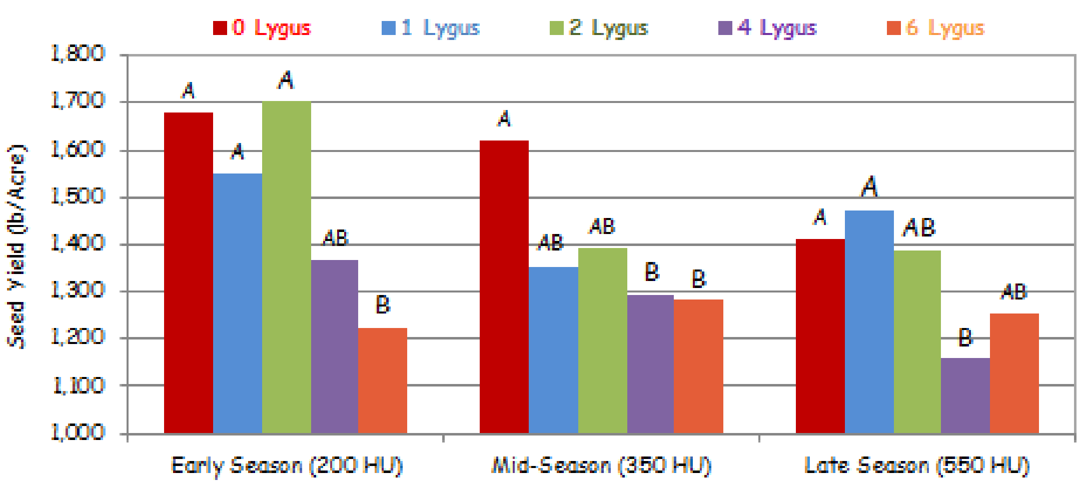

Figure 17. Cotton seed yield following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages at three cotton phenological stages, Lubbock, TX, 2015.

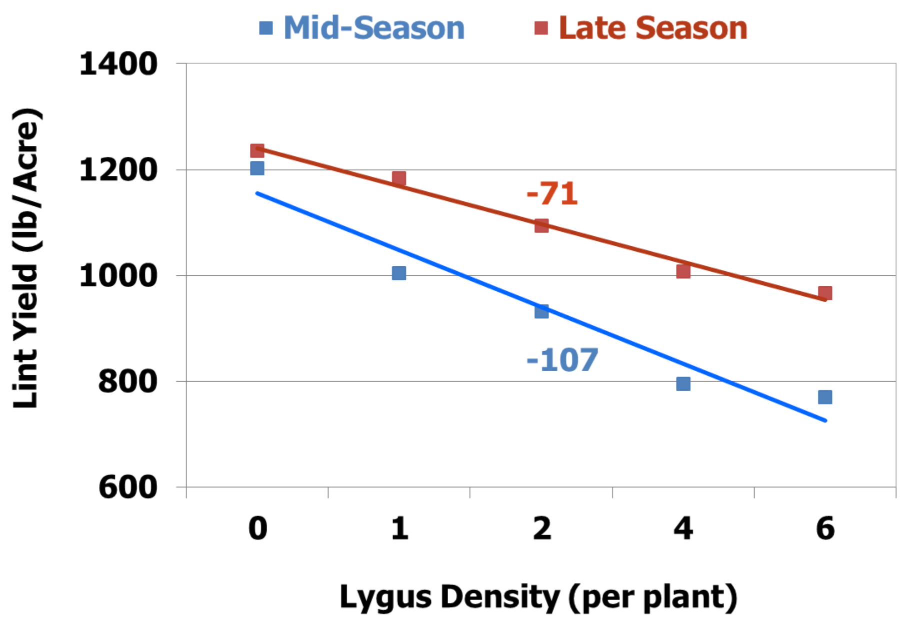

Figure 18. Regression analyses on average of four years of *Lygus* single, whole-plant cage study data (2012-2014) and 2015 data on multi-plant cage study showing the relationship between the amount of lint yield reduction and *Lygus* density augmentation per plant. Mid-season infestation, $y=1263.1 - 107.43x$ (R^2 = 0.94); Late season infestation, $y=1311.8-71.47x$ (R^2=0.99); x=Number of adult *Lygus* bugs augmented per plant, y=Lint yield (lb/acre).

Summary

There was a significant change in boll composition (boll profile) between the cotton plants at 350 and 550 HU from first flower. Despite a subtle variation between no-choice (cup-caged single boll feeding) versus choice (single-plant or multi-plant cages with access to all boll types for feeding) situations, it appeared that bolls were relatively safe at 28-30 mm diameter size or 350 HU, which was approximately equivalent to 2-wk old bolls. While year-to-year variation exists and the variation in boll susceptibility is expected across cropping system management (irrigation, planting date, fertility, etc.), maturing bolls should generally be safe from *Lygus* injury two weeks after white flower, especially for *Lygus* adults. *Lygus*-induced lint yield reduction for a given *Lygus* density was lower for late season compared to that for mid-season infestations in all four years of the study. Early season (200 HU) data were collected only during the 2015 season, so there were insufficient data to make specific recommendations for early season management. Averaged over four years, on a 1,200 lb/acre crop, artificial augmentation of 1, 2, 4, and 6 *Lygus* per plant at 350 HU after first flower reduced the cotton lint yield by 199, 271, 407, and 433 lb/acre, respectively, whereas the yield reduction values for the same *Lygus* densities were 52, 142, 229, and 269 lb/acre during the late season (550 HU from first flower). Thus, the *Lygus* yield reduction potential significantly decreased when cotton matured from 350 HU to 550 HU. These data clearly suggest that the maturing bolls are more tolerant to *Lygus* injury when the plant attains 550 HU from first flower. It is also possible that *Lygus* bugs may choose to feed on superfluous bolls or squares and the yield contributing fruits may not be significantly impacted by such late infestations. Because potential yield loss risks due to certain

Lygus density infestations vary with boll maturation profile, the *Lygus* management economic threshold should be optimized for a dynamic ET to accommodate for within-plant fruit maturity profiles. Regression analysis of our current four-year data suggests that *Lygus* adults could inflict maximum lint losses of 127 and 71 lb/acre, respectively, for mid- versus late season infestations of per unit (1 adult) *Lygus* per plant (Fig. 18). Although this study used up to 6 *Lygus* per plant to characterize the feeding behavior and to establish the treatment thresholds, our previous studies suggest that the survivorship of the field-collected and cage-released *Lygus* adults in the Texas High Plains is about 20-25%. Therefore, our highest actual density was set around 1-1.5 bugs per plant. Based on this study, *Lygus* management options for Texas High Plains cotton after the initiation of first flower are recommended as follows: 1) careful monitoring of cotton crop and adjacent habitats for *Lygus* abundance and movement behavior and avoidance of insecticide treatment for *Lygus* in cotton prior to 200 HU from first flower, 2) 2-4 *Lygus* per 6 row-ft for mid-season cotton, and 3) 4-6 *Lygus* per 6 row-ft for late season cotton.

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PROJECT FINAL REPORT
2012-2015

Cotton Incorporated Core Program

Project Number: 12-364

Characterization of Cotton Crop Response to Thrips Injury for Improved Thrips Management in Texas High Plains Cotton

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Characterization of Cotton Crop Response to Thrips Injury for Improved Thrips Management in Texas High Plains Cotton

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PROJECT SUMMARY

The western flower thrips, *Frankliniella occidentalis* Pergande, is a serious pest on seedling cotton in the Texas High Plains and other regions of the U.S. cottonbelt. Thrips are an early season pest which can cause severe damage to seedling cotton. First three weeks of seedling stage is important because thrips can cause significant damage during this period when plants are 1-3 true-leaf stage. Heavy infestations can cause leaves to shrivel, reduction in leaf chlorophyll content and leaf area, and ultimately significant yield reduction. The manipulation of thrips populations in a cotton field setting is very challenging and maintaining selected thrips densities on cotton seedlings in an open field condition are unmanageable. Nevertheless, it is essential to use field cages and confine known number of thrips per caged plants to obtain a desired thrips density. Specific objectives of this study were to: 1) evaluate cotton varietal response to natural colonization of thrips in open field studies, 2) greenhouse evaluation of cotton varietal response to thrips augmentation, and 3) design a field cage prototype to determine the cotton crop damage potential of the western flower thrips for developing an economic threshold. The ultimate goal of the research project was to develop new economic thresholds for thrips based upon plant response characteristics, validating or revising the current Texas High Plains thrips treatment threshold recommendations, and precisely characterizing the cotton crop response to various levels of thrips injury at different cotton seedling ages.

In the greenhouse study, 0, 0.5, 1 and 2 thrips per plant were released at 1- to 2- true-leaf stage. Twenty-two days following the release, seedlings were harvested, washed and thrips counted. Significantly higher thrips densities were observed from treatments where 1 or 2 thrips were released per seedling compared to 0.5 and control. Visual ranking values of plants from thrips densities 0 and 0.5 were significantly superior (i.e., less visual damage) compared to that from thrips densities 1 and 2. Similar densities were achieved in field cages via thrips release in NoThrips® cages to compensate for 80% field mortality. Significant numbers of thrips were recovered from all thrips-augmented treatments, with lowest numbers recovered from control plants. Leaf area was significantly higher in uninfested control compared to those in thrips-augmented treatments. Seedling health, measured by visual ranking, declined progressively with increased thrips densities. Thrips densities @ 0.5 thrips per plant or greater significantly reduced plant vigor. Thrips densities of 0.5, 1, and 2 per plant at early seedling stage all reduced lint yield significantly compared to that in uninfested control plots. Similar densities were achieved in field cages via thrips release in No-Thrips® cages to compensate for 80% field mortality. Thrips densities of 0.5, 1, and 2 per plant at early seedling stage all reduced lint yield significantly compared to that in uninfested control plots.

Introduction

Thrips are economically important pests in Texas cotton. Thrips can be found in cotton throughout the growing season, but cotton is most vulnerable to thrips damage for the first thirty days following planting and cotyledon emergence. In the U.S., thrips infested a cumulative area equaling 8.9 million acres in 2012 while thrips infested 3.8 million acres in Texas which caused a loss of approximately 9,000 bales in Texas (Williams 2013). Excessive feeding of thrips leads to the browning of leaves on the edges, development of a silvery color, or curling upward from the edges (Fig. 1). Western flower thrips, flower thrips, soybean thrips, onion thrips, and tobacco thrips are five common thrips species found in U.S. cotton (Cook *et al*. 2011). Albeldaño *et al.* (2008) have reported nine species of thrips from Texas cotton. Western flower thrips [*Frankliniella occidentalis* (Pergande)] is a key pest in Texas cotton (Greenberg *et al*. 2009) and causes severe damage to cotton seedlings in infested fields, which are generally vulnerable to thrips damage up to the 4-5 true leaf stage (Cook *et al*. 2011). Thrips cause leaf area destruction, delayed maturity, retarded plant growth and loss of apical dominance (Reed *et al.* 2001, Sadras and Wilson 1998, Harp and Turner 1976). Previous thrips surveys revealed at least eight thrips species in Texas cotton, but *Frankliniella occidentalis* (western flower thrips) and *Thrips tabaci* (onion thrips) are the most common species, comprising more than 75% of the thrips found in Texas cotton. The various thrips species in Texas, being difficult to identify, have typically been managed as a single complex, with a single approach being broadly applied. Differential damage potential and pesticide susceptibility among these species remain unexamined, but with the recent aldicarb (Temik®) discontinuation, their examination may become critical.

Lacking thrips-tolerant cotton cultivars, cotton growers primarily use insecticides to control thrips. While several seed treatment options are available, soil-applied aldicarb had been the most reliable and common method used for cotton seedling thrips control. With the discontinuation of aldicarb, cotton growers will need alternative thrips management techniques, especially in the Texas High Plains. Ideally, cotton growers should be empowered with the capability to estimate the daily cost of delaying foliar insecticide applications for controlling thrips, further empowering them to finely adjust and achieve their acceptable, sustainable economic injury level for maximum benefits and minimum costs. Proposed project outputs include information such as the specific relationship between the degree of thrips injury to cotton seedlings and the resulting plant response in terms of final yield and fiber quality, the specific cotton growth stage most vulnerable to thrips infestation, an accurate economic threshold for initiating thrips management actions, and the effect of infestation duration on cotton development and lint yield, all of which would be valuable to empower growers with such a capability, given EPA-mandated aldicarb discontinuation.

Foliar insecticide applications are likely to replace aldicarb, and are likely to increase in number. Given such an increase, and since information regarding specific thrips species, their damage potential, and how cotton responds is unavailable, the risk of excessive or inadequate insecticide use is likely to increase as well. Further, while Texas A&M AgriLife Extension currently provides general thrips management thresholds, such broadly-applicable thresholds are insufficient to address specific thrips species, different injury levels, infestation duration, and their effects on the cotton crop growth response and final yield potential. Therefore, the goal of this project is to develop applicable information which will empower producers to optimize the timing and extent of management actions to mitigate thrips damage while protecting the agroecosystem, maximizing yields, and minimizing production costs. In addition to benefitting producers, the outcome of this study will aid crop consultants and county IPM agents in making recommendations to improve thrips management in Texas High Plains cotton.

The manipulation of thrips populations in a cotton field setting is very challenging and maintaining selected thrips densities on cotton seedlings in an open field condition are unmanageable. Nevertheless, we must use field cages and confine known number of thrips per caged plant to get a desired thrips density. Specific objectives of the second year of this study were to: 1) evaluate cotton varietal response to natural colonization of thrips in open field studies, 2) greenhouse evaluation of cotton varietal response to thrips augmentation, and 3) design a field cage prototype to determine the cotton crop damage potential of the western flower thrips for developing economic thresholds. The ultimate goal of the research project is to develop new economic thresholds for thrips based upon plant response characteristics, validating or revising the current Texas High Plains thrips treatment threshold recommendations, and precisely characterizing the cotton crop response to various levels of thrips injury at different cotton seedling ages.

Figure 1. A) Adult western flower thrips, *Frankliniella occidentalis*, B) Severe damage caused by *F. occidentalis* to seedling cotton, C) Stunted cotton seedlings due to thrips injury.

Materials and Methods

Objective 1. Cotton cultivar response to natural colonization of thrips in the field

This study was conducted at the Texas A&M AgriLife Research farm located near Lubbock, Texas. The study was deployed in a randomized block design with four replications and six cultivar treatments. Experimental plots were eight 40-inch rows wide x 90 ft long and 5 ft alleys separated the plots. Six cotton cultivars (SSG-HQ-212-CT, DP 353, FM 1740 B2F, T12 07-7-1407 CT 1205, T12 07-7-1001 CT 1206, and PHY 367 WRF) were planted on May 9 (2013) and June 3 (2014). Each 8-row plot was further divided to two 4-row plots and each of the two 4-row plots was randomly assigned to a 'control' or 'sprayed' treatment. Thus, the entire study consisted of 48 experimental units (six cultivars x two treatments x four replications).

2013 Study. Cotton germination was delayed due to cooler soil temperatures, but the plant emergence was satisfactory in most plots. Poor crop stand on some experimental plots may be attributed to variations in cultivar seedling vigor rather than to the soil conditions. Plant stand counts were performed on May 23 and June 3 by counting all plants in 3 row-ft per row in all 48 plots. Thrips densities were monitored in all 48 plots using a ten-plant thrips washing technique. Thrips sampling dates were May 23 (pre-treatment), May 25, June 3, June 10, and June 17. An insecticide (Orthene® 97UP @ 3.0 oz/acre) was sprayed in all 24 'sprayed' treatment plots after each thrips sampling event on May 24, May 30, June 11, and June 18, and the entire test (all 48 plots) was sprayed with this insecticide on June 26. Insecticide treatment application was skipped after the thrips sampling event on June 3 due to spray logistic issues, but the residual insecticides from previous week's application kept the thrips populations suppressed until the insecticide application on June 11. Plant response to thrips injury was monitored by measuring plant height, shoot length, root length, total leaf area, and total dry biomass of cotton seedlings from each plot on June 24. A 10-ft section was marked on each of the two center rows within each plot and the flowering profile was monitored 2-3 times per week. This type of phenological monitoring began prior to the initiation of flowering and continued until crop cut-out. Flowering profiles were monitored on July 10, 12, 16, 19, 22, 24, 26, 30, August 13, and 30. The two 10-ft sections from the middle two rows (20 total row-ft/plot) that were designated for plant fruiting response were harvested to estimate the cotton lint and seed yields from each experimental plot.

Plant response to thrips injury was monitored by measuring shoot length, root length, shoot biomass, root biomass, total leaf area, and total dry biomass of cotton seedlings from each plot. The study area received approximately 3.0 inches of rain on July 16-17 which provided a much needed break from an extended drought. Nevertheless, the test plots received a full complement of irrigation and the test had not been exposed to a water-stress situation. Frequent cultivations kept the weeds under control as well. The crop received harvest-aid chemicals on October 9 and the crop was harvested on November 4, followed by sample ginning on November 20.

2014 Study. The study site received frequent rains that cooled the soil temperature. Thus, the early crop growth was extremely slow. Plant stand counts were performed on 13 June by counting all plants in 3 row-ft on each of the four rows in all 48 plots. Thrips densities were monitored in all 48 plots using a five-plant thrips washing technique in each plot. Thrips sampling dates were June 19 and 25, and July 8. An insecticide (Centric® @ 3 oz. per acre) was sprayed in all 24 'sprayed' treatment plots on June 23, but there were no visible thrips in subsequent sampling events to warrant additional spray applications. Harsh weather conditions followed by some unexplained herbicide drift injury on most of the conventional (non Roundup® Ready) cotton lines in the region around the third week of June and first week of July prevented the test crop from achieving normal growth. Test plots were ranked using a 1-10 scale (1 being dead and 10 being healthy plants) on 16 July for crop vigor. Cultivar PHY 367 WRF and FM 1740 B2F scored 8 and 7.5, respectively, while SSG-HQ-212 CT scored the lowest at 3.25. Overall, the stand counts were poor. Monsanto representatives visited the test site to ascertain the herbicide injury. Similar symptoms had been observed in other regions of the Texas High Plains on cotton cultivars that did not possess the Roundup® Ready technology. The crop vigor did not improve during much of the growing season. No further pesticide was applied because of low thrips presence and stunted growth of the crop. Nevertheless, the crop was harvested and ginned.

2015 Study. A field study was deployed in a randomized block design with four replications and five cultivar treatments. Experimental plots were eight 30-inch rows wide x 50-ft long and 5-ft alleys separated the plots. Five cotton cultivars (FM 958, 07-7-519, 07-7-1020, 07-7-1407, AllTex Atlas) were planted on June 4, 2015 at the Texas A&M AgriLife Research farm in Halfway, TX. Each 8-row plot was further divided to two 4-row plots and each of the two 4-row plots was randomly assigned to a 'control' or 'sprayed' treatment. Thus, the entire study consisted of 40 experimental units (five cultivars x two insecticide treatments x four replications).

Thrips densities were monitored in all 40 plots using a five-plant thrips washing technique. Thrips sampling dates were June 12, 24, and July 3. An organically approved insecticide (Entrust®) @ 2 oz/acre) was sprayed in all 20 'sprayed' treatment plots on June 15, 29, and July 3. Frequent rain events prevented from thrips colonization in the study site, which rendered the study a failure.

Objective 2. Cotton cultivar response to different thrips densities in the greenhouse

2013 Study. A greenhouse study was conducted to determine the maximum potential effect of different densities of thrips on seedling cotton. Six cotton varieties (07-7-1001 CT-1206, 07-7-1407 CT-1205, PHY367 WRF, SSG HQ212 NCT, FM 1740 B2RF and ST 5458B2RF) were planted in 16-oz Styrofoam cups on October 8, 2013. At the bottom of the Styrofoam cups, 1-3 small holes were made to allow for water drainage from the potting soil. The study was deployed in a completely randomized block design with four replications, six cultivars, and four thrips densities. Each experimental unit contained 6 plants. Thrips were field-collected from cotton and reared on green beans in the laboratory. Immature thrips were transported to the greenhouse in containers with green beans. A brush was used to dislodge thrips from the green beans onto the cotton seedlings. Every effort was made to release only immature thrips to avoid unintentional movement of thrips between treatments. Thrips densities released included: no thrips (control), ½ thrips per plant (e.g., one thrips per two plants), one thrips per plant, and two thrips per plant at the 1- to 2-true leaf stage. An automatic sprinkler system was programmed to water the plants three times per week for 8 minutes/cycle. In addition, supplemental water was manually applied as needed.

The greenhouse ambient air temperatures were recorded using a small iButton® datalogger (Maxim Integrated, San Jose, CA). Visual leaf tissue damage rankings of all plants were recorded prior to clipping. Ranking was based on a scale of 1-10 (1 = healthy plants and no damage symptoms and 10 = plants killed by thrips). Chlorophyll readings were also recorded using a chlorophyll meter to determine if treatments (thrips densities) and/or tested cotton varieties had an impact on chlorophyll levels. Leaf area from each treatment was also recorded using a leaf area meter to test whether leaf surface areas were influenced by the various thrips level treatments.

Thrips were allowed to feed and reproduce for three weeks (the duration that is equivalent to the western flower thrips lifecycle) before plants were clipped near the soil surface and placed into denatured ethyl alcohol. Later, the adult and juvenile thrips were quantified via a plant washing technique as follows: All six plants per unit were placed on a fine sieve and rinsed in water until all thrips could be dislodged from the leaves and terminals onto a very fine sieve (No. 150)**,** and then thrips were washed in a salt solution. Sand and heavy materials were removed from the bottom opening of the separatory funnel and thrips were placed on a filter paper. A vacuum system was used to remove extra water. Adults and juveniles were counted using a microscope at a 10X or higher magnification. Numbers of thrips from each treatment and variety were recorded and used in the analysis. Analysis of variance was used to determine the effect of thrips densities on cultivars.

2014 Study. The identical greenhouse study of 2013 was repeated in 2014. Six cotton cultivars were planted in 16-oz Styrofoam® cups. The study was deployed in a complete randomized block design with four replications. Each experimental unit contained six plants. Thrips were reared on green beans purchased from local grocery stores. Four densities of thrips released onto seedling cotton included: no thrips (control), ½ thrips per plant (one thrips per two plants), one thrips per plant, and two thrips per plant at 1- to 2-true leaf stage. On the control plants, Orthene® 97UP was applied twice weekly. Twenty-two days following the thrips releases, the plants were clipped near the soil surface and stored in 90% ethyl alcohol for the thrips washing procedure. Prior to clipping the plants, visual leaf damage rankings were conducted which was based on a 1-10 scale (1 = normal healthy plants and 10 = plants killed by thrips). Chlorophyll readings were recorded using a chlorophyll meter and leaf area from each treatment was recorded using a leaf area meter. Adult and juvenile thrips were quantified via washing technique and counted using a microscope at 10X or higher magnification. Analysis of variance was conducted to test if thrips densities had an effect on tested variables.

Objective 3. Developing thrips economic threshold for seedling cotton

2014 Study. Density-dependent threshold studies were conducted in seedling cotton at the Texas A&M AgriLife Research farm near Lubbock. Rectangular wooden-frame cages [98 cm (L) x 30 cm (W) x 44 cm (H)] with No-Thrips® screen were constructed and deployed in the field, with each cage enclosing 8-13 cotton seedlings (Fig. 2). Silicone caulk was used to attach no-thrips screen to the wooden frame. A sheet metal flashing (1-1½-in width) was attached at the bottom of the cage to restrict thrips movement from the bottom of the cage. A temperature sensor was kept inside the cage to record the internal cage temperatures (Fig. 2).

Freshly collected adult thrips, primarily western flower thrips, were released at various densities to generate a damage gradient across density treatments. After the thrips were released and the plants caged, thrips were allowed to feed for 5-10 days and then the cages were removed. Two plants from each cage were removed and washed to retrieve thrips to estimate the thrips survival. Within 24 h of cage removal, thrips augmented plots were sprayed with Orthene® 97UP to kill all remaining thrips. Remaining plants were kept insect-free throughout the remainder of the growing season; these plants were harvested and ginned for lint yield estimation.

Three separate studies were conducted to capture within-season variation in seedling response to various thrips density treatments. Experimental protocols were identical in all three tests.

Figure 2. Wooden-framed No-Thrips® cages installed in the field and release of thrips densities for threshold study (left); thrips infested cotton seedlings (right).

Test I. Cultivar ST 5458 B2RF, without the seed treatment for thrips management, was planted on May 15, 2014. Field cages were deployed and six thrips density treatments were released onto plants on June 6 when the cotton seedlings were at the 1-2 true-leaf stage. Six density treatments included 0, 1, 2, 4, 6, and 10 thrips per plant, replicated five times (30 total cages). Within four days of thrips release, the test site received 2.5” of rainfall. Therefore, we allowed the thrips exposure to continue for about 10 days before removing the cages. Two plants from each cage were harvested and washed to retrieve thrips. After removal of cages, thrips augmented rows were sprayed with Orthene® 97UP.

Test II. Only 100 m from the *Test I* site, *Test II* was conducted on the same cotton cultivar using the same cages. This cotton cultivar ST 5458 B2RF was planted on June 3 and the test was deployed on June 18 at the 1-2 true-leaf stage. Thrips densities included 0, 1, 2, 4, 6, and 10 thrips per plant plus an uncaged control. Cages were removed on June 23 and two plants from each cage were clipped at the base and washed to retrieve thrips. After removal of cages, thrips augmented rows were sprayed with Orthene® 97UP.

Test III. Immediately after *Test II* was terminated, *Test III* was deployed in the same experimental field only 50 feet away from *Test II* using the same cages. Thrips cages were deployed and thrips were released on 25 June. Because the seedlings were at the 4-5 true-leaf stage, thrips release densities were increased. Treatments included 0, 2, 4, 10, 20 and 30 thrips per plant. Cages were removed on July 1, removed two plants per cage to retrieve thrips via plant washing, and sprayed Orthene® 97UP to kill augmented thrips on remaining plants.

Following removal of the cages, all plots were regularly monitored such that the study site could remain relatively pest-free for the remainder of the season. Plant-mapping data were collected and the cotton lint from the plants was harvested to quantify the crop response to various levels of thrips infestations.

Density-dependent threshold studies were conducted in seedling cotton near Lubbock. Rectangular wooden-frame cages [98 cm (L) x 30 cm (W) x 44 cm (H)] with No-Thrips® screen were constructed and deployed in the field that enclosed 8 seedlings per cage (Fig. 2). Silicon was used to attach no-thrips screen to the wooden frame. A thin metal flashing (1-1½-in width) was attached at the bottom of the cage to restrict thrips movement from the bottom of the cage. A temperature sensor was kept inside the cage to record the temperatures.

2015 Study. The study design was near-identical to the 2014 study, except for thrips density adjustment in 2015.

Three separate studies were conducted to capture within-season variation in seedling response to various density treatments. Nevertheless, the experimental protocols were identical in all three tests. **Test I.** Cultivar ST 4946 GLB2 was planted on May 27, 2015. Field cages were deployed and thrips density treatments applied on June 6 when cotton seedlings were at the cotyledon stage. Six density treatments included 0, 1, 2, 4, and 6 thrips per plant, replicated six times (total 30 cages). Thrips were allowed to feed for 5 days and cages were removed on June 12, and then thrips release rows were treated with Orthene® 97UP. **Test II.** Only 100 m from Test I experimental site, Test II was conducted on the same cotton cultivar. This cotton was planted on May 27 and test was deployed on June 12 when seedlings were about 2 true-leaf stage. Thrips density treatments included 0, 1, 2, 4, and 6 thrips per plant. Cages were removed on June 19. After removal of thrips cages, thrips augmented rows were sprayed with insecticide Orthene® 97UP. **Test III.** Immediately after Test II was terminated, Test III was deployed in the same experimental field only a short distance away from Test II. Thrips cages were deployed and thrips released on June 19. Because the seedlings were at about 4-5 true-leaf stage, density treatments were 0, 5, 10, 15, 20, and 25 thrips per plant. Cages were removed on June 26. From all three tests, two plants per cage were removed and washed for thrips density estimation. These test plots were monitored throughout the growing season for any other pests and maintained the test areas relatively pest free for the remainder of the season. Test plots were harvested the following week to quantify the crop response to various levels of thrips infestations.

In addition to field-cage studies, an attempt was made to compare thrips cage studies with open-field releases. Three sets of cage studies (noted above) were matched with identical uncaged tests next to each study simultaneously. One week after each release, Orthene® 97UP was sprayed to keep plants free from thrips. Harvested cotton was ginned and lint and seed yields were recorded.

Results and Discussion

Objective 1. Cotton cultivar response to natural colonization of thrips in the field

2013 Study. Visual thrips counts did not significantly vary between treatments or cultivars. Stand counts between treatments were also non-significant; however, plant counts were significantly higher in CT1205, CT1206, DP353 and PHY376 compared to FM1740 and SSGHQ. Cultivar DP353 and PHY367 had significantly more thrips in control plots than sprayed plots (Fig. 3). However, drastic varietal difference in plant growth and yield masked the subtle difference in thrips tolerance across these tested varieties. No significant thrips population densities or lint yield differences were found between the insecticide-treated and untreated control portions of the other four cultivars. Cultivar DP353 had the longest flowering period and peak flowering occurred later in the season compared with other cultivars examined (Fig. 4). In both treated and control plots, the highest number of white flowers were observed in PHY367 on July 30 (Fig. 4) and peak flowering continued from mid-July through August. Several significant differences were observed between plant biomass and cultivar treatments (P<0.1) in control and sprayed plots (Tables 1 and 2); however, interactions between insecticide and cultivar treatments were non-significant. Significantly lower lint yield in untreated control plots (P<0.1) was observed between sprayed and control plots in DP353 and PHY367 which might be due to presence of significantly more thrips in control plots than insecticide-sprayed plots in these two cultivars (Fig. 5). Significant differences in seed yield was observed between sprayed and control plots in DP353 only, whereas no significant differences in seed yield were observed between sprayed and control plots in other cultivars (Fig. 6).

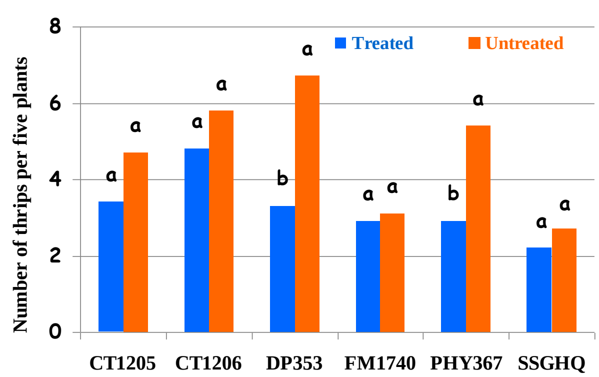

Figure 3. Thrips densities recovered using whole-plant washing procedure, 2013.

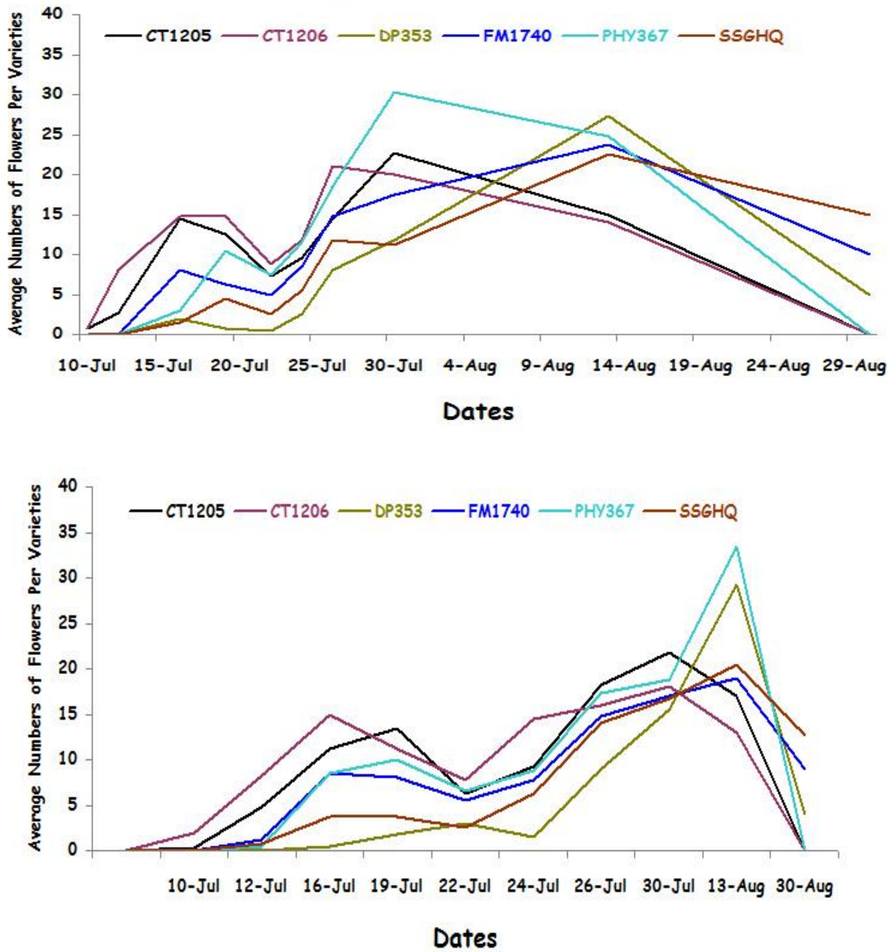

Figure 4. Flowering profile of cotton cultivars in untreated control (upper panel) and insecticide sprayed plots (lower panel), 2013.

Table 1. Varietal variation in selected plant parameters observed in control plots, Lubbock, TX, 2013.

	Varieties/Lines					
Plant Parameters	CT1205	CT1206	DP353	PHY367	FM1740	SSGHQ
Shoot length (cm)	9.10a	8.97a	8.32a	8.37a	7.90a	6.52a
Root length (cm)	17.35a	16.47a	14.32a	16.37a	16.25a	14.07a
Shoot biomass (g)	2.06a	2.36a	1.42ab	1.31ab	1.67ab	0.94b
Root biomass (g)	1.76ab	2.05a	1.06bc	1.20bc	1.49abc	0.93c
Leaf biomass (g)	4.69ab	5.50a	3.73ab	3.04b	3.94ab	2.56b
Leaf area (cm^2)	135.6ab	163.41a	134.19ab	103.22ab	114.86ab	85.15b
Leaf chlorophyll	54.39a	53.60a	49.75a	55.12a	55.24a	51.14a

Table 2. Varietal variation in selected plant parameters observed in sprayed plots, Lubbock, TX, 2013.

	Varieties/Lines					
Plant Parameters	CT1205	CT1206	DP353	PHY367	FM1740	SSGHQ
Shoot length (cm)	8.32ab	8.97ab	8.72ab	9.47a	8.25ab	6.22b
Root length (cm)	19.57a	19.19ab	15.35b	17.50ab	15.90ab	16.10ab
Shoot biomass (g)	2.88a	2.47a	1.90ab	2.23ab	1.58ab	0.88b
Root biomass (g)	2.44a	2.15a	1.40ab	2.02a	1.56ab	0.91b
Leaf biomass (g)	6.61a	6.29a	4.77ab	4.59ab	3.85ab	2.70b
Leaf area (cm^2)	163.83a	170.01a	162.86a	128.96a	111.14a	73.19a
Leaf chlorophyll	53.91a	54.38a	51.47a	54.64a	53.30a	51.10a

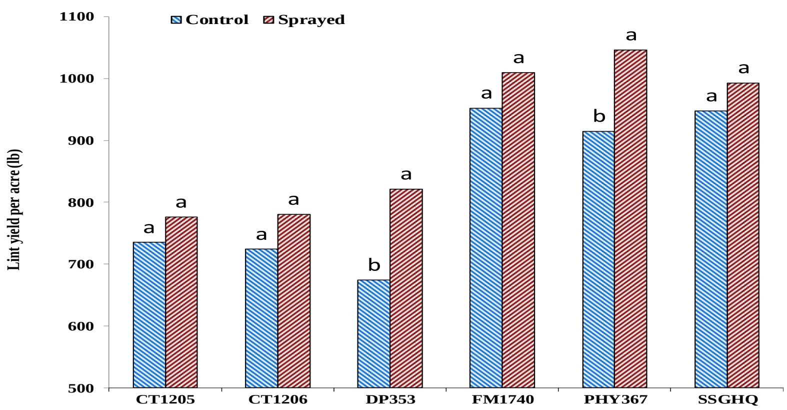

Figure 5. Lint yield (lb per acre) across tested cultivars and breeding lines, 2013.

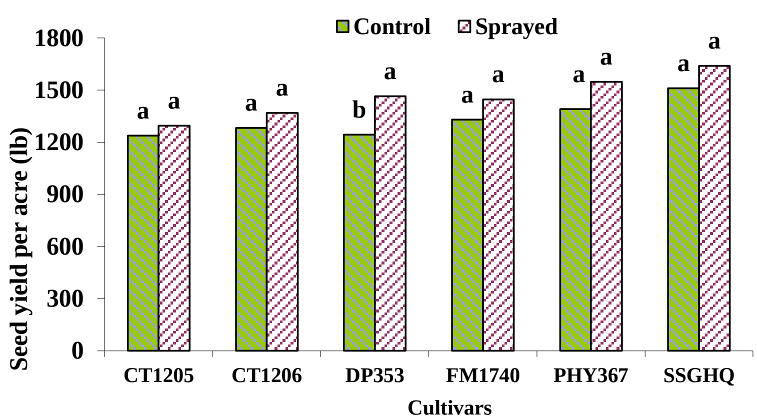

Figure 6. Seed yield (lb per acre) across tested cultivars and breeding lines, 2013.

During this study, we observed that field colonization of thrips was low during the study period, varied with cultivars, with DP353 attracting the most adult thrips and lowest densities observed in FM1740 and SSGHQ (Fig. 3).

2014 Study. Because the plant growth was compromised (see Material and Method section above) for this study for much of the early growing season, thrips colonization did not occur. As a result, the study was reduced to a simple agronomic comparison of tested cultivars and germplasms. The test plots were harvested on December 15, 2014 and ginned on January 12, 2015. Lint yield varied significantly among tested cultivars, but the seed yield did not vary among the cultivars (Fig. 7).

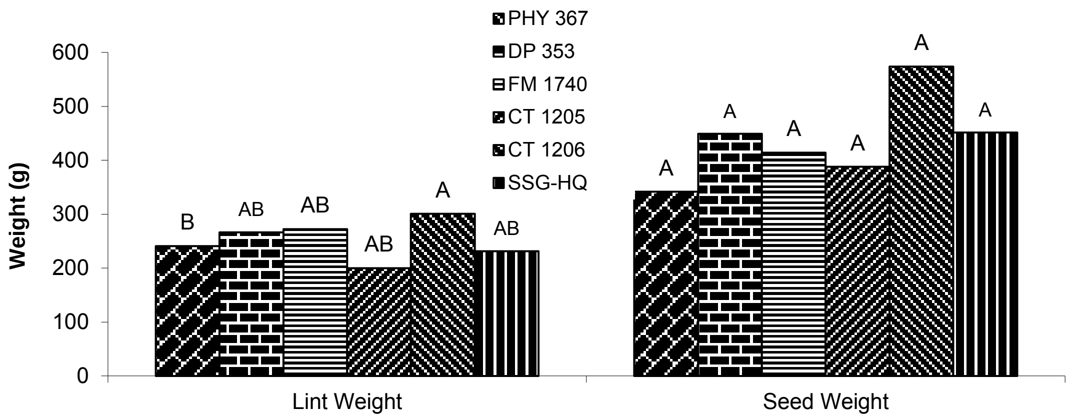

Figure 7. Lint and seed yield (lb per acre) across tested cultivars and breeding lines, 2014.

Objective 2. Cotton cultivar response to different thrips densities in the greenhouse

In 2013, several factors were significant between released thrips densities and thrips numbers recovered. A significant number of thrips (adults + immatures) were recovered between densities 0, 0.5, 1 and 2 (Fig. 8). For both adult and immature thrips numbers, thrips release density 0 had the lowest numbers of thrips retrieved compared to the thrips augmented treatments, indicating that the thrips movement across treatments was minimal. Total thrips retrieved were the highest at 1 thrips per plant treatment, followed by 2 thrips per plant, 0.5 thrips per plant, and the lowest number in uninfested treatment, all significantly different from each other (Fig. 8).

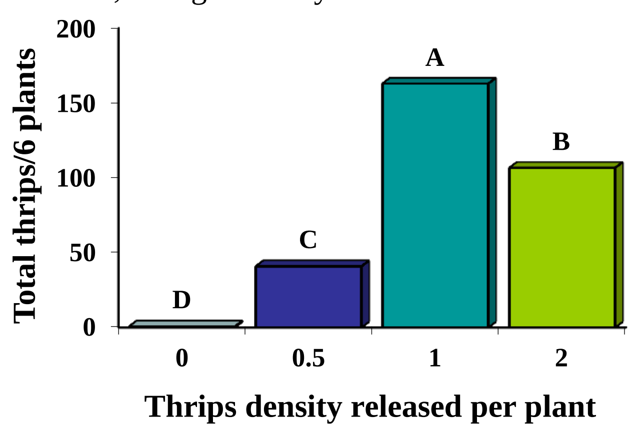

Figure 8. Recovery of total thrips (adult and immature) from seedling cotton using a plant washing technique following a greenhouse study, 2013.

Adult thrips numbers retrieved after three weeks of study were highest in 0.5 and 1 density treatments, followed by 2 thrips per plant, and the lowest numbers in uninfested treatment (Fig.

9). Immature thrips densities increased to 157 and 104 per 6-plant treatments at 1 and 2 thrips per plant densities, respectively, whereas 0.5 thrips per plant resulted in 32 thrips per 6-plant (Fig. 10). No significant differences were found between cultivars on recovered total thrips (adults + immatures), immatures only or adults only. In 2014, total thrips were significantly higher in the 2 thrips per plant release treatment, followed by 1 and 0.5 thrips per plant treatments, and an insignificant number in the uninfested treatment (Fig. 11).

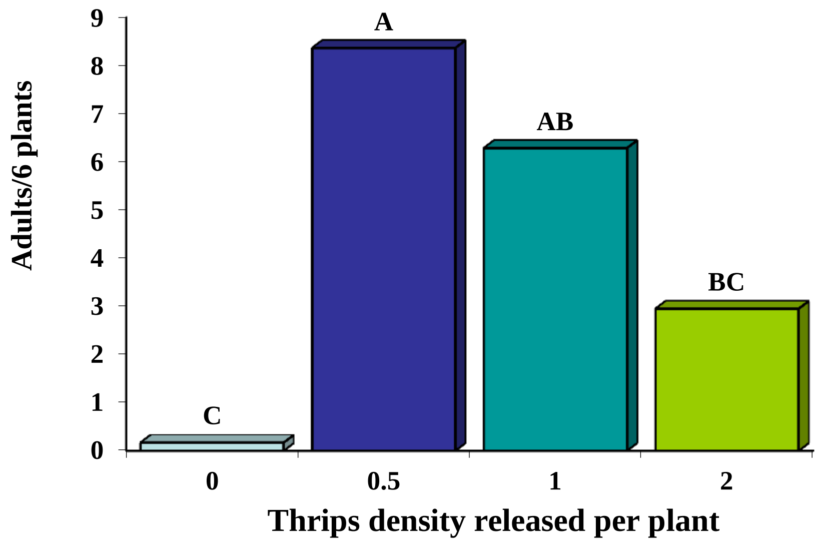

Figure 9. Recovery of adult thrips (22 days after initial thrips releases) from seedling cotton using a planting washing technique in a greenhouse study, 2013.

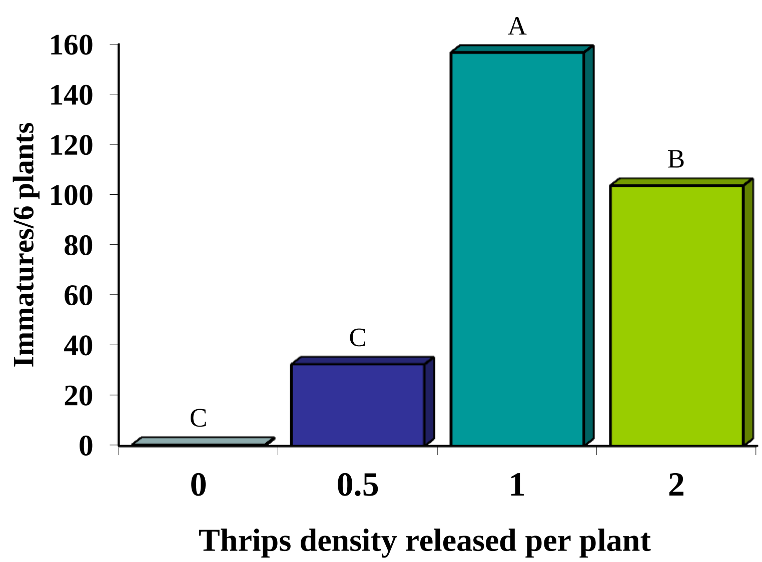

Figure 10. Recovery of immature thrips from seedling cotton using a washing technique in a greenhouse study, 2013.

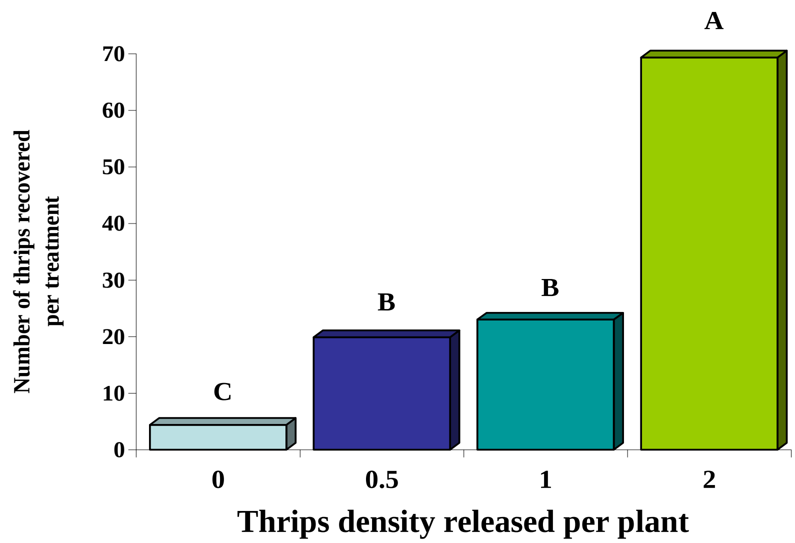

Figure 11. Recovery of total thrips (adult and immature) from seedling cotton using a plant washing technique following a greenhouse study, 2014.

*Leaf area***.** Leaf surface area measurements were significant between thrips density 0 and both 0.5 and 2 treatments; however, no significant differences in leaf area were recorded between thrips release densities of 0 and 1 per plant; and densities 1 and 2 (Fig. 12). Additionally, no significant differences were found in leaf area reduced by thrips among the cultivars tested. There was a clear indication that thrips infestations, regardless of the densities, tended to reduce the leaf surface area in seedling cotton.

Visual ranking. Significant differences were observed in visual ranking of the cotton seedlings between thrips densities released (P = 0.0001); however, no significant differences (P>0.05) were recorded in visual ranking between cultivars. Visual injury ranking was significantly lower (significantly less injury) in thrips densities 0 and 0.5 compared with that in thrips densities 1 and 2; however, no significant differences (P>0.05) were recorded in visual ranking between thrips densities 1 and 2 (Fig. 13). It is noteworthy that 0.5 thrips per plant exerted significantly higher injury, based on visual ranking, compared with that in no-thrips control plants.

Chlorophyll readings. In 2014, no significant differences were observed in chlorophyll readings of the indicator leaf on seedlings between thrips densities released (P>0.05) but various significant differences (P<0.05) were recorded in chlorophyll readings between cultivars tested (Fig. 14). Cultivar CT-1206 showed the highest chlorophyll readings, which were significantly different from ST 5458B2RF, PHY 367WRF and HQ212NCT. No significant differences (P>0.05) in chlorophyll levels were recorded among cultivars CT-1205, CT-1206 and 1740B2RF. Also, no significant differences (P>0.05) in chlorophyll levels were recorded among ST 5458B2RF, PHY 367WRF and HQ212NCT. Chlorophyll readings were not consistent between 2013 and 2014 across cultivars tested (Fig. 14).

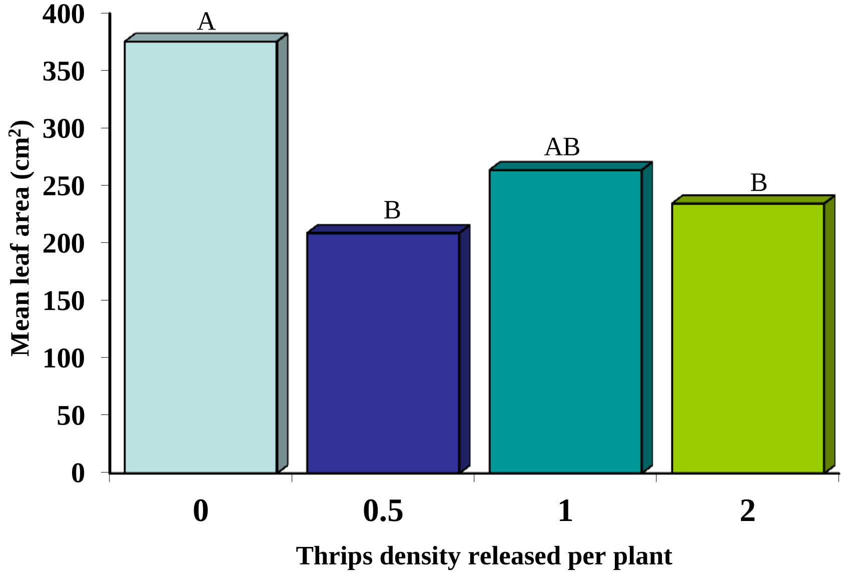

Figure 12. Effect of western flower thrips injury on leaf surface area of the cotton seedlings at various thrips densities in a greenhouse study, 2013.

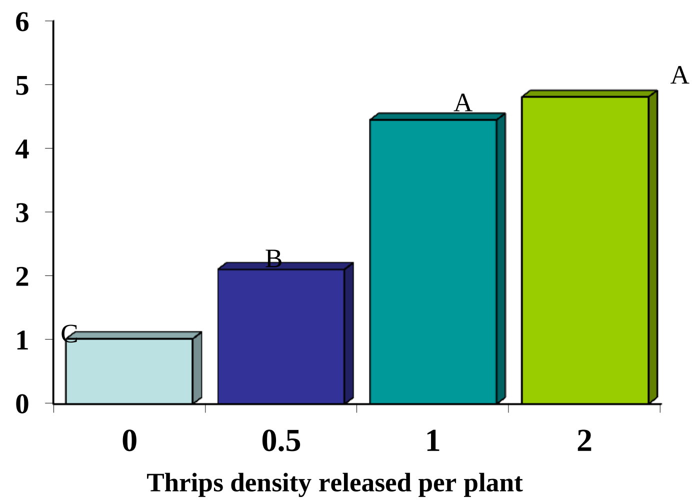

Figure 13. Effect of western flower thrips injury on visual leaf damage ranking of the cotton seedlings at various thrips densities in a greenhouse study, 2013.

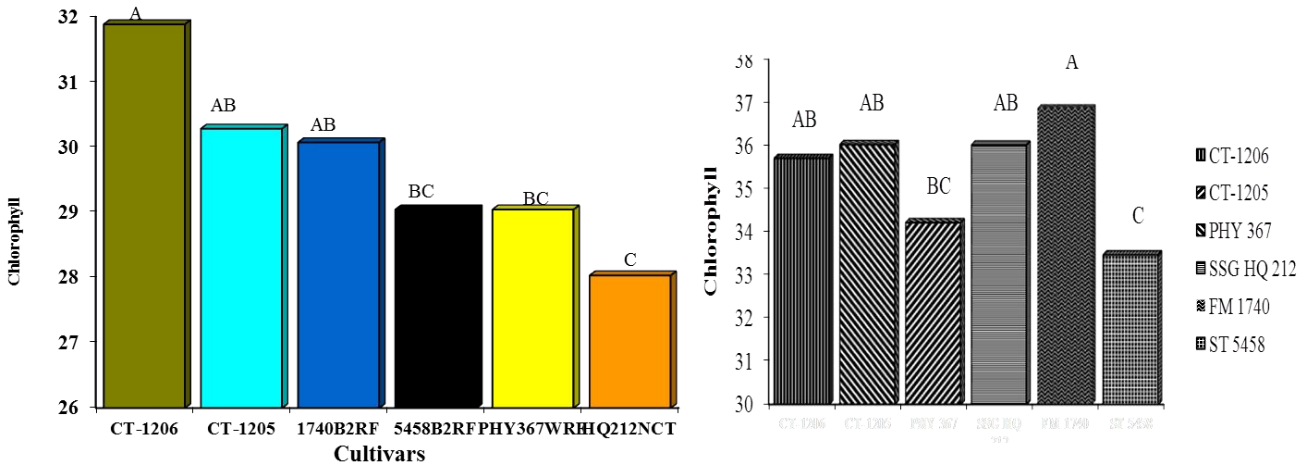

Figure 14. Effect of western flower thrips injury on chlorophyll readings of the cotton seedlings of selected cultivars in a greenhouse study, 2013 (left) and 2014 (right).

Objective 3. Determine the cotton crop damage potential of the western flower thrips for developing economic thresholds

2014 Study. No-thrips® cages appeared to contain thrips in the field cages better than any of the other field cage materials (fabrics) that we have used in previous studies. Different materials and designs were used in the past, including 1) transparent plastic cup cage, 2) wire mesh sleeve cage, 3) opaque plastic cylinder, 4) transparent plastic jar without ventilation, and 5) transparent plastic jar with ventilation (Fig. 15). None of these methods were suitable for thrips studies in the field because of the excessive temperature buildup inside the cages, plus material of the screen was unable to contain the thrips. However, the No-Thrips® cage design provided a satisfactory performance.

Figure 15. Cage types evaluated previously: 1) transparent plastic cup cage, 2) wire mesh sleeve cage, 3) opaque plastic cylinder, 4) transparent plastic jar without ventilation, and 5) transparent plastic jar with ventilation.

Despite our preliminary study showing a satisfactory thrips retention in the No-Thrips® cage, 5-day post-release thrips retrieval was much lower than expected in all three studies. We speculated that a frequent rain and cool/wet weather might have attributed to this lower thrips retrieval from the cages. It was also suspected that there might have been a greater mortality once they were released into the cages. We do not believe that the large number of thrips escaped from the cages, but a small number of escapes is always a possibility. Despite the low rate of retrieval, it appears that the thrips feeding had exerted some effect on the plants, resulting in reduced yield. On the first test, all five thrips augmented treatments had lower average lint yields (749 lb/acre in 6 thrips/cage treatment to 964 lb/acre in 4 thrips/cage treatment) compared to that in control cages (1145 lb/acre), although the values were not statistically significant owing to a large variance in the data (Fig. 16). Test II also suggested that thrips feeding occurred, resulting in lower plant height and smaller main-stem diameter in all thrips augmented treatments compared to that in control cages (Fig. 17). Nevertheless, the thrips feeding, if any, during the seedling stage in this study did not significantly impact lint yields (Fig. 17). Test III was conducted when plants were near the end of the thrips tolerant stage: 5-6 true-leaf stage with good crop health. Therefore, a significant yield-reducing effect of thrips augmentation was not expected. Nevertheless, thrips augmented treatment cages had numerically lower yield compared to that in control cages (Fig. 18).

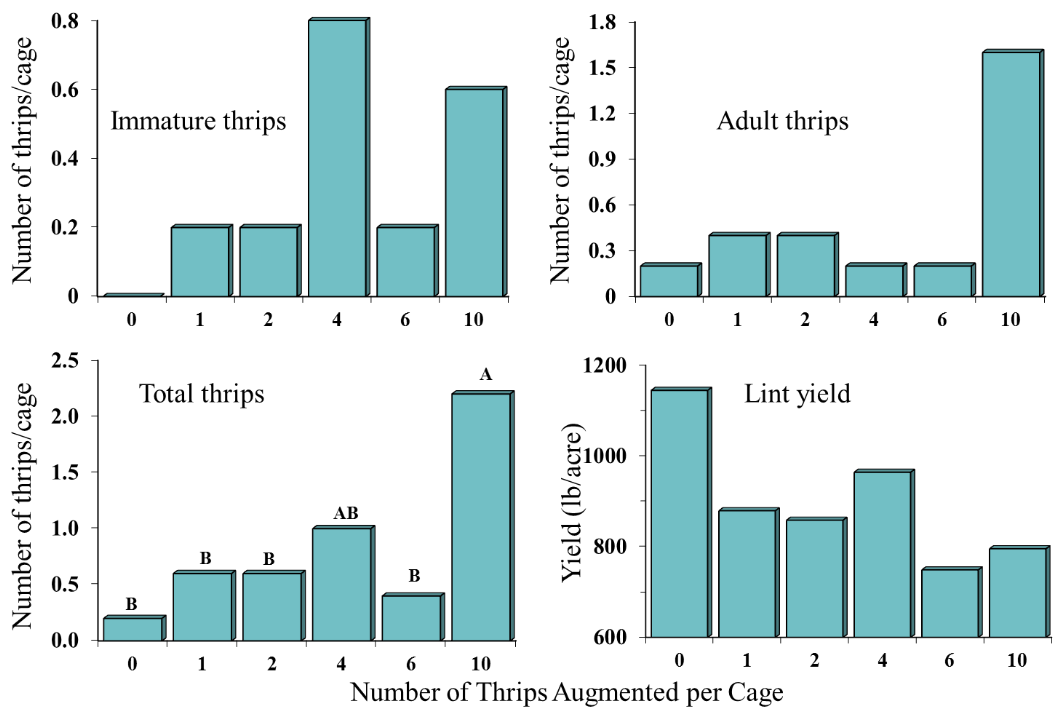

Figure 16. Number of thrips recovered at 5-day post-release into field cages and lint yield from cotton infested with varying densities of thrips in No-Thrips® cages during the 1-2 true-leaf stage, Lubbock, Texas, 2014 (*Study I*).

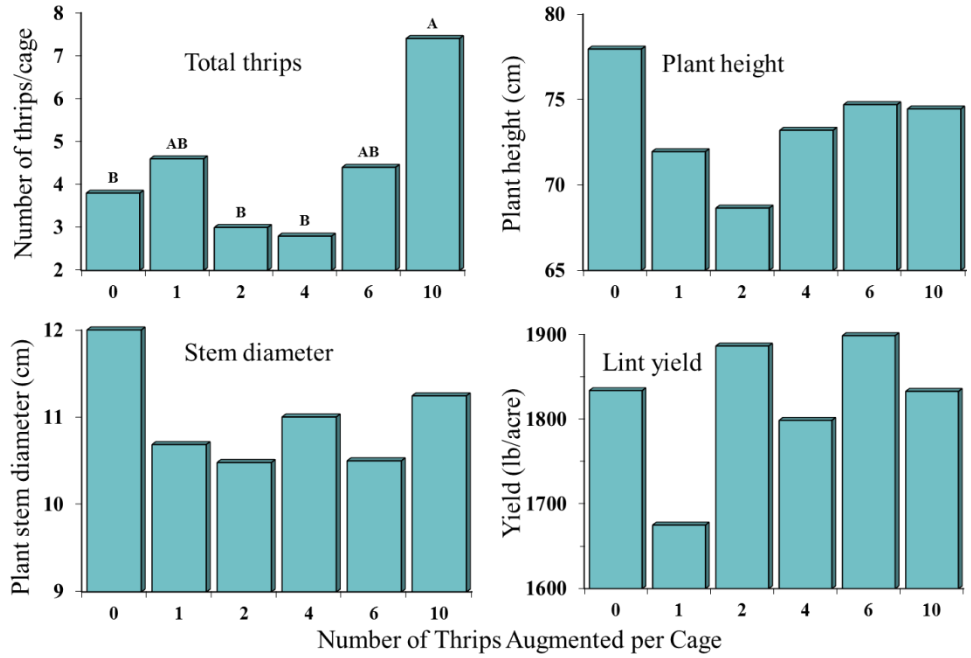

Figure 17. Number of total thrips (immatures plus adults) recovered at 5-day post-release into the field cages, plant height, stem diameter, and lint yield from cotton infested with varying densities of thrips in No-Thrips® cages during the 1-2 true-leaf stage, Lubbock, Texas, 2014 (*Study II*).

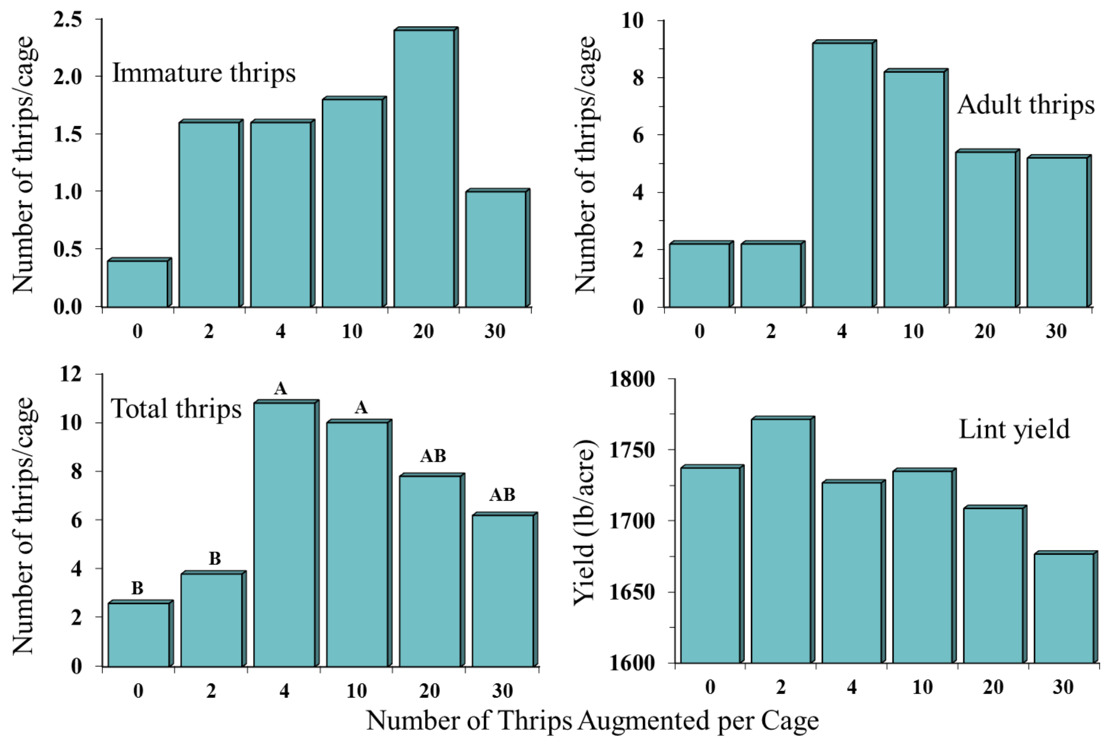

Figure 18. Number of thrips (immature, adult, and total) recovered at 5-day post release into the field cages and lint yield from cotton infested with varying densities of thrips in No-Thrips® cages during the 5-6 true-leaf stage, Lubbock, Texas, 2014 (*Study III*).

2015 Study. As noted earlier, the 2015 growing season was marked by heavy and frequent rainfall events, especially during the seedling period when cotton was most prone to thrips colonization and vulnerable to thrips injury. As a result, retrieval of thrips 7 days post-release, as a proxy for thrips colonization, was very low. Thrips releases at 5-6 true-leaf stage, which is generally considered as the cotton stage that is no longer susceptible to thrips injury, appeared to colonize thrips on plants, but these numbers were insignificantly low for this crop stage (Fig. 19). Seedling growth and the total foliage surface area increased significantly when crop stage advanced from 0-1 to 1-3 and then from 1-3 to 5-6 true-leaf stage (Fig. 20), rendering the crop less susceptible to thrips injury as crop stage advanced. Because thrips densities were low and there were no measurable symptoms of thrips feeding, cotton lint yield did not generally vary across thrips density treatments, regardless of the seedling stage that the infestations were augmented. Nevertheless, cotyledon stage did appear to show some significant yield reduction or a trend toward lint yield penalty in thrips augmented treatments, except the highest density treatment had no yield difference with that in control plots (Fig. 21).

The two-year multi-plant cage study suggested that thrips density-dependent threshold studies can be conducted in the Texas High Plains using the No-Thrips® cages. However, several considerations may be necessary to accomplish the stated objectives of developing treatment thresholds based on such data. First, 2-3 more years of density-dependent data need to be collected to establish thrips feeding potential and plant response to thrips feeding. Second, factors responsible for thrips mortality in cages need to be examined more thoroughly, stage-specific thrips feeding potential need to be characterized to account for thrips age structure in the population.

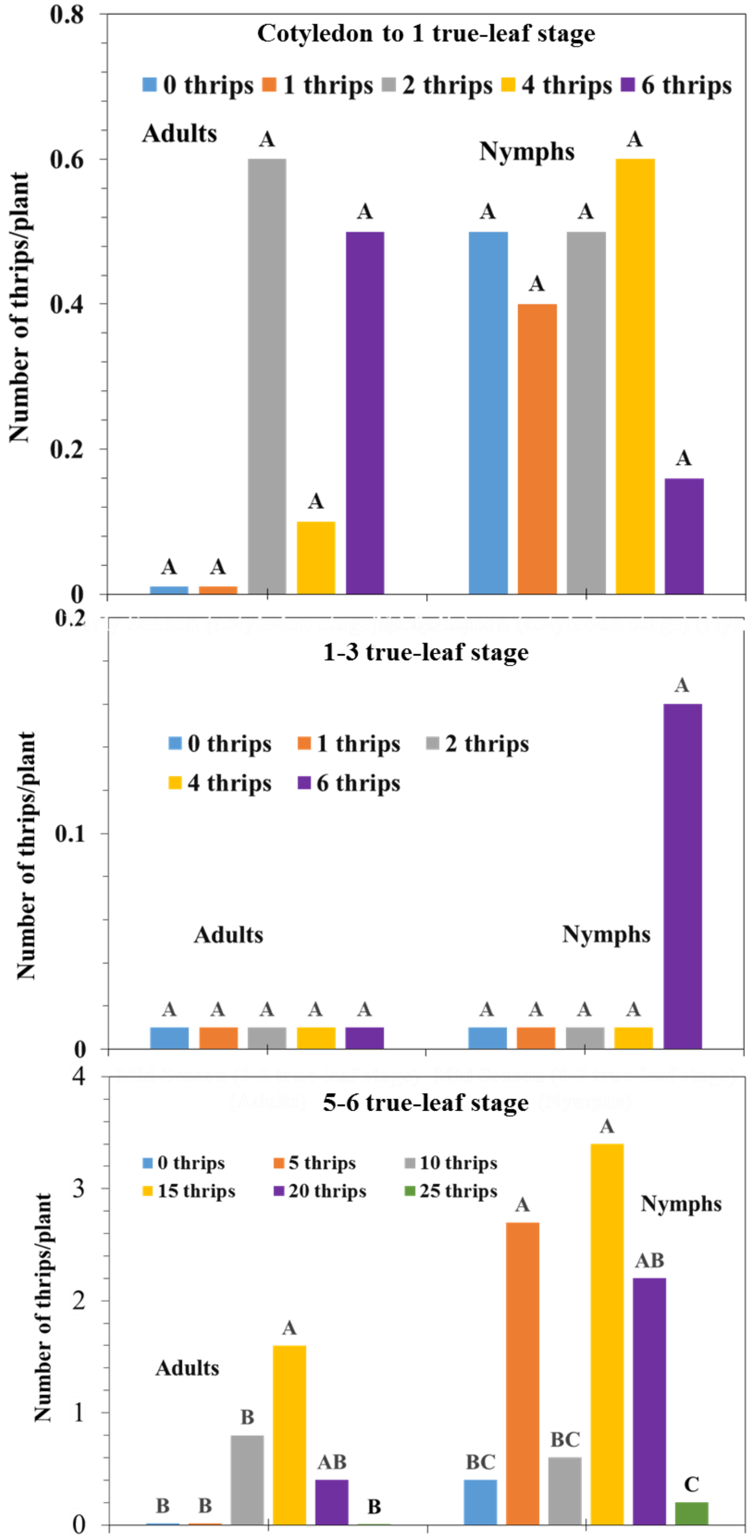

Figure 19. Number of thrips retrieved from cotton seedlings 7-day post-release into No-Thrips® field cages at three seedling stages, Lubbock, Texas, 2015.

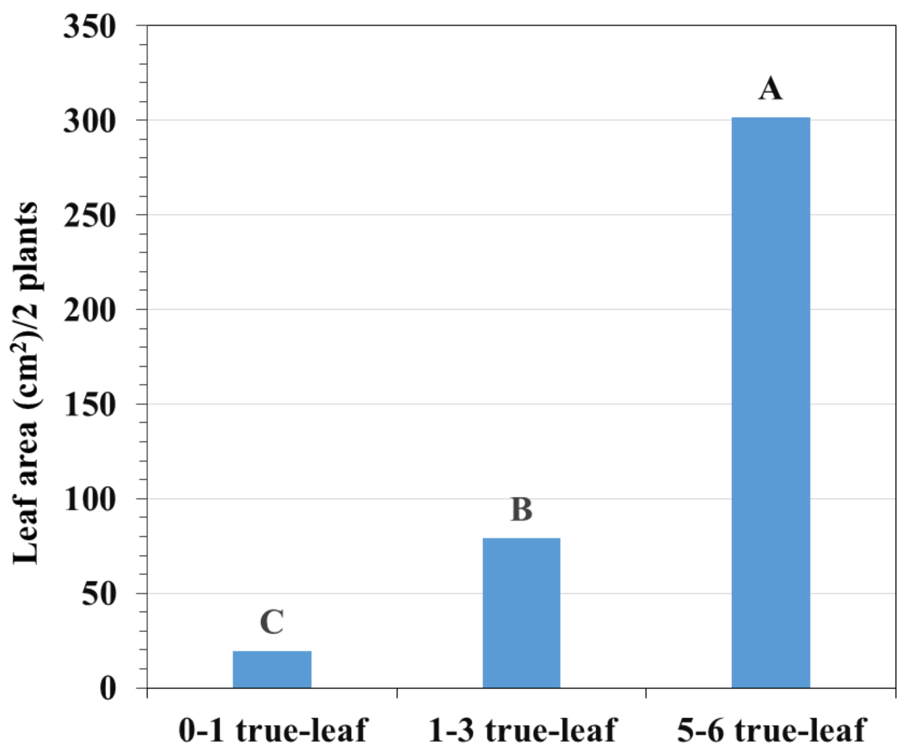

Figure 20. Leaf area measurements of seedling cotton at three different seedling stages (<1, 1-3, and 5-6 true-leaf stage), Lubbock, TX, 2015.

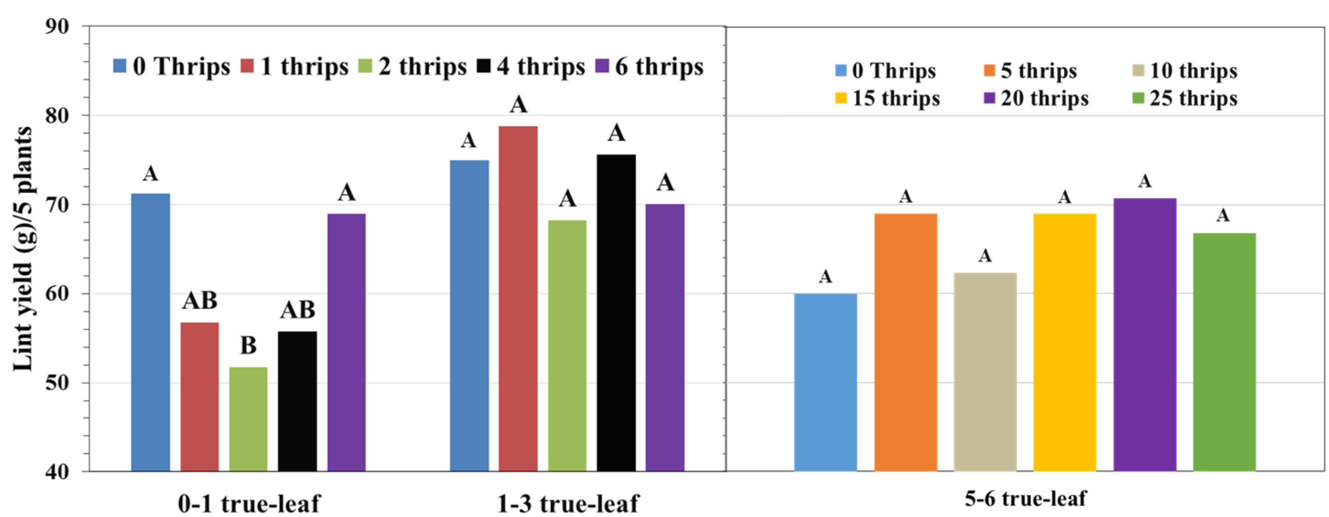

Figure 21. Effect of various densities of thrips releases during three different seedling stages (<1, 1-3, and 5-6 true-leaf stage) on cotton lint yield, Lubbock, TX, 2015.

This four-year project aimed to characterize the thrips feeding behavior and establish a thrips density-dependent damage relationship of seedling cotton. The project invested the first two years to devise thrips field research techniques via examining several field cage options to effectively enclose thrips in the field. We are fairly confident in our multi-plant field cage with NoThrips® screen material for thrips studies in the field. However, the harsh environmental conditions in the Texas High Plains region during the early cotton growth stage, characterized by cool weather and high wind speeds associated with sandstorms, prevented this project from achieving the type of data that were envisioned. Nevertheless, the 2013 and 2014 data from the greenhouse study clearly showed the importance of thrips management in early seedling stage (Fig. 22), but the field validation of the greenhouse study was adversely influenced by harsh weather conditions. The 2014 field cage study resulted in a reasonable relationship between field-augmented thrips densities and lint yield (Fig. 23). However, field augmented thrips densities failed to establish thrips on the plant and did not result in measurable injuries to cotton seedlings in 2015.

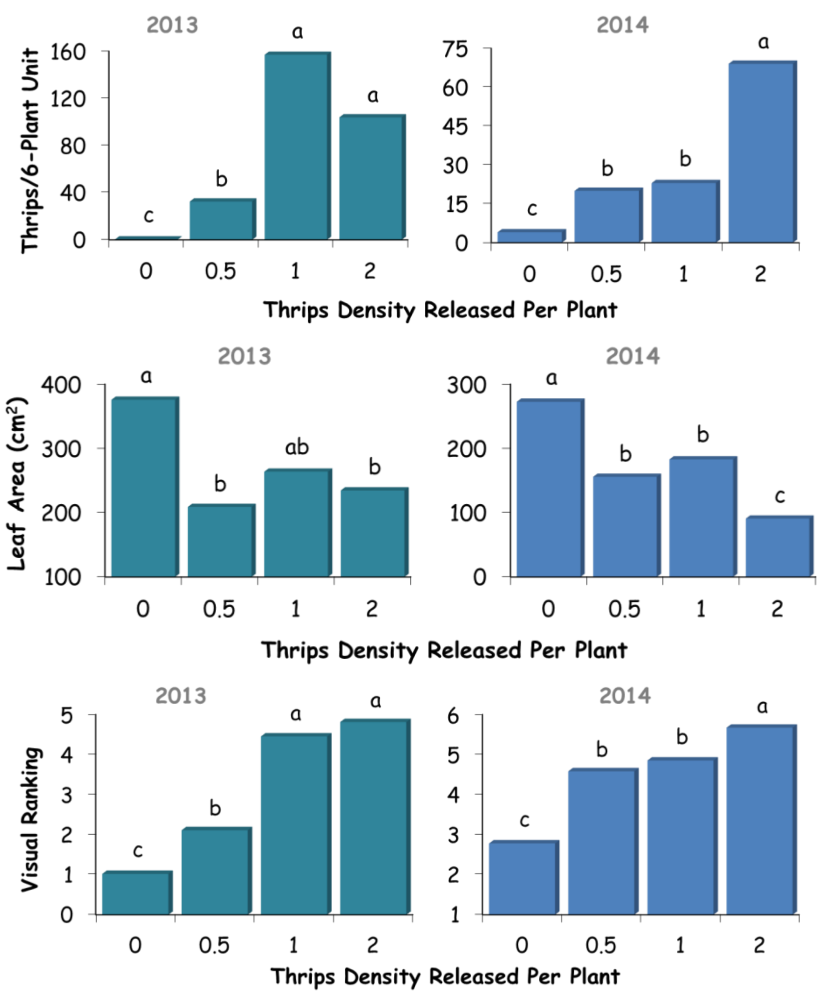

Figure 22. Thrips densities retrieved, effect of thrips on seedling growth (leaf area), and visual ranking of the cotton seedlings following thrips infestation in the greenhouse, 2013-2014, Lubbock, TX.

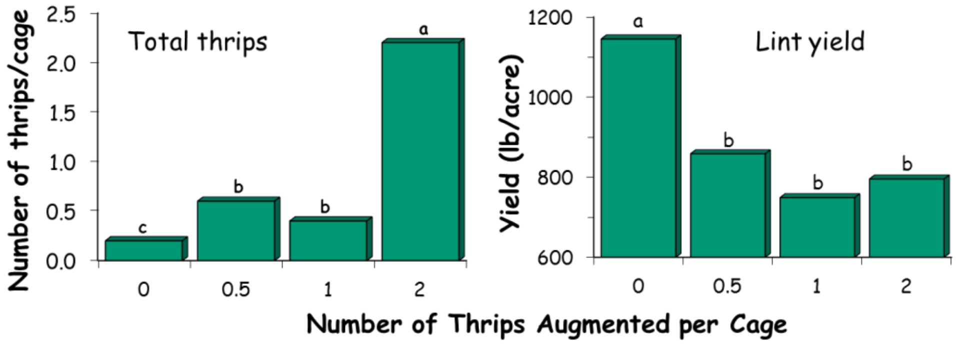

Figure 23. Field validation of the greenhouse study on thrips density augmentations and resulting lint yield reduction, 2014, Lubbock, TX.

Acknowledgement

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Thrips Management in Texas High Plains Cotton

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Thrips Management in Texas High Plains Cotton

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PROJECT SUMMARY

Thrips are the top-ranked economic insect pests in Texas High Plains cotton. Thrips can be found in cotton throughout the crop season, but cotton is most vulnerable to thrips damage within the first 30 days following seedling emergence. Lacking thrips-resistant cotton cultivars leaves no option for cotton growers but to primarily use insecticides for thrips management. While several seed treatment options are available, soil-applied aldicarb had been the most reliable and common method used for cotton seedling thrips control until the discontinuation of aldicarb in 2012. Foliar-applied insecticides such as spinosads, organophosphates, and neonicotinoids are the obvious alternatives, but since these insecticides may negatively impact the agroecosystem via long-term excessive use, their use must be optimized for effectiveness against thrips and minimal ecological impacts. Objectives of this project were to: 1) evaluate the foliar insecticide application frequency in managing thrips in seedling cotton, and 2) evaluate the efficacy, residual performance, and economic competitiveness of selected products in thrips management.

The experiment was conducted at three Texas locations (Hale County, Swisher County, and Wilbarger County) to represent cotton fields surrounded by variable vegetation/crop complexes and thrips population pressure in cotton. Thrips populations subjected to various foliar insecticide treatment regimes and thresholds were monitored on cotton cultivar FM 4946GLB2. Insecticide treatments included: 1) untreated check, 2) one foliar application at cotyledon stage (100% seedling emergence), 3) foliar applications at 100% seedling emergence and 1-2 true leaf stage, 4) foliar applications at 100% seedling emergence, 1-2 true leaf stage, and 3-4 true leaf stage, 5) foliar applications at 1-2 true leaf stage and 3-4 true leaf stage, 6) foliar treatments based on the current action threshold (1 thrips per true leaf), and 7) foliar treatments based on 50% of the current action threshold. Orthene® 97UP at a rate of 3.0 oz/acre was used for all foliar applications. Seed treatment (thiamethoxam [Avicta®], imidacloprid [Aeris®]) and foliar (Orthene®, Bidrin®, Vydate®) insecticide treatments were evaluated for their efficacy and cost effectiveness in managing thrips populations in cotton relative to an untreated control.

The 2015 growing season was marked by cool and wet spring, frequent rain events during early growing season, dry mid-summer, and warm fall. Thrips were unable to colonize in any of our test sites due to frequent rain events. Overall, Halfway (Hale Co.) site had higher thrips abundance than at Chillicothe (Wilbarger Co.) site, but the densities were far below economic threshold level of 1 thrips per leaf. Thrips began to colonize at the seed treatment and foliar insecticide study site near Halfway by the first sampling date, but the densities did not sustain due to rain events. On average, neonicotinoid seed treatments (imidacloprid and thiamethoxam) and foliar insecticide treatments both significantly reduced thrips populations compared to that in untreated control plots; all five insecticide products provided similar level of thrips population suppression. Thrips populations did not develop at the Chillicothe site. Lint yield did not vary across treatments at the Chillicothe site where no thrips infestations occurred. Lint yield was significantly lower in imidacloprid treatment plots compared to that in thiamethoxam plots at Halfway, but we found no biological basis for such difference. Thrips densities were similar and much below ET level in all insecticide treatments, so the yield was expected to be similar across all treatments.

Introduction

Thrips are the top-ranked economic insect pests in Texas High Plains cotton. Thrips can be found in cotton throughout the growing season, but cotton is most vulnerable to thrips damage within the first 30 days following seedling emergence. In Texas, an average of 4.5 million acres of cotton is infested with thrips annually, and approximately $1.2 million is spent annually to control thrips in cotton. Thrips are economically damaging to Texas cotton, and results in an average of 70,000 bales lost each year, equivalent to $33 million (Williams 2013).

Previous thrips surveys revealed at least eight thrips species in Texas cotton, but *Frankliniella occidentalis* (western flower thrips) and *Thrips tabaci* (onion thrips) are the most common species, comprising more than 75% of the thrips found in cotton (Albeldano et al. 2008). The various thrips species in Texas, being difficult to identify, have typically been managed as a single complex, with a single approach being broadly applied. Differential damage potential and pesticide susceptibility among these species remain unexamined.

Lacking thrips-resistant cotton cultivars leaves no option for cotton growers but to primarily use insecticides for thrips management. While several seed treatment options are available, soil-applied aldicarb (Temik®) had been the most reliable and common method used for cotton seedling thrips control until the discontinuation of aldicarb insecticide in 2012. Foliar-applied insecticides such as spinosads, organophosphates, and neonicotinoids are the obvious alternatives, but since these insecticides may negatively impact the agroecosystem via long-term excessive use, their use must be optimized for effectiveness against thrips and minimal environmental impacts. Information is crucial in achieving such minimization, and an understanding of cotton crop responses to various levels of thrips-induced injury throughout seedling development would be valuable for decision-making related to implementation of thrips management actions.

Ideally, cotton growers should be empowered with the capability to estimate the daily cost of delaying foliar insecticide applications for controlling thrips, further empowering them to finely adjust and achieve their acceptable, sustainable economic injury level for maximum benefits and minimum costs. Specific objectives of this project were to generate: 1) information on commercially available, effective and alternative chemical products for thrips management, and 2) information on economically viable delivery methods for chemical control (e.g., seed treatment versus foliar application) of thrips under variable growing conditions and pest pressure. Such information is expected to empower Texas High Plains cotton growers to address thrips management in a timely and cost effective manner.

Material and Methods

Objective 1. Evaluating the foliar insecticide application frequency in managing thrips in seedling cotton under variable levels of pest pressure.

The experiment was conducted at three Texas locations (Hale County, Swisher County, and Wilbarger County) to represent cotton fields surrounded by variable vegetation/crop complexes and thrips population pressure in cotton. Thrips populations subjected to various foliar insecticide treatment regimes and thresholds were monitored on cotton cultivar FM 4946GLB2. The individual experimental plots were 4 rows by 50 feet. Insecticide treatments along with their assigned treatment numbers included: 1) untreated check, 2) one foliar application at cotyledon stage (100% seedling emergence), 3) foliar applications at 100% seedling emergence and 1-2 true leaf stage, 4) foliar applications at 100% seedling emergence, 1-2 true leaf stage, and 3-4 true leaf stage, 5) foliar applications at 1-2 true leaf stage and 3-4 true leaf stage, 6) foliar treatments based on the current action threshold (1 thrips per true leaf), and 7) foliar treatments based on 50% of the current action threshold. Orthene® 97UP at a rate of 3.0 oz/acre was used for all foliar applications.

A plant washing technique was used for collecting and estimating the thrips densities at each study location weekly until the cotton was no longer considered susceptible to thrips damage. Five cotton seedlings were selected randomly from each plot as a sample unit. Plants were clipped at the base and placed in a .9451-L jar containing approximately 100 ml of 70% ethanol. Samples were taken back to the laboratory and were processed using the washing technique described by Burris et al. (1990). The jar was filled with 500 ml of tap water and 10 ml of household bleach, and one drop of liquid detergent was added to break the surface tension of the washing solution. The jar was agitated vigorously for 30 seconds, and the contents were poured into a No. 25 sieve (U.S.A. standard testing sieve, Sargent Welch Scientific, Buffalo, NY) on the top of a No. 230 sieve, and the sieves were rinsed to dislodge any remaining thrips. Plants were discarded and the sediment was backwashed with 70% ethanol into a 10 cm diameter Büchner funnel lined with a standard drip-coffee filter. The liquid was then suctioned off using a water faucet vacuum aspirator. The coffee filter with its contents was examined under a stereomicroscope, and both adult and immature thrips were counted and recorded separately.

Study Site I - Wilbarger Co. (Chillicothe). Cotton trial was planted on June 2, 2015. The first thrips sampling was conducted on June 10, followed by the application of spray treatments on the same day. Because no thrips were detected on this study site, treatments #6 and #7 were not triggered. Rain events prevented the sampling crew to access this study site at a regular weekly interval, but the second sampling was conducted on June 23, followed by the application of spray treatments on the same day. Again, frequent rain events prevented thrips colonization and no thrips were detected at this site. While this test was considered 'failed' in regards to providing relevant information to address our research goal, the crop was terminated with harvest-aids and harvested on November 3.

Study Site II - Hale Co. (Halfway). Cotton trial was planted on May 27. The first thrips sampling was conducted on June 8, followed by the application of spray treatments on the same day. Because no thrips were detected on this study site, treatments #6 and #7 were not triggered. Second sampling was conducted on June 18, followed by the application of the spray treatments. The area-wide frequent rain events prevented the thrips colonization at this test site as well. As a result, the third sampling on June 26 also failed to detect any economically relevant thrips densities to trigger treatments #6 and #7. While this test was also considered 'failed' in terms of providing relevant information to address our research goal, the crop was terminated with a boll opener (Boll Buster® 1 quart per acre) and a defoliant [ET® (pyraflufen) 1.25 oz per acre] in a tank mix on October 14 and the test was hand-harvested on November 8, 2014.

Study Site III - Swisher Co. Cotton trial was planted on June 3. The first thrips sampling was conducted on June 12, followed by the application of spray treatments on the same day. Because no thrips were detected on this study site, treatments #6 and #7 were not triggered. Second sampling was done on June 24, followed by the application of the spray treatments. The area-wide frequent rain prevented the thrips colonization at this site as well. As a result, the third sampling on July 3 also failed to detect any economically relevant thrips densities to trigger treatments #6 and #7. This test was completely failed due to recurring weather events. The test was not harvested.

Objective 2. Evaluating the efficacy, residual performance, and economic competitiveness of selected products in thrips management

Seed treatment (thiamethoxam, imidacloprid) and foliar (Orthene®, Bidrin®, Vydate®) insecticide treatments were evaluated for their efficacy and cost effectiveness in managing thrips populations in cotton relative to an untreated control. The study was conducted at two different locations within the Texas High Plains and one location in the Rolling Plains to represent cotton fields surrounded by variable vegetation/crop complexes and thrips population pressure in cotton. Cotton cultivar 'FM 4946GLB2' was planted (Hale Co., May 27; Wilbarger Co., June 2; Swisher Co., June 5). Treatment plots with foliar applications were planted with the 'base' (minimal seed treatment for warehouse storage purposes only) seed and the foliar applications were based on Texas A&M AgriLife Extension Service treatment thresholds for thrips. Adult and immature thrips were sampled by thrips washing of 5 cotton seedlings, once pre-treatment and then weekly for three times after the treatment deployment at Halfway (Hale County) location and two times at Chillicothe (Wilbarger County) location. Plans were laid-out for plant damage ratings and leaf area measurements, but no significant thrips pressure occurred in any of the three study locations due to frequent, heavy rain events during the early growth stage of cotton, except for some low density thrips at the Halfway site. Even without the thrips infestation, we kept the tests at Halfway and Chillicothe locations for harvesting to evaluate the effect of seed treatments on yield. Test plots were harvested on November 3 and 8 in Chillicothe and Halfway, respectively. Harvested samples were ginned and lint samples have been sent to Cotton Incorporated for fiber analysis.

Results and Discussion

Objective 1. Evaluating the foliar insecticide application frequency in managing thrips in seedling cotton under variable levels of pest pressure.

The 2015 growing season was marked by cool and wet spring, frequent rain events during the early cotton growing season, dry mid-summer, and warm and open fall. Because the cotton seedling stage, the susceptible stage for thrips infestation and injury, received frequent rain events, thrips were unable to colonize in any of our test sites. Overall, the Halfway site had higher thrips abundance than at Chillicothe site, but the densities were far below the current Extension recommended economic threshold level of 1 thrips per leaf. At Halfway, average thrips densities ranged from 1 to 3 thrips per 5-seedling sample on June 8, but the density quickly declined by the next sampling date. There were no significant differences in aphid densities across seven foliar application treatments (Fig. 1). The Chillicothe study site had no measurable thrips densities.

Because the thrips densities were very low (Halfway) to non-existent (Chillicothe), lint yield did not significantly vary with foliar application treatments that were targeted toward thrips population suppression (Fig. 2). Lint yield was lower at Halfway compared to that at Chillicothe across all treatments.

Objective 2. Evaluating the efficacy, residual performance, and economic competitiveness of selected products in thrips management

Thrips began to colonize at the seed treatment and foliar insecticide study site in Halfway by the first sampling date. On June 17 (second sampling date), thrips abundance increased and marginally reached the economic threshold of 2 thrips per 2-leaf seedling cotton (Fig. 3). However, a heavy rain event after the second sampling date reduced thrips densities in all treatment plots to near zero. Thrips failed to recolonize beyond that point. On average, neonicotinoid seed treatments (imidacloprid and thiamethoxam) and foliar insecticide treatments both significantly reduced thrips populations compared to that in untreated control plots (Fig. 3); all five insecticide products provided similar level of thrips population suppression. Insecticide treatments significantly increased leaf area compared with that in control plots, except for Vydate (Fig. 4). Thrips populations did not develop at the Chillicothe site.

Lint yield did not vary across treatments at the Chillicothe site where no thrips infestations occurred. Lint yield was significantly lower in imidacloprid treatment plots compared to that in thiamethoxam plots at Halfway (Fig. 5), but we found no biological basis for such difference. Thrips densities were similar and much below ET level in all insecticide treatments, so the yield was expected to be similar across all treatments.

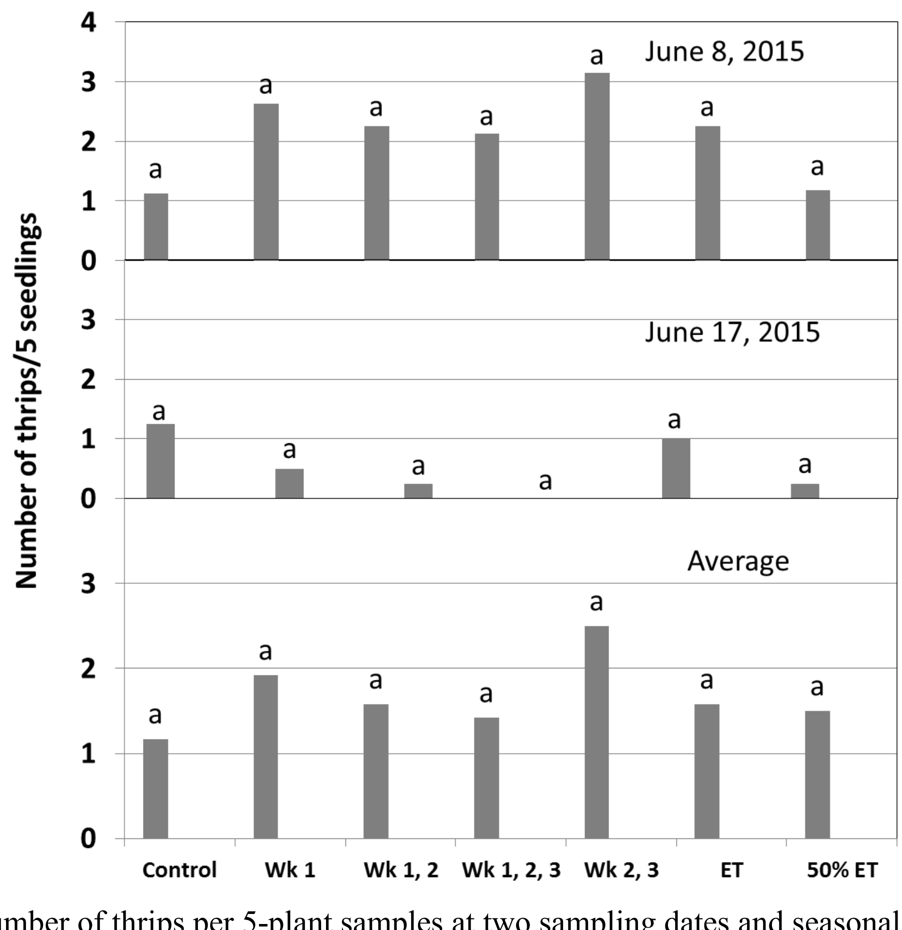

Figure 1. Number of thrips per 5-plant samples at two sampling dates and seasonal average thrips densities at Halfway as affected by foliar application of Orthene® 97UP at different application frequencies in managing thrips in seedling cotton, 2015.

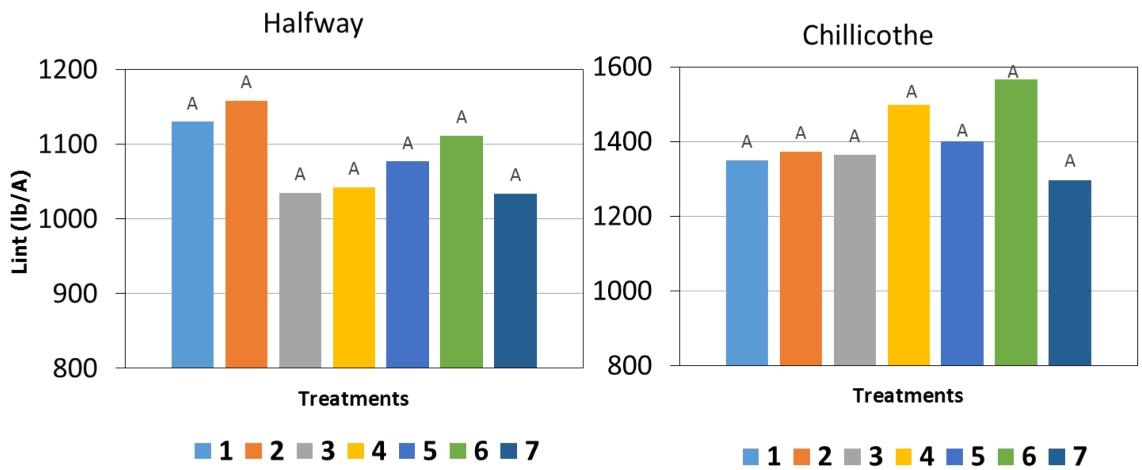

Figure 2. Lint yield (lb/acre) as influenced by foliar applications of Orthene® 97UP at different application frequencies in managing thrips in seedling cotton at two locations. Insecticide treatments numbers are as follows: 1) untreated check, 2) one foliar application at cotyledon stage (100% seedling emergence), 3) foliar applications at 100% seedling emergence and 1-2 true leaf stage, 4) foliar applications at 100% seedling emergence, 1-2 true leaf stage, and 3-4 true leaf stage, 5) foliar applications at 1-2 true leaf stage and 3-4 true leaf stage, 6) foliar treatments based on the current action threshold (1 thrips per true leaf), and 7) foliar treatments based on 50% of the current action threshold. Halfway and Chillicothe, 2015.

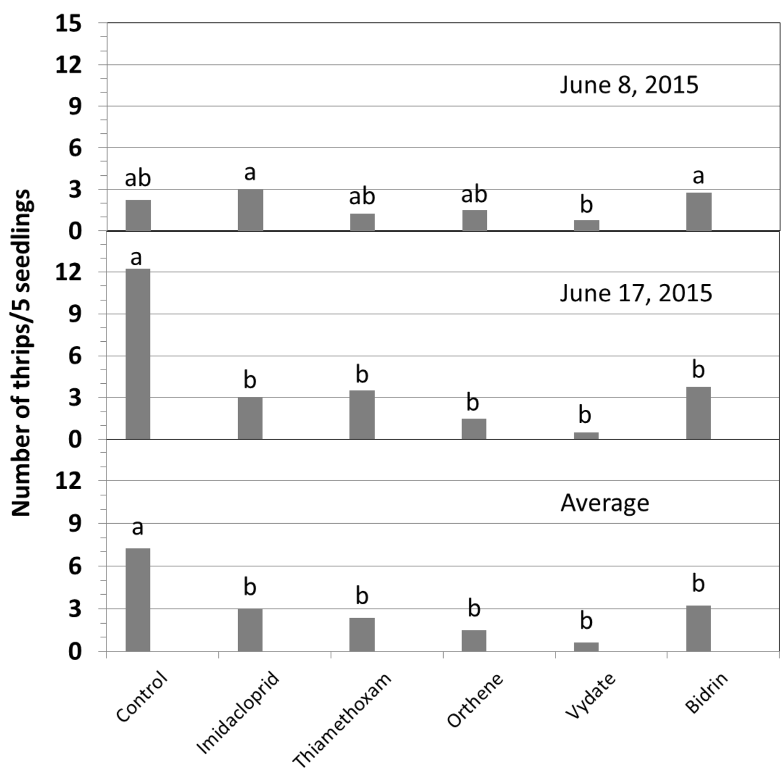

Figure 3. Number of thrips per 5-plant samples at two sampling dates and seasonal average thrips densities at Halfway as affected by seed treatment and foliar applications of selected thrips management products, 2015.

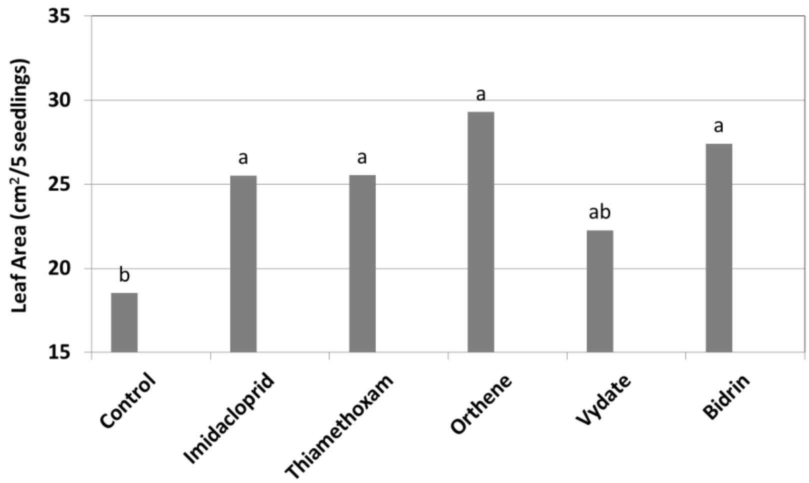

Figure 4. Total leaf area per 5-seedling sample as influenced by seed treatments and foliar applications of selected thrips management products, Halfway, TX, 2015.

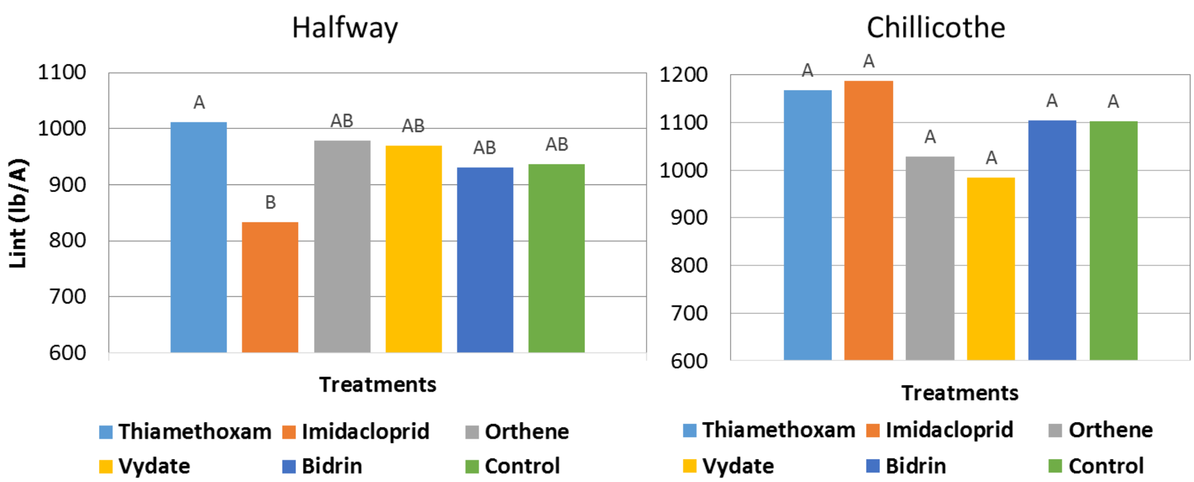

Figure 5. Lint yield (lb/acre) as influenced by seed treatments (thiamethoxam and imidacloprid) and foliar applications (Orthene®, Vydate®, and Bidrin®) of selected thrips management products at two locations, 2015.

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Seasonal abundance patterns of bollworm, tobacco budworm, and beet armyworm moths in the Texas High Plains

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INTRODUCTION

A long-term study (14 years and continuing) study has been conducted in the southern Texas High Plains (THP) region to investigate the year-around weekly moth flight activity patterns of cotton bollworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), and beet armyworm, *Spodoptera exigua* (Hübner).

These three species are important cotton pests in the southern Texas High Plains, which is recognized as the most intensive cotton growing region of the world (Fig. 1). In this region, the bollworm is classified as an important economic pest while the tobacco budworm and beet armyworm are classified as occasional pests.

The regional adoption of cotton and corn crop cultivars incorporating *Bt* technology has been instrumental in reducing the current threat of these lepidopteran pests, yet diminishing underground water availability for irrigation is necessitating lower crop inputs, such as genetically modified seed costs, for our increasing dryland crop production acreage.

MATERIALS & METHODS

Study Duration: March 2002 to Present

Study Sites: Lubbock County, Texas

Pest Species Monitored: Cotton bollworm, tobacco budworm, and beet armyworm

Survey Protocol:

Nine pheromone traps [3 lepidopteran species monitored X 3 study sites (replications)] were placed in Lubbock County representing the approximate center of the southern Texas High Plains (Fig. 1). The three sites were selected and one trap for each pest species was placed, then baited and monitored weekly (growing season) to twice monthly (non-crop months) throughout the year. Trap types included: 1) Texas pheromone trap (Fig. 2A, Hartstack et al. 1979) for bollworms and tobacco budworms, and 2) Bucket traps (green, Fig. 2B) for beet armyworms. Pheromone was secured from a single source (Trece®, Inc., Adair, OK). Trapping sites were selected and recorded their GPS coordinates.

RESULTS & DISCUSSION

Seasonal abundance and flight patterns of cotton bollworm, tobacco budworm, and beet armyworm moths were determined based upon captures in pheromone traps monitored all months of the year. For each species, the ongoing 14-year trapping study has been sub-divided into four successive periods, including: 1) 2002-2005, 2006-2009, 2010-2013, and 2014-2015, representing boll weevil eradicated and beginning of *Bt* cotton adoption in THP, low *Bt* cotton acreage (<50%), majority *Bt* cotton (70%), and the current two years, respectively.

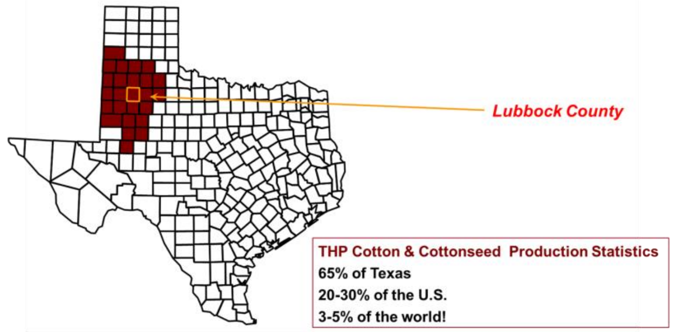

Figure 1. Texas High Plains pheromone trapping study site, Lubbock County, TX, 2002-2015.

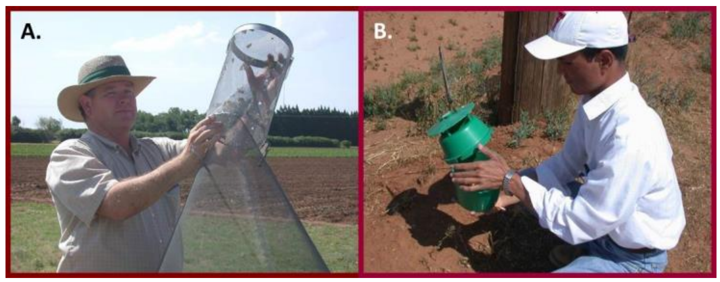

Figure 2. S. Carroll services a Texas pheromone (TP) trap to monitor bollworm and tobacco budworm moths (A). R. Shrestha counts beet armyworm moths in a green bucket trap (B).

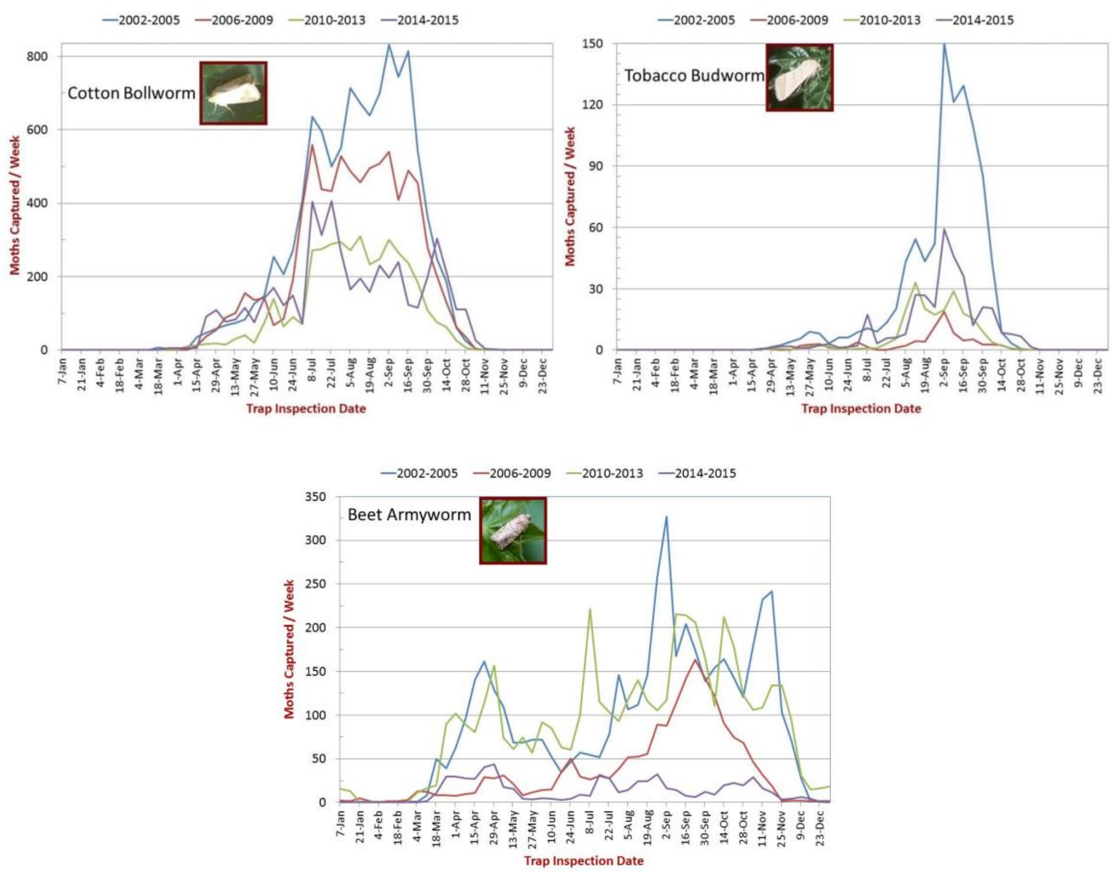

Figure 3. Number of bollworm (top-left), tobacco budworm (top-right), and beet armyworm (bottom-center) moths captured per week, averaged across four selected 2-4 consecutive-year groupings spanning the 14-year study, Lubbock County, TX, 2002-2015.

Cotton Bollworm. The cumulative annual number of bollworm moths captured per trap averaged 10,618, 7,970, 4,071 and 5,014 for 2002-2005, 2006-2009, 2010-2013, and 2014-2015, respectively. The observed trend suggests a decreasing, yet high bollworm numbers during years 2002 to 2009, followed by a leveling off of numbers beginning in 2010 to the present. Fig. 3 (top-left panel) clearly illustrates this trend of decreasing trap captures during the first 8 years, followed by lower, yet fairly level bollworm captures from 2010 to 2015. Interestingly, although bollworm numbers decreased over time, the flight profiles remained quite similar over the four periods.

Tobacco Budworm. The cumulative annual number of tobacco budworm moths captured per trap averaged 953, 87, 209 and 354 for 2002-2005, 2006-2009, 2010-2013, and 2014-2015, respectively. Higher numbers of tobacco budworm moths were trapped during the early 2002-2005 period and then numbers decreased and have remained fairly low in the past 10 years with the exception of one 2014-2015 peak in early September (Fig. 3, top-right). Although the number of trapped budworm moths varied between the four defined periods, the overall flight activity patterns had somewhat similar profiles with activity starting in late April, peak activity during early August to early October and most trap response ending by late October.

Beet Armyworm. The cumulative annual number of beet armyworm moths captured per trap averaged 4,651, 1,790, 4,596, and 656 for 2002-2005, 2006-2009, 2010-2013, and 2014-2015, respectively. Although beet armyworm moths were often captured during all months of the year, they were primarily active during the period of mid-March to early December (Fig. 3, bottom-center panel). Unlike decreasing bollworm and tobacco budworm numbers since the beginning of the study, no obvious population trends are evident. For example, high cumulative trapped beet armyworm numbers were observed during two separate periods of 2002-2005 and 2010-2013. The lowest numbers have been observed during the current years.

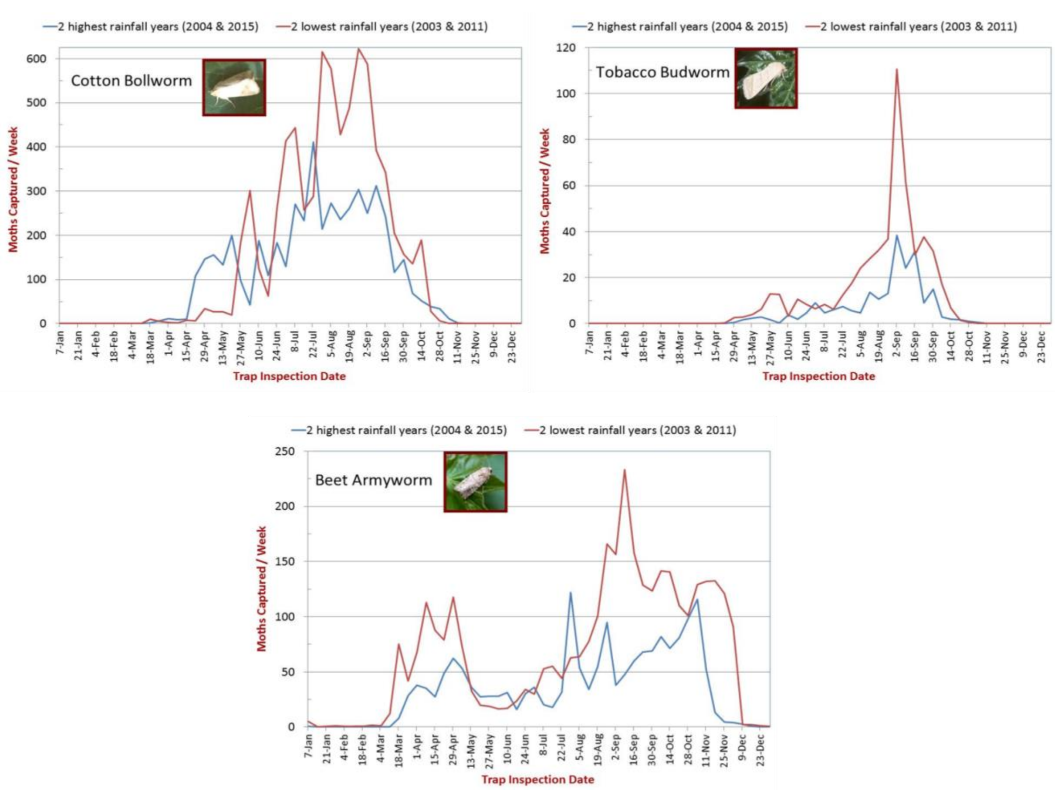

Figure 4. Cotton bollworm (top-left), tobacco budworm (top-right), and beet armyworm (bottom-center) moth seasonal flight profiles for: 1) Two study years with the highest rainfall (2004 & 2015), and 2) Two lowest rainfall years (2003 & 2011). Lubbock County, 2002-2014.

Influence of annual rainfall on moth abundance and flight profiles. Within the 14-yr study period, cumulative annual rainfall ranged from 5.7-in. to 33.3-in. The two years with the lowest rainfall were 2003 (8.8-in.) and 2011 (5.7-in.), while the two highest rainfall years were 2004 (33.3-in.) and 2015 (29.5-in.). For each pest species, the seasonal abundance and flight profiles are plotted for the two highest and two lowest rainfall years (Fig. 4).

*Cotton Bollworm***.** The overall timing of the flight profiles were similar between high and low rainfall years, except in regard to the magnitude of the peak numbers of moths captured (Fig. 4, top-left panel). The highest cumulative number captured per trap per year was 7,254 for the low rainfall years, while the numbers in highest rainfall years declined by 31.0% to 5,005 moths.

*Tobacco Budworm***.** Again, the overall timing of the flight profiles was similar between high and low rainfall years, but more budworm moths were captured during the low rainfall years (Fig. 4, top-right panel). The highest cumulative number captured per trap per year was 533 for the low rainfall years, while the cumulative number in the highest rainfall years declined by 58.5% to 221 moths.

*Beet Armyworm***.** During the low rainfall years, the beet armyworm flight profile started earlier and also extended later into the early winter period as compared to the flight active periods observed during the high rainfall years (Fig. 4, bottom-center panel). The highest cumulative number of beet armyworm moths captured per trap per year was 3,398 for the low rainfall years, while the numbers in highest rainfall years declined by 47.8% to 1,773 moths.

ACKNOWLEDGMENTS

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Pheromone Trapping of *Helicoverpa* Moth Species in the Texas High Plains: Investigation of Possible Old World Bollworm Detection

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INTRODUCTION

An ongoing one-year study has been conducted in Lubbock County to investigate the seasonal moth flight activity patterns of *Helicoverpa* spp. captured on the following trapping treatments: 1) Two different types of traps, and 2) Species-specific pheromones obtained from several sources. The investigated insects included *Helicoverpa zea* (Boddie), a common key pest of numerous U.S. and Texas High Plains crops, plus a conceivable detection of a new invasive 'old world' pest, *Helicoverpa armigera* (Hübner), commonly referred to as the old world bollworm (OWB).

A comprehensive published collection of *H. armigera* information can be found at the following link: https://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/NPRG_H_armigera.pdf. This publication reports recent OWB detections by scientists in Brazil (2013), Paraguay (2013), Argentina (2014), and most recently from Bolivia, Uruguay, Puerto Rico, and the U.S. (Florida). The old world bollworm closely resembles the widespread cotton bollworm, *H. zea*. The adult male moths of the two species have slight morphological differences that can be used by trained individuals to distinguish the two species apart, yet the larvae of these closely related species cannot be distinguished apart, except via DNA analysis. To complicate matters related to species identification and monitoring, the laboratory synthesized *H. armigera* pheromone incorporated into lures produced in order to attract old world bollworms to traps is also very attractive to *H. zea*. For this report, the numbers of male *Helicoverpa* moths captured per trap per day are reported, but genus species complex breakdowns for each pheromone type or trap type have not been processed to date, due primarily to the difficulty and time requirements of identification. We plan to conduct the dissection studies in 2016 in an attempt to detect any old world bollworm invasion to Texas.

The primary objectives of the following study were to: 1) Investigate the effectiveness of species-specific pheromone lures obtained from two vendors, and 2) Determine the efficiency of two different trap designs in capturing *Helicoverpa* spp. moths. Ultimate goal of the project is to dissect sub-samples of these trapped moths towards examining for old world bollworm introduction to Texas.

MATERIALS & METHODS

Study Duration: Year 1 - August 2015 to November 2015

Study Sites: Four sites in Lubbock County, Texas

Targeted Pest Species Monitored:

1) Cotton bollworm [*Helicoverpa zea* (Boddie)]
2) Old world bollworm [*Helicoverpa armigera* (Hübner)]

Trap Types Tested: 1) Texas trap
2) Bucket trap (green)

Pheromone Types and Sources:

1) Cotton bollworm (*H. zea*) lures from Trece®, Inc.

2) Old world bollworm (*H. armigera*) lures from Trece®, Inc.
3) Old world bollworm (*H. armigera*) lures from USDA Cooperative Agricultural Pest Survey (CAPS) Program

Treatments:
1) Texas Trap w/ Trece® *H. armigera* lure
2) Texas Trap w/ Trece® *H. zea* lure
3) Bucket Trap w/ Trece® *H. zea* lure
4) Bucket Trap w/ Trece® *H. armigera* lure
5) Bucket Trap w/ USDA 'CAPS' *H. armigera* lure

Trapping Protocol:

Five pheromone traps, each representing one of the study treatments (listed above), were randomly assigned positions at each of four separated Lubbock County sites, all located along a 21-mile east/west stretch of highway FM 1294 in northern Lubbock County. At each site, the five traps were evenly spaced apart by positioning them near five consecutive electrical utility poles. Placing the test traps near utility poles protected the traps from interfering with normal farming operations in the adjacent fields. These study traps were monitored and the captured moths counted approximately weekly during the study period of August to November. All traps were re-baited with fresh lures approximately every two weeks. Exact trapping site locations were determined via GPS coordinates.

RESULTS & DISCUSSION

For each trap inspection interval (x-axis), Figure 1 illustrates the mean number of adult male moths captured per trap per day in traps representing combinations of two trap designs, two species-specific pheromones, and two sources for the pheromone lures. The chart clearly shows that during periods of high *Helicoverpa* flight activity, Texas (TP) traps captured much higher numbers of moths as compared to the Bucket traps, while during periods of reduced flight activity (e.g., cooler late-season period), the differences between all five treatments, regardless of pheromone source or trap design, were relatively small. Without exception, bucket traps baited with USDA CAPS *H. armigera* lures attracted fewer moths than identical traps baited with Trece® *H. armigera* lures, which suggests the Trece® formulated lure may have superior properties.

During periods of elevated moth flight activity, Texas traps baited with Trece® *H. armigera* pheromone lures tended to attract and capture more moths than *H. zea* baited traps (Figure 2). During inspection intervals with diminished moth flight activity, the *H. armigera* and *H. zea* baited Texas traps both captured very few, yet similar numbers of moths.

Figure 3 illustrates, as mentioned earlier, that the green Bucket traps baited with Trece® formulated *H. armigera* lures (blue line) consistently captured more moths than identical traps baited with the USDA CAPS *H. armigera* lures (red line). When one compares the number of moths captured on Bucket traps baited with Trece® *H. armigera* lures versus Bucket traps baited with Trece® *H. zea* lures, the trends are not as consistent as seen with the Texas traps, but in most cases the *H. armigera* baited traps capture slightly higher numbers of moths than the *H. zea* baited traps. It should be noted that most (or all) of the moths captured in traps baited with *H. armigera* lures are likely *H. zea* moths; we plan to conduct dissection on sub-samples of these collected moths to determine if *H. armigera* were captured in our study.

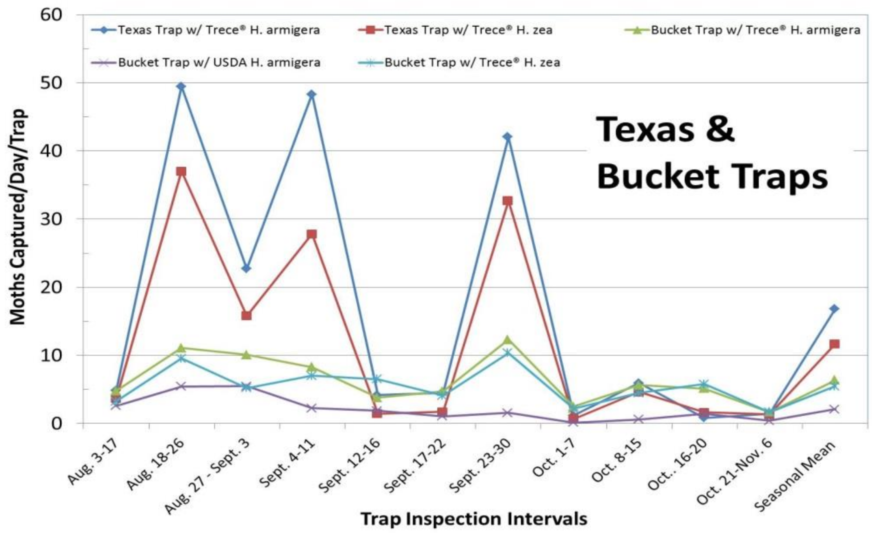

Figure 1. Average number of *Helicoverpa* spp. male moths captured per trap per day, Lubbock County, TX, 2015.

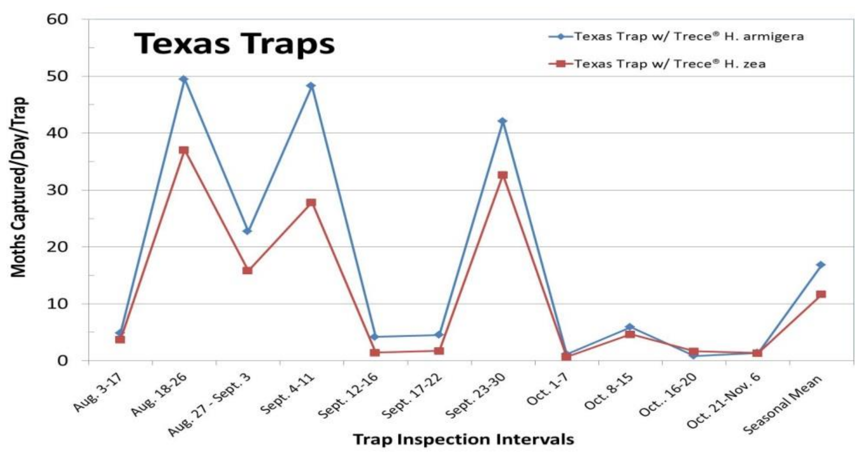

Figure 2. Average number of *Helicoverpa* moths captured per day per trap, baited with two Trece® sourced lures, one for *H. armigera* monitoring and other for *H. zea*, Lubbock County, TX, 2015.

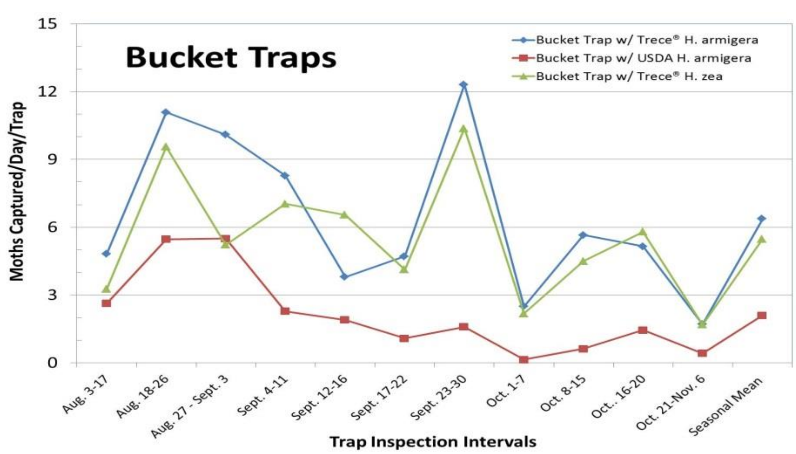

Figure 3. Average number of *Helicoverpa* spp. male moths captured per day per trap on green Bucket traps, Lubbock County, TX, 2015.

EFFICACY OF NEONICOTINOID SEED TREATMENTS ON THRIPS IN COTTON

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Abstract

Seed treatments are common and effective pest control methods in many crop systems. Cotton seed treatments are often used for control of early season pests. A market shift in seed treatments to neonicotinoid formulations followed the phasing out of aldicarb (Temik®). Imidacloprid and thiamethoxam (Cruiser®) are two commonly used insecticide cotton seed treatments, but concern lies with the possibility of varying degrees of efficacy of these seed treatments on the different thrips species. The common thrips species that infest cotton seedlings are tobacco thrips (*Frankliniella fusca*), flower thrips (*Frankliniella tritici*), western flower thrips (*Frankliniella occidentalis*), and onion thrips (*Thrips tabaci*), and each of these exhibit different degrees of susceptibility to various insecticide formulations. It is necessary for us to evaluate the thrips species composition in Texas cotton, as well as the impact of thiamethoxam and imidacloprid seed treatments on those species. The evaluated locations throughout Texas included: Chillicothe, College Station, Halfway, Kress, Lamesa, and Wall. These are representative sample areas of the High Plains, Rolling Plains, and Central Texas areas. Thrips populations were low in Chillicothe, Lamesa and Wall, but there were fewer thrips in treated seeds for most sampling dates in College Station, Halfway and Kress. Imidacloprid treated seed resulted in greater yield than the control in College Station, which was the only harvested location with thrips populations exceeding treatment threshold (one visible thrips per true leaf) levels. Greenhouse evaluations of thiamethoxam and imidacloprid seed treatments for western flower thrips provided information on maximum potential efficacy of these products in a more controlled environment.

Introduction

Thrips are an important early season insect pest on cotton throughout the U.S. cotton belt. In Texas, the thrips complex generally ranks first in terms of cotton lint yield loss due to insect pests (Williams 2013). Until 2011, thrips were commonly managed by using in-furrow applications of aldicarb (Temik®) and growers achieved satisfactory control. However, the removal of Temik® from the market due to concern over its possible environmental impact has forced cotton producers to resort to other available insecticide seed treatments. These seed treatment insecticides primarily belong to one insecticide group, the neonicotinoids. Currently, there are two neonicotinoid insecticides available for use as cotton seed treatments. Although both insecticides (imidacloprid and thiamethoxam) belong to the same group, their physical and chemical properties vary, which may result in differences in efficacy of these two products on the target insect pest (thrips). Therefore, region-specific evaluation of different seed treatment products is necessary to determine their effectiveness in managing thrips populations in respective regions. Also, a product may not be equally effective on all the prevalent thrips species impacting cotton across Texas' production regions. For example, a thrips population may be composed of individuals from different species. The common thrips species that infest cotton seedlings are tobacco thrips (*Frankliniella fusca*), flower thrips (*Frankliniella tritici*), western flower thrips (*Frankliniella occidentalis*), and onion thrips (*Thrips tabaci*). Each of these thrips species could have variable levels of tolerance or susceptibility to each of the available insecticides. Western flower thrips populations, for example, have been found to be resistant to both organophosphates and pyrethroids in Australia (Herron *et al*. 1996). Similarly, resistance to pyrethroids within western flower thrips populations has been documented from Missouri and California (Zhao *et al*. 1995, Immaraju *et. al*. 1995). Historically, western flower thrips have shown to develop resistance to insecticides, as evident from the previous examples and a number of other reports from around the world. Therefore, it is possible that western flower thrips in cotton could develop resistance to the neonicotinoid seed treatments over time. While western flower thrips are a potential candidate for developing resistance to neonicotinoid seed treatments, there are already reports of resistance development to thiamethoxam in tobacco thrips populations as documented by several researchers from the mid-south of the U.S. (Stewart 2013).

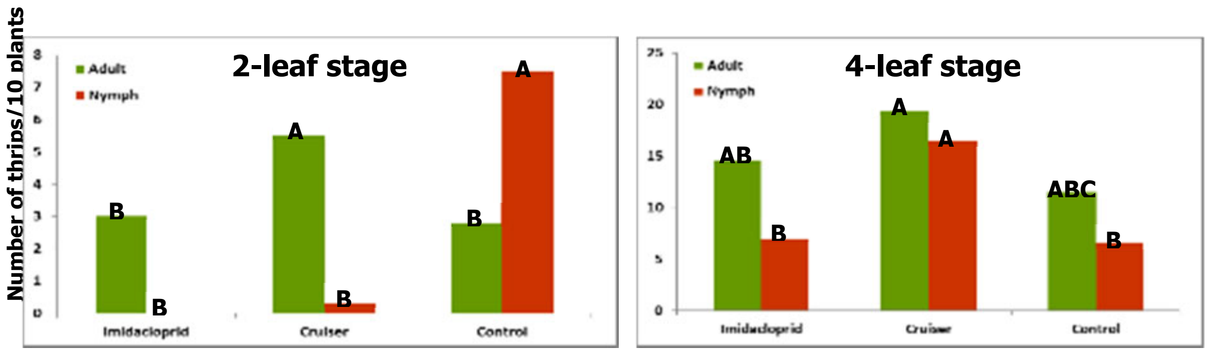

Fig. 1. Number of thrips recorded on cotton seedlings resulting from two different neonicotinoid insecticide treated seed plantings along with an untreated control; all three treatments evaluated at two plant growth stages.

The thiamethoxam (Cruiser®) resistance in tobacco thrips is a great concern for U.S. cotton growers; not only in the mid-south, but also in Texas. There are also some suggestions that thrips populations in south Texas, which is dominated by tobacco thrips, is less susceptible to Cruiser® (thiamethoxam) as compared to imidacloprid (Fig. 1). The above graphs represent the results from a trial conducted in Matagorda Co., TX by Dr. Roy Parker and observed that the thiamethoxam (Cruiser®) treated cotton plots had more thrips compared to the seedlings resulting from seed treated with imidacloprid (Gaucho Grande®). It is imperative for us to evaluate this product (thiamethoxam) more closely in different Texas cotton production regions in order to detect any possible resistance development in our thrips populations. The two objectives of this study will effectively address the possibility of varying degrees of the neonicotinoid seed treatment efficacy on different species. Specifically, surveying the thrips species present in Texas fields will provide information on the current and changing species composition of thrips in Texas cotton, while comparing the seed treatments in different locations will give us direct information on the current level of efficacy of the seed treatment insecticides, especially thiamethoxam. The final goal of this project is to generate information regarding thrips populations in Texas cotton production regions so that our regional growers and consultants have useful information on what thrips species are most likely to occur in their fields and what available effective products be used to control those specific thrips species.

Materials and Methods

Greenhouse Trials

To evaluate the efficacy of thiamethoxam and imidacloprid in a controlled environment, we conducted a greenhouse study. Two weeks prior to planting the cotton seeds, we planted solid trays of wheat in order to build the western flower thrips populations within the greenhouse. Metro-mix® was used as the potting medium for both wheat and cotton. Prior experiments in the greenhouse had highest success with planting one tray of wheat for every 2 trays of cotton, so we planted 48 trays of wheat to ensure adequate thrips populations. We planted 288 FM 1944 GLB cotton seeds (one seed per experimental cup), 96 of each treatment (thiamethoxam, imidacloprid, and control). The seedlings were completely randomized within trays containing 18 seedlings total, 6 of each treatment. Samples of 4 trays were taken at 7 days after emergence (DAE), 14 DAE, 21 DAE and 28 DAE. The samples were taken by cutting the base of the seedlings, and placing 6 seedlings of each treatment in a quart-sized mason jar containing 70% ethanol. Thrips were counted from these samples using the thrips washing method (Burris *et al.* 1989).

Field Trials

The field trials consisted of three treatments (thiamethoxam, imidacloprid, and control), with 4 replications. Plot size was 4 rows wide by 50 feet long, with 5 feet alleys separating the plots. Each trial consisted of 12 plots, randomized within the replication (block). The sites for the field trials were all located in Texas, the locations as follows: Kress (Swisher county), Halfway (Hale county), Lamesa (Dawson county), Wall (Tom Green county), Chillicothe (Hardeman county), and College Station (Burleson county). All trials were conducted with sufficient irrigation. The cotton variety used was the same as we used in the greenhouse, FM 1944 GLB. This cotton variety was chosen as it is suited to the regions we chose for trial sites, as well as having some tolerance to root-knot nematodes. No nematicide was applied to the seeds in order to avoid any possible interaction with the insecticides we are testing. Planting dates were adjusted in each location according to the location's recommendation, and sampling took place at the cotyledon, 1-2 true-leaf and 3-4 true- leaf stages of the plants. Ideally, sampling was to take place at 7-day intervals, but inclement weather and other management logistics forced us to deviate from the 7-day intervals in some cases. During sampling, 10 random seedlings from each plot were placed in a quart-sized mason jar and taken to the lab to be processed using the thrips washing method (Burris *et al.* 1989). We recorded the number of thrips larvae, adults and the total number of thrips from each plot, and placed the adults in 70% ethanol for species identification later.

Results and Discussion

In the greenhouse, we found no significant difference between treatments at any time intervals, but over the first 3 weeks the thrips populations increased. At the 3rd true leaf stage, we had about 42 thrips per seedling. Soil selection likely impacted our results, and this trial is to be repeated using field soil rather than a potting soil mix in 2015 (Fig. 2).

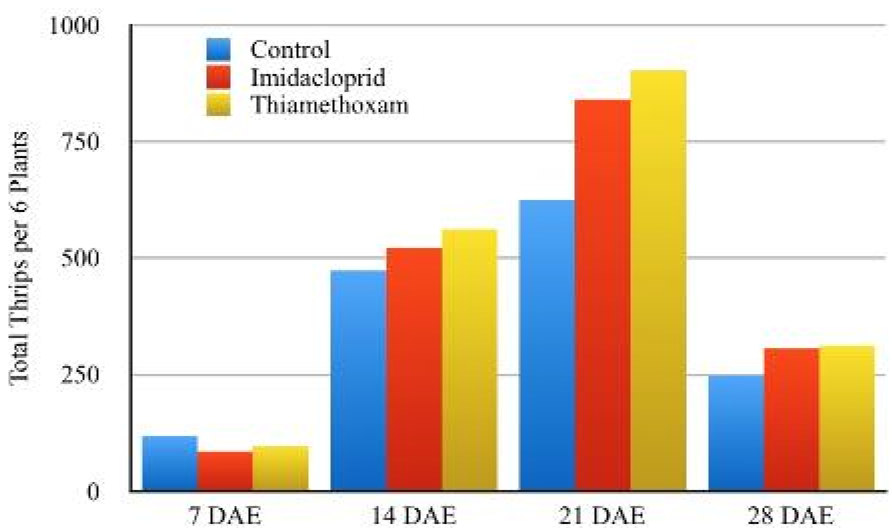

Fig. 2. Total number of thrips per 6 plants in the greenhouse at 7 DAE, 14 DAE, 21 DAE and 28 DAE. There are 3 separate treatments, a control, imidacloprid, and thiamethoxam. There was no significant difference between treatments at any sampling date.

At the farthest north location, the Kress site, our untreated control plots had over 20 thrips per plant at the 1st true leaf stage, while the treated plants had about 20 total thrips on all 10 seedlings. The 2nd sampling date followed cool and wet weather, and we saw no difference in thrips numbers between treatments. This field site was replanted to sorghum shortly following the 2nd sampling date due to a poor stand after adverse weather (Fig. 3).

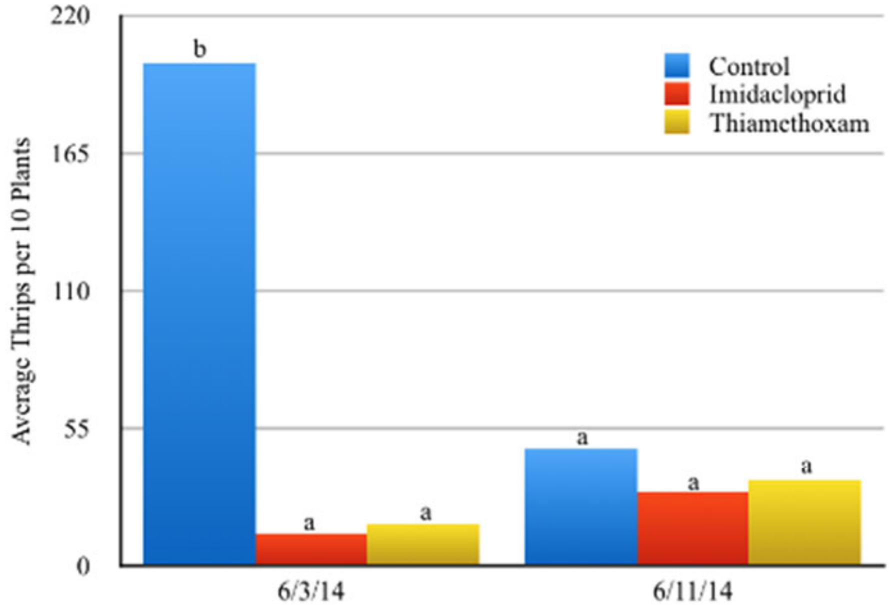

Fig. 3. Total number of thrips per 10 plants at 2 separate sampling dates at the Kress site. There are 3 treatments, a control, imidacloprid, and thiamethoxam at each sampling date. The trial was planted on 5/13/14. Letters note significant difference between treatments within sampling dates.

At the Halfway site, we observed differences between the two seed treatments and the control at the first 2 sampling dates. The control plants for the first 2 sampling dates had about 1.3 thrips per plant, but by the third sampling date, the thrips populations had decreased, and were no longer significantly different (Fig. 4).

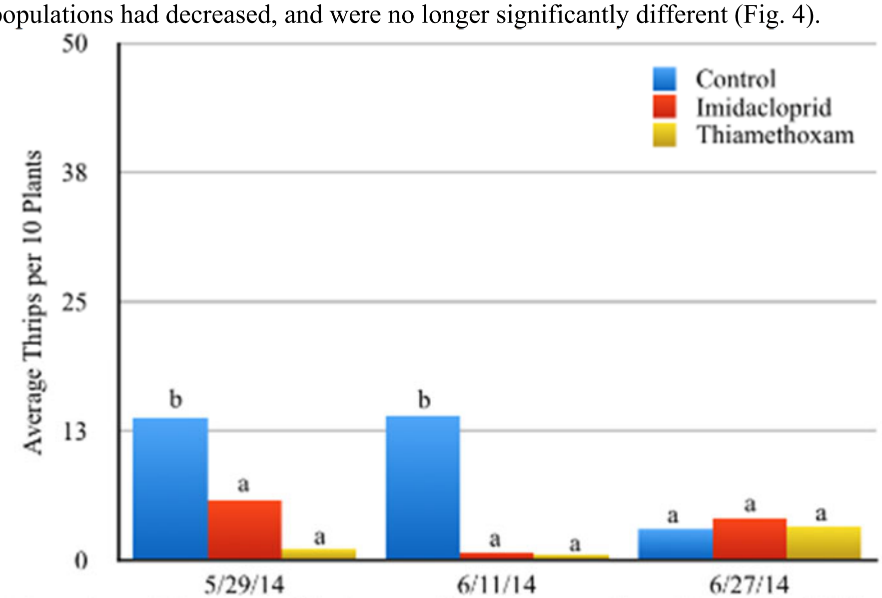

Fig. 4. Total number of thrips per 10 plants at 3 separate sampling dates at the Halfway site. There are 3 treatments, a control, imidacloprid, and thiamethoxam at each sampling date. The trial was planted on 4/6/14. Letters note significant difference between treatments within sampling dates.

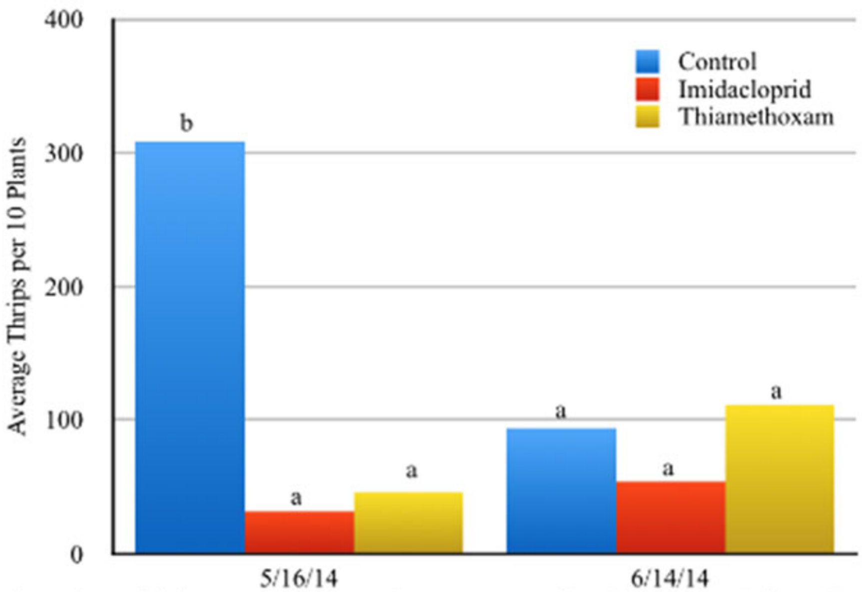

Fig. 5. Total number of thrips per 10 plants at 2 separate sampling dates at the College Station site. There are 3 treatments, a control, imidacloprid, and thiamethoxam at each sampling date. The trial was planted on 4/25/14. Letters note significant difference between treatments within sampling dates.

In College Station, we observed a difference between control and the 2 seed treatments at the first sampling date. Thrips populations had declined by the 2nd sampling date, following cool weather and heavy rainfall (Fig. 5). The Wall, Lamesa, and Chillicothe sites showed no difference between any of the treatments, but we also did not observe many thrips at any of these sites. (Figs. 6-8).

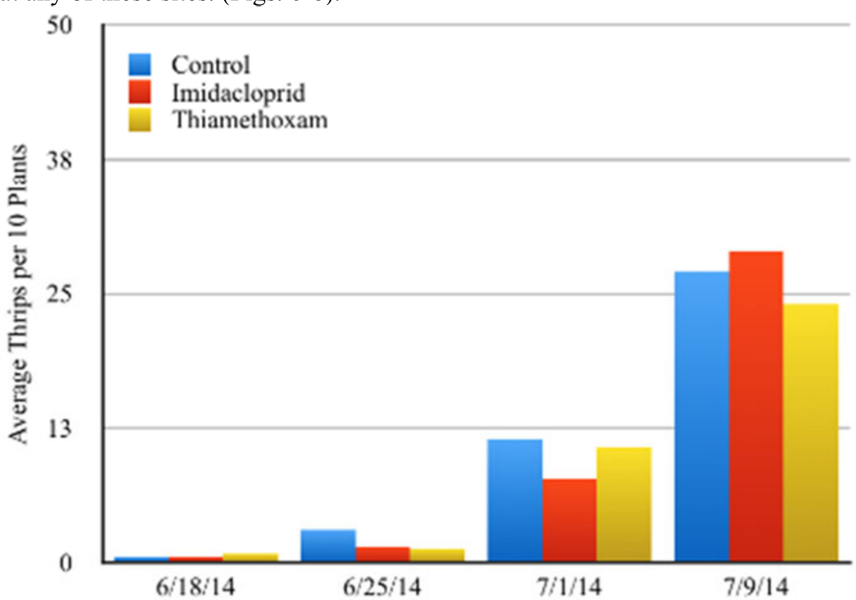

Fig. 6. Total number of thrips per 10 plants at 2 separate sampling dates at the Wall site. There are 3 treatments, a control, imidacloprid, and thiamethoxam at each sampling date. Lack of letters shows that there was no significant difference between treatments within sampling dates.

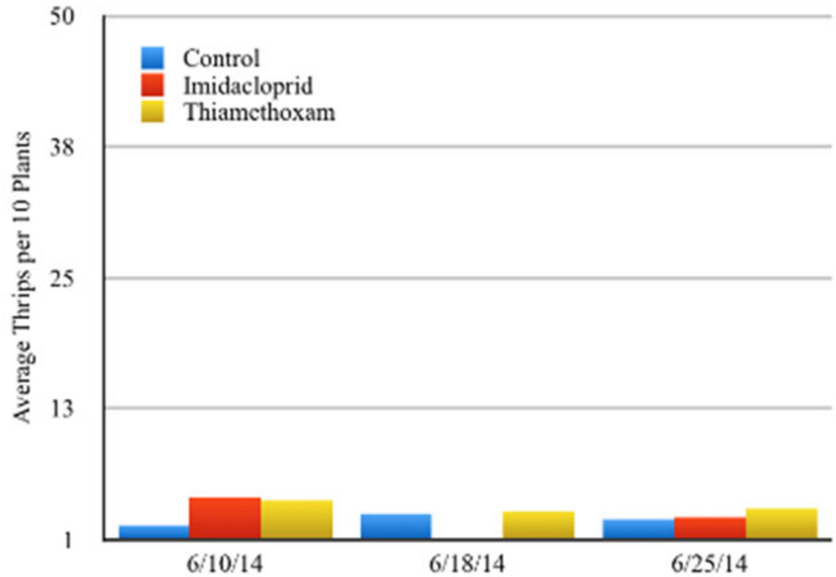

Fig. 7. Total number of thrips per 10 plants at 2 separate sampling dates at the Lamesa site. There are 3 treatments, a control, imidacloprid, and thiamethoxam at each sampling date. Lack of letters shows that there was no significant difference between treatments within sampling dates.

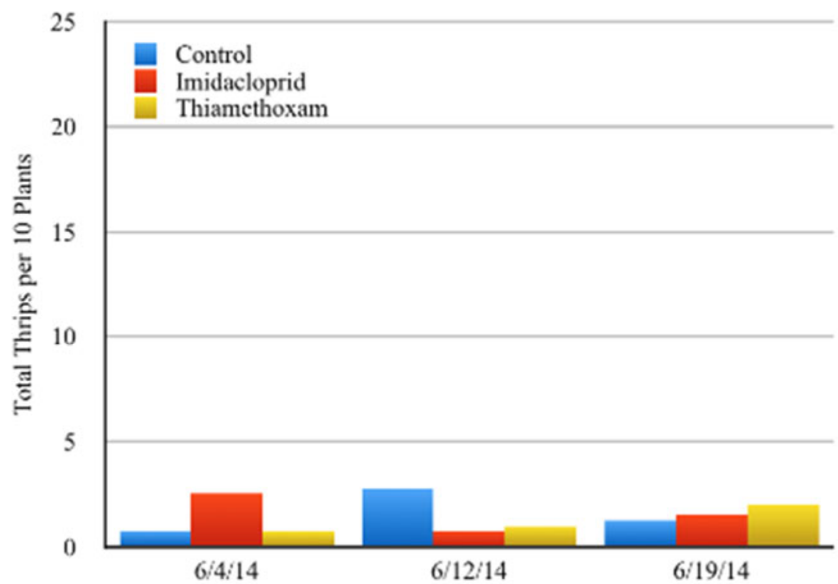

Fig. 8. Total number of thrips per 10 plants at 2 separate sampling dates at the Chillicothe site. There are 3 treatments, a control, imidacloprid, and thiamethoxam at each sampling date. Lack of letters shows that there was no significant difference between treatments within sampling dates.

The College Station site was the only one that reached the threshold for thrips at any point during the season. This was also the only site we observed a difference in yield between the treatments. Imidacloprid treated plots produced a higher yield than did the control plots, and thiamethoxam showed no significant difference compared to imidacloprid or the control (Fig. 9).

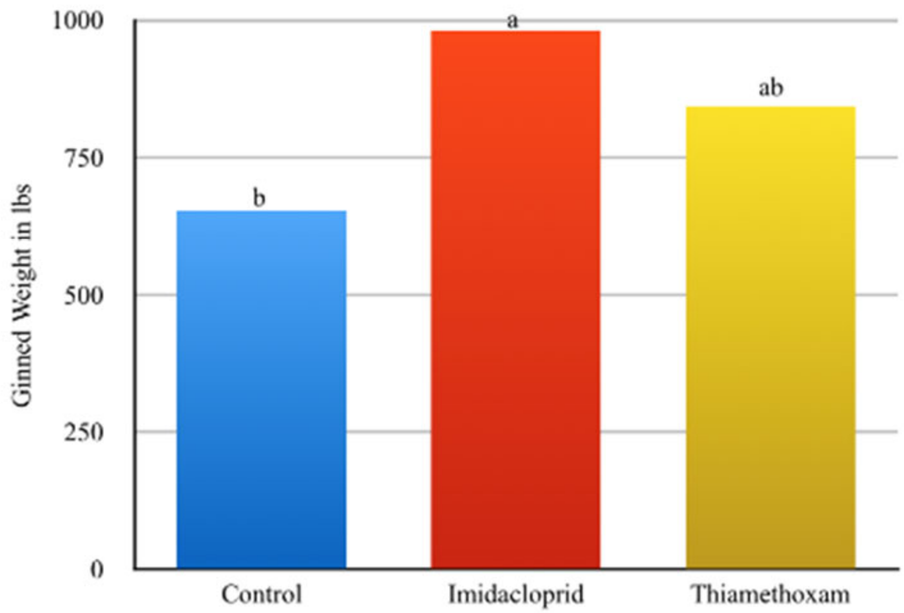

Fig. 9. Yield per acre in pounds from the College Station site. A 38% turnout was used to calculate the approximate ginned weight. Letters note significance between treatments.

Summary

In conclusion, the seed treatments reduced thrips populations in Kress, Halfway, and College Station. The efficacy of these treatments decreased over time, as expected. Only one location, College Station, had thrips populations to reach threshold and influence yield significantly. At the College Station site, imidacloprid treated seed outyielded the control. Our greenhouse data did not show significant treatment effects, likely due to methodological reasons with potting soil selection. In the future, we plan to repeat the greenhouse trials with field soil instead of potting soil, and repeat the field trials in the same locations next year.

Acknowledgements

This work was conducted as part of Kate Harrell's MS thesis research. KH would like to thank the Plains Cotton Growers association, Cotton Incorporate and the Texas A&M Agrilife Extension service for their support of this project. In addition to my committee members, I'd also like to thank Mark Arnold, Steven Biles, Stan Carroll, Tommy Doederlein, Jeffery Drymalla, Jeff Harrell, Cole Miller, Rick Minzenmayer, Richard Norman, Pat Porter, Blayne Reed, Cesar Valencia, Kerry Siders, Leslie Wells, and Wending Zhou for their help with this research.

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BREEDING VALUE OF HOST PLANT THRIPS RESISTANCE FOR NEW CULTIVAR DEVELOPMENT

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Abstract

Thrips (Thysanoptera: Thripidae) management is an important component of cotton production on the Texas High Plains. With the loss of systemic aldicarb insecticides, host plant resistance can be a valuable tool for mitigating thrips injury to cotton seedlings. Understanding the nature and breeding value of such resistance is key for its utilization in cultivar development. Field and greenhouse trials were conducted in 2011-2014 to evaluate genetic segregation and estimate broad sense heritability (H^2) and actual gain from selection (G_s). In 2011, an interspecific F_2 population was grown in a greenhouse trial to evaluate genetic segregation of the trait. Individual plants were evaluated for thrips feeding injury at 4-5 true leaves, utilizing a visual damage rating scale. Phenotypic data were continuously distributed, and subsequent chi-square analyses confirmed that the data did not fit known single- or two-gene segregation ratios ($P < 0.05$). A similar trial was repeated at the field level in 2012, utilizing a different interspecific F_2 population. Chi-square analyses again suggested quantitative inheritance of thrips resistance ($P < 0.05$). Therefore, in 2013-2014, five families, derived from interspecific crosses, were evaluated in greenhouse trials to estimate the H^2 of thrips resistance. Individual parent and F_2 plants were planted and visually assessed for thrips damage. H^2 values ranged 40-70%, depending on the family. F_2 and F_3 populations, resulting from an interspecific cross, were evaluated at the field level in 2012 and 2013, respectively, to estimate actual gain per cycle of selection. A 10% selection intensity (SI) resulted in an approximate 9% gain per cycle of selection; 5% SI resulted in a 22% gain and 1% SI resulted in only a 3% gain. Given the potential sensitivity of this trait to environmental conditions, a 5% SI appeared to optimize actual genetic gain, whereas 1% SI was likely too intense. Selection intensity should therefore be adjusted according to ambient thrips populations in a given location, to ensure greater capture of genetic effects through selection. These results suggest that host plant thrips resistance in cotton is quantitatively-inherited, with moderately high heritability. Significant genetic improvement can be thereby achieved through visual selection, depending on selection intensity in a given cycle.

UTILIZATION OF NO-THRIPS® CAGES TO QUANTIFY THRIPS-INDUCED LOSSES TO COTTON IN THE TEXAS HIGH PLAINS

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Texas produces 55% of U.S. cotton, of which, approximately 66% is produced in the Texas High Plains region. In 2013, arthropods caused a 2.27% cotton yield loss in the U.S. Thrips infested 6.7 million acres of cotton in the U.S. in 2013, with 2.4 million acres infested in Texas. This infestation caused a 0.56% yield loss due to thrips and ranked third among arthropod-caused losses, with ca. 7,000 bales lost to thrips in Texas (Williams 2014). The western flower thrips, *Frankliniella occidentalis* Pergande, is a major thrips pest on seedling cotton in Texas. Thrips are an early season pest which can cause severe damage to seedling cotton. Thrips cause damage to seedling cotton and excessive feeding leads to browning of leaves on the edges, develop a silvery color, or curl upward from the edges and cause the loss of leaf chlorophyll and leaf area. Thrips are very small insects which make it harder to study them in enclosures. The objective of this study was to evaluate No-Thrips® cages (Green-Tek, Edgerton, WI) to study thrips behavior.

This study was conducted at the Texas A&M AgriLife Research farm located near Lubbock, Texas. The study was deployed in a randomized block design with five replications and six treatments. Cotton cultivar ST 5458B2RF was planted on June 3, 2014. Rectangular wooden-frame cages [98 cm (L) x 30 cm (W) x 44 cm (H)] with No-Thrips® screen were constructed and deployed in the field, with each cage enclosing 8-13 cotton seedlings (Fig. 1). Silicone caulk was used to attach No-Thrips® screen to the wooden frame. A thin metal flashing (2.54-3.8 cm) was attached at the bottom of the cage to restrict thrips movement from the bottom of the cage. A temperature sensor was kept inside the cage to record the internal cage temperatures. Freshly collected adult thrips, primarily western flower thrips, were released at various densities to generate a damage gradient across density treatments. Six thrips density treatments were released onto plants on June 18 when the cotton seedlings were at the 1-2 true-leaf stage. Six density treatments included 0, 1, 2, 4, 6, and 10 thrips per plant, replicated five times (total 30 cages) plus an uncaged control. Cages were removed on June 23 and two plants from each cage were clipped at the base, secured in a glass jar, and washed to retrieve thrips. After removal of cages, thrips augmented rows were sprayed with Orthene® 97. The remaining plants from the caged sections were maintained relatively insect-free for the remainder of the growing season, and were harvested upon crop maturity for lint yield. This study was repeated for three phenological stages of cotton, but only one data set is presented in this paper.

Figure 1. Wooden-framed field cage covered with No-Thrips® screen for threshold study (left); Installation of thrips cages in the field and release of thrips densities (right).

No-thrips® cages appeared to hold thrips in the field cages better than any of the other field cage materials (fabrics) that we have used in previous studies. Different materials and designs were used in the past with very little success. Those designs included 1) transparent plastic cup cage, 2) wire mesh sleeve cage, 3) opaque plastic cylinder, 4) transparent plastic jar without ventilation, and 5) transparent plastic jar with ventilation (Fig. 2). None of these methods were suitable for thrips studies in the field because of the excessive temperature buildup inside the cages, plus material of the screen was unable to contain the thrips. However, the No-Thrips® cage design provided a satisfactory performance. Unfortunately, heavy rainfall within 48-hour of the thrips cage release severely comprised our test in this study. Unusually cool weather during the thrips exposure period resulted in low thrips survival and reduced feeding on the cotton seedling tissues (Fig. 3).

Figure 2. Cage types evaluated previously: 1) transparent plastic cup cage, 2) wire mesh sleeve cage, 3) opaque plastic cylinder, 4) transparent plastic jar without ventilation, and 5) transparent plastic jar with ventilation.

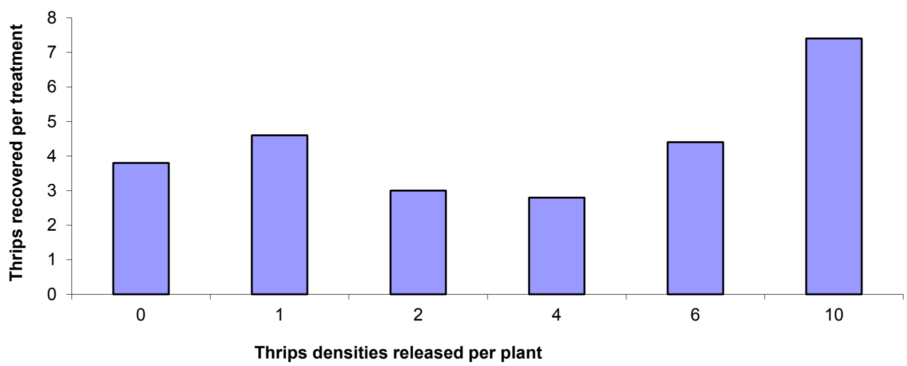

Figure 3. Recovery of thrips from cotton seedlings 5-days post-release of varying densities of thrips in No-Thrips® cages.

Although the thrips retrieval post-release was very low, it appears that the thrips feeding had exerted some effect on plant, resulting in reduced plant height and smaller main-stem diameter in all thrips augmented plants compared to that in control cages (Figs. 4-5). Nevertheless, the thrips feeding, if any, during the seedling stage in this study did not significantly impact lint or seed yields (Figs. 6-7).

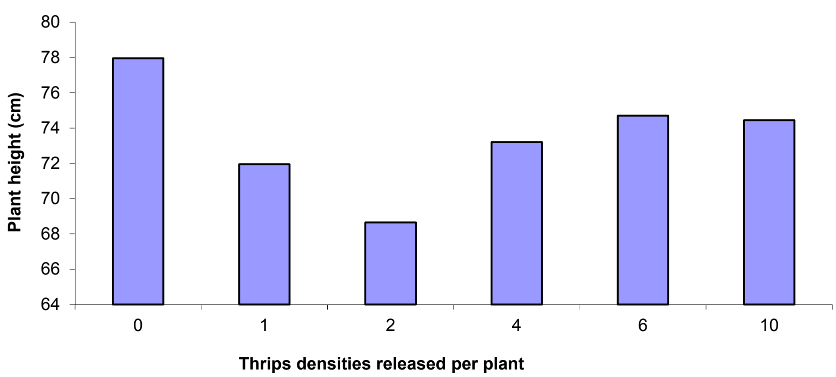

Figure 4. Plant height measurements of cotton plants at full maturity that were infested with varying densities of thrips in No-Thrips® cages during the seedling stage.

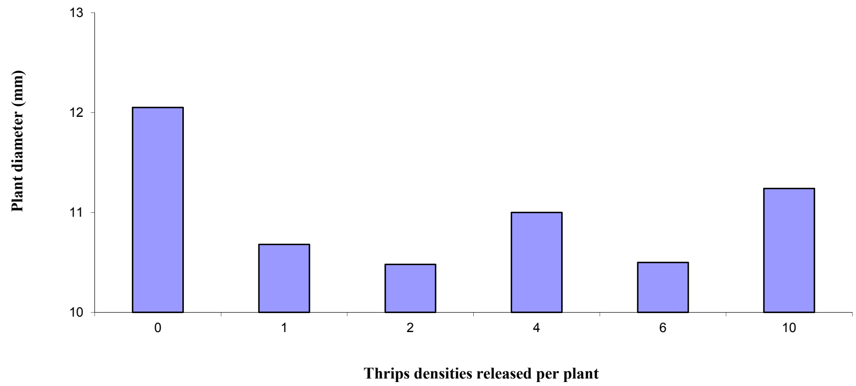

Figure 5. Plant diameter measurements of cotton plants at full maturity that were infested with varying densities of thrips in No-Thrips® cages during the seedling stage.

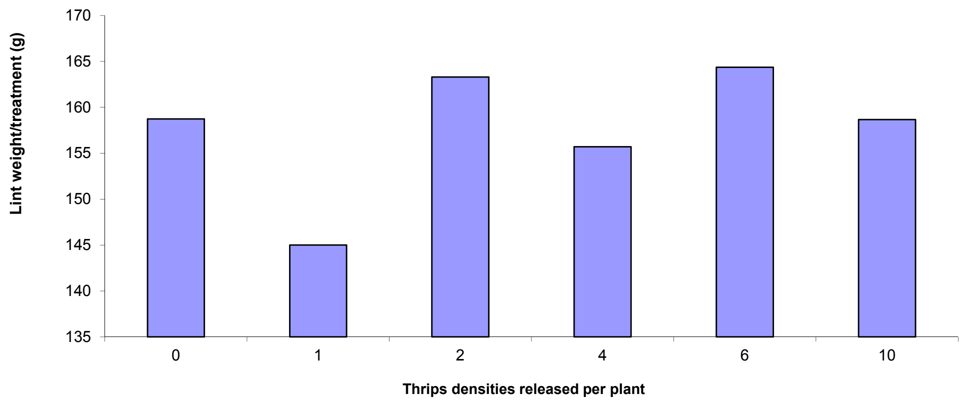

Figure 6. Lint weight from cotton infested with varying densities of thrips in No-Thrips® cages during the 1-2 true-leaf stage, Lubbock, Texas, 2014.

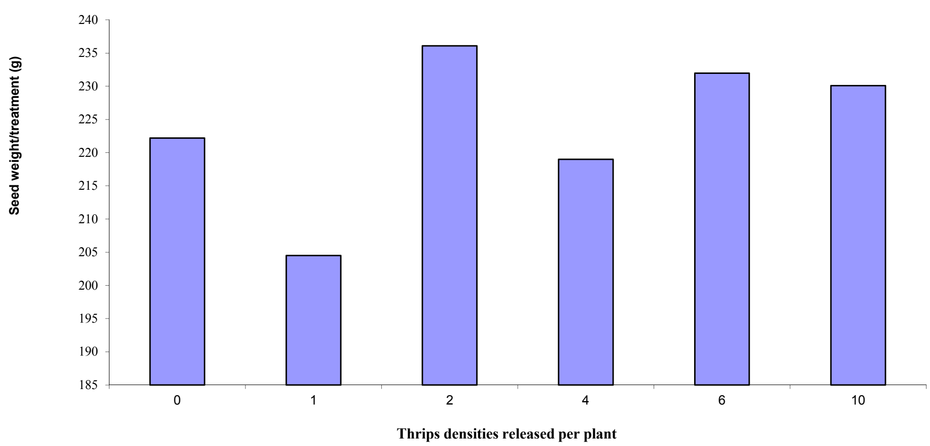

Figure 7. Seed weight from cotton plants infested with varying densities of thrips in No-Thrips® cages during the 1-2 true-leaf stage, Lubbock, Texas, 2014.

USE OF A MODIFIED - VIRAL GENE TO CONFER RESISTANCE AGAINST APHIDS IN TRANSGENIC PLANTS

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Abstract

Use of chemical insecticides, the predominant control method thus far, has resulted in environmental damage, pest resurgence, and negative effects on non-target species. Genetically modified crops offer a promising alternative, and *Bacillus thuringiensis* toxin genes have played a major role in this respect. However, to overcome insect tolerance issues and to broaden the target range, it is critical to identify alternative insecticidal toxins working through novel mechanism. Our group has identified a coat protein kinase from Chilo iridescent virus (CIV) that has insecticidal activity. The CIV toxin, expressed in yeast systems, induces 50% mortality in cotton aphids and 100% mortality in green peach aphids (GPAs). Our hypothesis is cloning this viral kinase gene into plants will generate transgenic lines toxic for aphids and other pests. Expression of foreign genes in plants is often complicated by codon usage, mRNA instability, translational efficiency, and proteolytic degradation. Therefore, the viral toxin gene was codon optimized to favor translation and stability in planta. This optimized viral gene was stably transformed into *Arabidopsis* plants. The stable lines expressing this toxin induced moderate to very high mortality in GPAs and significantly affected its population growth. The aphidicidal potential of these transgenic *Arabidopsis* lines will be presented. Our long term goal is to generate insect-resistant cotton using the viral gene. This will ultimately yield transgenic cotton cultivars resistant to pests other than caterpillars and therefore be more profitable for US farmers in an increasingly competitive global market.

LATE SEASON *LYGUS HESPERUS* MANAGEMENT IN TEXAS COTTON

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Abstract

A three-year field cage study was conducted to characterize the density-dependent boll feeding potential of *Lygus hesperus* in Texas High Plains cotton. *Lygus* damage to cotton bolls at various *Lygus* densities was determined. Individually caged cotton plants were exposed to 4 levels of *Lygus* (0, 1, 2 and 4 or 6 adults per cage) for one week when plants were at two selected boll development stages (350 and 550 HU >60 °F after first flower). When the crop matured from 350 HU to 550 HU after first flower, the percentage of bolls vulnerable to *Lygus* feeding damage was reduced from 50% to 30%. Averaged over three years, artificial augmentation of 1, 2, 4, and 6 *Lygus* per plant at 350 HU after first flower reduced the cotton lint yield by 137, 313, 422, and 516 lb/acre, respectively, whereas the yield reduction values for the same *Lygus* densities were 66, 191, 213, and 415 lb/acre during the late season (550 HU from first flower). Thus, the *Lygus* yield reduction potential decreased by 52, 39, 50, and 20% for 1, 2, 4, and 6 *Lygus* per plant infestation when cotton matured from 350 HU to 550 HU. A detailed understanding of *Lygus* boll feeding biology and behavior will be highly valuable in improving *Lygus* management decisions during the different boll developmental stages.

Introduction

Cotton, *Gossypium hirsutum* L., is a major cash crop in the U.S. and worldwide. The U.S. is the world's third largest cotton producer and the U.S. cotton industry is valued at more than $25 billion per year. In Texas, approximately six million acres of cotton have been planted annually in recent years, and Texas is the largest cotton producing state (Williams 2013). *Lygus hesperus* is an important economic pest of cotton in some regions of the United States and it is an emerging pest of Texas High Plains cotton. In Texas, over 2 million acres of cotton were infested by *Lygus* in 2012 (Williams 2013). *Lygus* can cause severe cotton square loss, anther damage, and seed damage depending upon the crop growth stage the infestation occurs. Both adult and nymphal stages of *Lygus* can inflict damage to cotton fruiting structures. *Lygus* late-instar nymphs are capable of inflicting greater internal damage to maturing bolls than are adults, and this was especially true for 1-2 week old (150-250 HU) bolls (Jubb and Carruth 1971, Parajulee *et al.* 2011). In the Texas High Plains region, *Lygus* generally infest cotton fields during the latter part of the cropping season, thus causing damage primarily to the cotton bolls. Following the introduction of *Bt*-technology (Bollgard® cotton), outbreaks of lepidopteran pests have been drastically reduced, and in recent years, secondary piercing-sucking pests such as *Lygus* are of increasing concern to Texas High Plains producers (Parajulee *et al.* 2008).

Cotton boll profiles change as the crop matures, and as a result, the number of *Lygus* susceptible and/or tolerant bolls to *Lygus* damage also change. As boll maturity profiles change, *Lygus* boll selection and feeding behavior may also change which can result in different levels of crop injury and yield loss. There is a strong relationship between boll maturity and *Lygus* feeding damage, thus understanding the boll maturation profile and characterizing *Lygus* damage risk dynamics is important. The objective of this study was to quantify the yield loss caused by 4 different levels of *Lygus* infestations (0, 1, 2 and 4 or 6 *Lygus* adults per plant). The overall goal is to better understand the boll feeding biology and behavior of *Lygus hesperus* in order to further develop a dynamic economic threshold for improved *Lygus* management in Texas High Plains cotton.

Materials and Methods

A field study was conducted to quantify the effect of *Lygus* density and infestation timing on cotton yield and fiber quality. Cotton cultivar ST 5458B2RF was planted on May 18 (2012), May 22 (2013), and May 15 (2014) in a drip-irrigated field with 40-inch row spacing at the Texas A&M AgriLife Research farm located near Lubbock, Texas. The targeted seeding rate was 56,000 seeds per acre. On June 2, the 2012 study was treated with Orthene® 97S for thrips at a rate of 3.0 oz per acre and with Cornerstone Plus® herbicide (41% glyphosate) at 32 oz per acre for weed management, whereas the 2013 and 2014 study plots did not receive insecticide interventions for thrips control and weeds were removed via cultivation and hand-hoeing.

The field study was laid out in a split-plot randomized block design with three replications, two main plot factors [two cotton boll developmental stages (early boll development and late boll development)], and four subplot factors [four levels of *Lygus* infestation (control or zero bugs, one bug/plant, two bugs/plant, and four or six bugs/plant)]. There were a total of 24 experimental units. Each experimental unit had 8 cotton plants as subsamples (3 used for damage assessment and 5 for yield and quality assessment). A total of 192 whole-plant sleeve-caged cotton plants (three blocks x two cotton boll stages x four *Lygus* densities x eight subsamples) were used for this study (Fig. 1).

Figure 1. Field deployment of whole-plant cages for *Lygus* threshold study, Lubbock, TX, 2012-2014.

The cotton field study site was closely monitored and kept virtually arthropod pest-free until cages were deployed on July 24, July 29, and July 28 in 2012, 2013, and 2014, respectively. When the cotton plants reached the target maturity level (350 HU after first flower on August 7, August 13, and August 17 in 2012, 2013, and 2014, respectively, and 550 HU after first flower on August 21, August 29, and August 27 in 2012, 2013, and 2014, respectively), lab-reared *Lygus* were released into the whole-plant sleeve-cages at the rates of 0, 1, 2, and 4 bugs/plant in 2012 and 2013; the infestation densities were changed to 0, 2, 4, and 6 bugs/plant in 2014 to increase the damage intensity. *Lygus* adults were collected from nearby alfalfa field or from alfalfa in adjacent counties and acclimatize in the laboratory for 48 hours before using them for the boll feeding experiment. Cotton plants were exposed to the *Lygus* adults for ~7 days, after which time, the insects were killed via a pesticide application. Three randomly selected cotton plants from each plot were cut and brought to the laboratory on August 13, August 19, and August 27 for the 350 HU and August 29, September 2, and September 5 for the 550 HU plots in 2012, 2013, and 2014, respectively. The cotton crop was defoliated by spraying FOLEX® 6EC (12 oz per acre) and a boll opener (Ethephon® 6; 32 oz per acre) in a tank mix in all three years of the study. After the crop was ready to harvest, the remaining 5 caged plants from each plot, which had been maintained pest-free, were harvested manually to evaluate the lint yields and fiber quality. Harvested single-plant samples were ginned individually via table-top gin and samples were analyzed for fiber quality (HVI) parameters at Cotton Incorporated. Data from the whole-plant cage study were summarized by calculating average and standard errors. ANOVA, GLM model (SAS Institute 2010) was used to evaluate the treatment effects (α=0.1) and treatment means were compared by LSMEAN procedure.

Results and Discussion

In general, as expected, *Lygus* augmentation reduced the lint yield compared to that in uninfested control cages (Figs. 2-4). However, the damaging effect of *Lygus* was more pronounced during mid-season (350 HU from first flower) compared to that in late season (550 HU from first flower) for all three years of the study. Although year-to-year variation existed, *Lygus* augmentation of 1 adult per cage did not significantly decrease the lint yield, but the higher densities reduced the yield significantly compared to that in uninfested cages.

In 2012, artificial augmentation of 1, 2, and 4 *Lygus* bugs per plant at 350 HU after first flower reduced the cotton lint yield by 116, 425, and 580 lb/acre, respectively, whereas the yield reductions for the same *Lygus* densities were 125, 149, and 185 lb/acre during the late season (550 HU from first flower) (Fig. 2).

In 2013, cotton lint yields in mid-season plots (cages) were much lower than in 2012, but the augmentation of 1, 2, and 4 *Lygus* bugs per plant reduced the cotton lint yield by 157, 106, and 281 lb/acre, respectively (Fig. 3). While these lint yield reduction values were not statistically significant, owing to greater variation in data, the trend was convincingly supportive of a clear influence of *Lygus* augmentation on yield reduction and the data trend was similar to that in 2012. Overall, lint yield was higher in late-season test plants compared to that in mid-season test plants, but the augmentation of 1 *Lygus* per plant did not result in significant yield reduction, whereas 2 and 4 *Lygus* per plant reduced 143 and 159 lb/acre, respectively (Fig. 3).

In 2014, augmentation of 2, 4, and 6 *Lygus* per plant at 350 HU after first flower reduced the cotton lint yield by 407, 406, and 516 lb/acre, respectively, whereas the yield reductions for the same *Lygus* densities were 282, 295, and 415 lb/acre during the late season (550 HU from first flower) (Fig. 4). Overall yield in 2014 was higher than in 2012 and 2013, but the damage inflicted by 2 and 4 *Lygus* per plant on mid-season cotton was comparable to that for 2012, whereas the damage inflicted in late season cotton was higher in 2014 compared to that in 2012 or 2013.

Lygus-induced lint yield reduction for a given *Lygus* density was lower for late season compared to that for mid-season infestation in all three years of the study (Figs. 2-4). These data clearly suggest that the maturing bolls are more tolerant to *Lygus* injury when the plant attains 550 HU from first flower. It is also possible that *Lygus* bugs may choose to feed on superfluous bolls or squares and the yield contributing fruits may not be significantly impacted by such late infestation. Because potential yield loss risks due to certain *Lygus* density infestations vary with boll maturation profile, the *Lygus* management economic threshold should be optimized for a dynamic ET to accommodate for within-plant fruit maturity profiles.

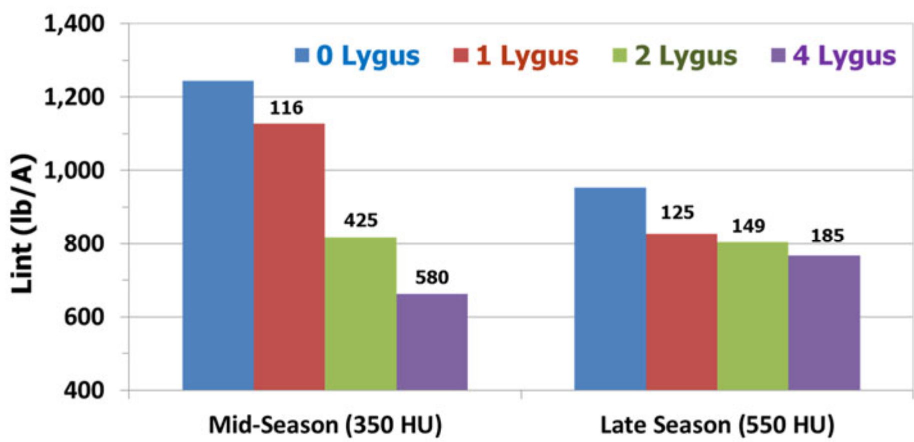

Figure 2. Influence (pounds of yield reduction) of varying levels of *Lygus* infestations on lint yield at two crop phenological stages, Lubbock County, TX, 2012.

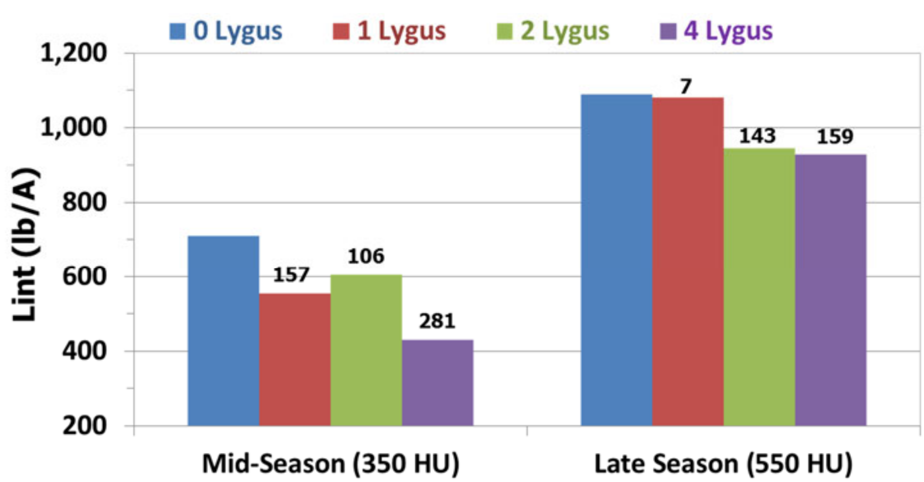

Figure 3. Influence (pounds of yield reduction) of varying levels of *Lygus* infestations on lint yield at two crop phenological stages, Lubbock County, TX, 2013.

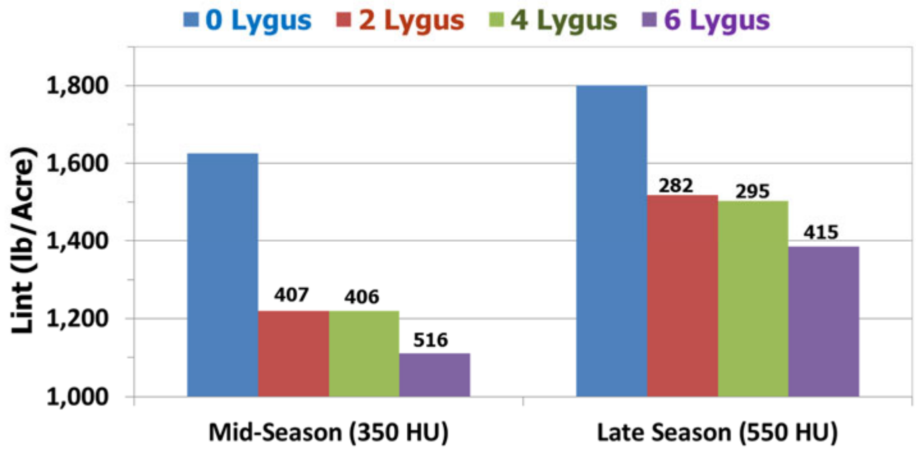

Figure 4. Influence (pounds of yield reduction) of varying levels of *Lygus* infestations on lint yield at two crop phenological stages, Lubbock County, TX, 2014.

Averaged over three years, artificial augmentation of 1, 2, 4, and 6 *Lygus* per plant at 350 HU after first flower reduced the cotton lint yield by 137, 313, 422, and 516 lb/acre, respectively, whereas the yield reduction values for the same *Lygus* densities were 66, 191, 213, and 415 lb/acre during the late season (550 HU from first flower). Thus, the *Lygus* yield reduction potential decreased by 52, 39, 50, and 20% for 1, 2, 4, and 6 *Lygus* per plant infestation when cotton matured from 350 HU to 550 HU. Late season *Lygus* management program may consider decreasing *Lygus* damage potential as season progresses and adjust the economic threshold (ET) accordingly. We are currently developing dynamic ET for *Lygus* management in the Texas High Plains.

Acknowledgements

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UPDATE ON BOLLWORM PYRETHROID RESISTANCE MONITORING

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Abstract

Polyphagous bollworms are potentially exposed to pyrethroid insecticides during each generation. Since cotton is a host during the latter part of the growing season, any resistance developed during the season will reduce control realized in cotton. Pheromone traps have been used sporadically since the late 1980s throughout the cotton belt to collect male moths for testing resistance to a pyrethroid insecticide. Testing was conducted across the cotton belt in a coordinated fashion from 2007-2014 using a concentration of 5 µg/vial of cypermethrin as the diagnostic dose. Overall survival during 2014 was 18.8%, which was somewhat higher than recent years. However, resistance was not uniform across all states. Louisiana and Virginia have regularly had higher survival than all other states during recent years. This year Georgia joined them with all having yearly average survivorship between 30 and 35%. In contrast, Missouri, South Carolina and Tennessee all had average survival of less than 10%. The other states fell between these extremes.

Introduction

Bollworm, *Helicoverpa zea*, is a pest in numerous crops where it may be exposed to pyrethroid insecticides. Since it can have 5 or more generations per year in the southern U.S., it has the potential to develop large populations and insecticide resistance has the potential to develop and spread rapidly. One to two of these generations occur in cotton, causing substantial economic loss. Because pyrethroid insecticides are relatively inexpensive, they are often the first choice of growers for foliar control of bollworms. Knowledge of the susceptibility of bollworms to pyrethroid insecticides is therefore critical to effective management of this pest.

Monitoring pyrethroid resistance in bollworms has been conducted for numerous years, beginning in 1988 in a few states and then coordinated throughout the cotton belt in 1989-1990 (Rogers et al. 1990). Since then monitoring has continued at various levels every year. Regional data from previous years can be found in earlier Beltwide cotton conference proceedings (Martin et al. 1999, 2000, Payne et al. 2001, 2002, Musser et al. 2010, 2011, 2013). During this time the bioassay methodology has remained consistent. Male moths are captured in a pheromone trap and placed in a glass vial that was previously treated with insecticide. Mortality is recorded after 24 h. A concentration of 5 µg cypermethrin / vial has been used with baseline survival generally less than 10% (Martin et al. 1999).

Materials and Methods

Hartstack pheromone traps were placed in various locations in ten states across the cotton belt from VA to TX. Pheromones (Luretape with Zealure, Hercon Environmental) were changed every 2 weeks. Some traps were monitored at least weekly from May until September, but most were monitored over a shorter period when bollworms were abundant and cotton was susceptible to bollworm feeding. Healthy moths caught in these traps were subsequently tested for pyrethroid resistance. Moths were individually placed in 20 ml scintillation vials that had been previously coated with 0 or 5 µg cypermethrin per vial. Vial preparation for all locations except Louisiana was done at Starkville, MS and shipped to cooperators as needed throughout the year. Louisiana data are from vials prepared in Louisiana. In addition to rates of 0 and 5 µg cypermethrin per vial, Louisiana also tested survival at 10 µg cypermethrin per vial. At all locations, moths were kept in the vials for 24 h and then checked for mortality. Moths were considered dead if they could no longer fly. Reported survival was corrected for control mortality (Abbott 1925).

Results and Discussion

A total of 8815 moths were assayed during 2014. The fewest moths (169) were tested in North Carolina while the most moths (2539) were tested in Louisiana. Average survival to the 5 µg cypermethrin / vial concentration was 18.8% in 2014 (Table 1), which was the highest rate of survival since 2007 (Fig. 1). As has been consistently observed in the past, survival during July was higher than during previous months. While late season moths are often more susceptible, survival rates during 2014 were maintained during August and September.

Table 1. Bollworm survival to 5 µg cypermethrin per vial in 24-h vial tests during 2014.

State	May	June	July	Aug	Sep	Overall	Total bollworms tested
AR	3.6	8.3	26.2	8.0	14.3	14.3	990
GA		8.3	20.8	42.1	21.6	30.4	787
LA	7.1	25.0	31.4	43.2	52.2	33.3	2539
MS	18.9	11.8	12.3	9.1		13.8	1178
MO				10.2	7.3	9.3	597
NC			24.4			24.4	169
SC		0.0	10.6	2.7	0.0	5.3	605
TN			7.6			7.6	261
TX		11.5	22.3	16.0	16.2	16.8	1220
VA			27.7	33.9	31.6	32.4	649
Average	9.9	10.8	20.4	20.7	20.5	18.8	8815

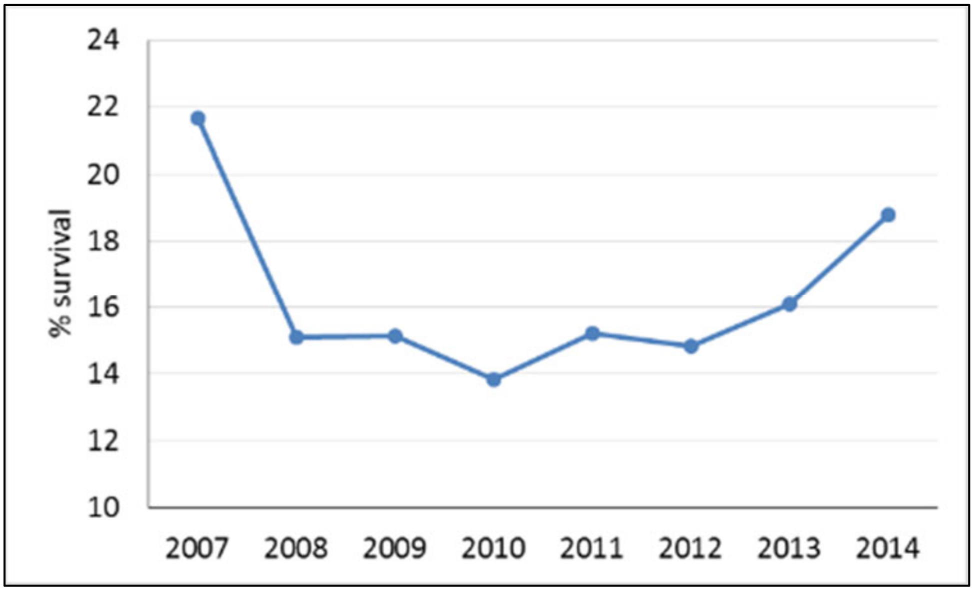

Fig. 1. Beltwide bollworm average survival per year at 5 µg cypermethrin per vial from 2007 – 2014.

Most states had survival rates similar to previous years, put survival in Georgia was sharply higher during 2014, making average survival in Georgia for the year similar to Louisiana and Virginia, the two states that have had the least susceptible moths during the last several years (Fig. 2). Whether this is a one-year spike like observed in 2007, or a long-term change in susceptibility remains to be seen. North Carolina has also had higher survival than most states each of the last two years, so it may be that pyrethroid resistance in bollworms is becoming more common along the eastern coast of the U.S.

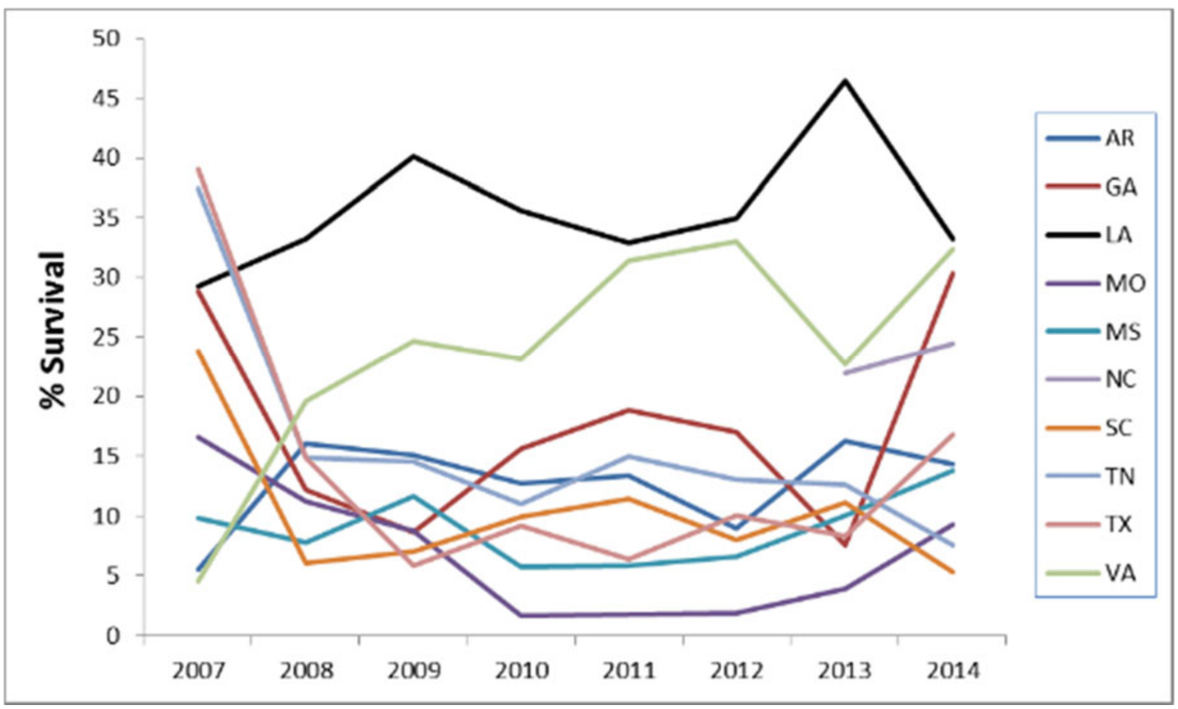

Fig. 2. Average bollworm survival by state per year at 5µg cypermethrin per vial from 2007 – 2014.

A comparison of bollworm susceptibility in Louisiana at both 5 µg and 10 µg cypermethrin, reveals that the relationship between these concentrations is not the same throughout the year. While survival during May and June was similar at both concentrations, survival continued to increase throughout the year at 5 µg, but stayed steady between 20% and 30% survival at 10 µg (Fig. 3). For a point of reference, tobacco budworm was considered resistant to pyrethroids when there was 30% survival of the moths at the 10 µg concentration. Louisiana stayed near this line most of the year, and larval control of bollworms with pyrethroids is considered erratic.

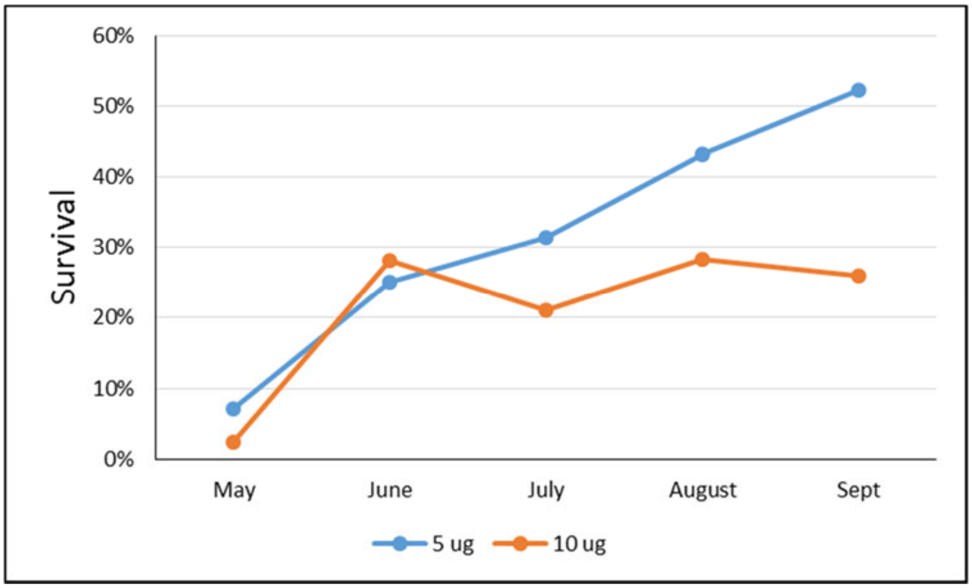

Fig. 3. Monthly bollworm survival at 5 µg and 10 µg cypermethrin per vial in Louisiana during 2014.

Bollworm adults are considered highly mobile (Lingren et al. 1994, Beerwinkle et al. 1995), which would suggest that pyrethroid resistance would quickly spread from one region to another. However, pyrethroid resistance has persisted in LA and VA for numerous years while populations in adjacent states remain largely susceptible. Field control of bollworm larvae is inconsistent throughout many parts of the cotton belt, so it is likely that numerous resistance genes are present in populations. It is likely that resistance is associated with high fitness costs, so resistance is reduced every winter, and spreads to new regions more slowly than expected. However, monitoring from 1998-2000 found average survival rates of less than 10%, while average current survival is approaching 20% and exceeds 30% in some states. Even though pyrethroids may not be applied as frequently in cotton as in the past, there are still enough applications made in the landscape to slowly decrease pyrethroid susceptibility, making the selection of this class of chemistry for targeting bollworms a risky decision.

Conclusions

Pyrethroid susceptibility in bollworms over the cotton belt appears to be slowly decreasing, but the rate of decline is not uniform. Louisiana and Virginia have had the lowest susceptibility for several years. Georgia has similar survival to pyrethroids during 2014. Average survival on 5 µg cypermethrin over the entire cotton belt rose to 18.8% during 2014, which was the highest survival observed since 2007.

Acknowledgements

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Moisture Conditions for Laboratory Rearing of Cotton Fleahoppers[1] from Overwintered Eggs Laid on Woolly Croton Plants

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Abstract. The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), is an economic pest of Texas cotton, *Gossypium hirsutum* L., that feeds on and causes abortion of early-stage squares. Cotton fleahopper eggs are laid in late fall and overwinter on woolly croton, *Croton capitatus* Michx. Cotton fleahoppers terminate diapause in early spring in response to minimum required temperature and moisture conditions. A laboratory study quantified the effects of different amounts of moisture (soaking durations of field-collected dead woolly croton plants) on the emergence of cotton fleahopper nymphs from diapaused eggs. Five moisture treatments evaluated were: 1) 24-hour initial soaking and no further moistening of the substrate for the remainder of emergence duration (T_1); 2) 2-hour initial soaking followed by daily mist spraying of the substrate (T_2); 3) 2-hour initial soaking followed by 30-minute soaking for the next 7 days and thereafter mist spraying daily (T_3); 4) 2-hour initial soaking followed by 30-minute soaking for the next 7 days and thereafter dipping the substrate in water daily (T_4); and 5) soaking for 15 minutes every other day (T_5). Emergence of nymphs started 6 days after initial incubation in T_3, while the latest emergence was recorded from T_2. Peak nymphal emergence was recorded 12-days after incubation. Significantly more (P = 0.05) nymphs emerged from T_4 (n = 425) and T_3 (n = 404) than from T_1 (n = 173), T_2 (n = 290), or T_5 (n = 293). To maximize fleahopper emergence from overwintered eggs in a laboratory, it was recommended that egg hatching be activated by soaking host substrate (croton) for 30 minutes daily for about 7 days and keeping the substrate moist throughout the emergence period.

Introduction

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), is an important pest of cotton, *Gossypium hirsutum* L., in Texas and Oklahoma, and an occasional pest in Arkansas, Louisiana, New Mexico, and other states in the mid-South (Walker et al. 1970, Esquivel and Esquivel 2009). Cotton fleahopper is a small insect with piercing-sucking mouthparts that feeds on early-stage (pinhead) cotton squares, causing shedding of affected squares and potential yield loss (Reinhard 1926, Almand 1974, Parajulee et al. 2006). At least 160 plant species in 35 families including pinkladies, *Oenothera speciosa* Nutt.; upright prairie

[1](Hemiptera: Miridae)

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coneflower, *Ratibida columnifera* (Nutt.) Woot. & Standl.; silverleaf nightshade, *Solanum elaeagnifolium* Cavanilles; and woolly croton, *Croton capitatus* Michx. are hosts for the cotton fleahopper (Snodgrass et al. 1984, Esquivel and Esquivel 2009). Woolly croton is a host of fleahopper adults throughout the growing season and for overwintering eggs (Almand 1974). In late fall, cotton fleahoppers lay eggs that overwinter on host plants until early spring. Warm temperatures and spring rainfall events activate diapaused eggs to emerge as nymphs. In a laboratory, Saunders (1983) terminated diapause by controlled temperature, light, and moisture. Despite its intriguing overwintering biology and host-associated differentiation (Barman et al. 2012), information is limited on the ecology of cotton fleahopper overwintering in a semi-arid environment such as the Texas High Plains.

While cotton fleahoppers frequent cotton in most cotton-growing states, they are considered pestiferous primarily in Texas. In Texas, the cotton fleahopper is more prevalent in the Coastal Bend and Brazos Valley but is an equally important economic pest on the Texas High Plains, Rolling Plains, and in the Trans-Pecos region. While the cotton fleahopper can significantly reduce the yield of cotton lint (Williams 2013), timing of cotton fleahopper infestation and cotton cultivar and inputs (e.g., irrigation and fertility) could affect the severity of damage and the compensatory response of the plant to injury (Chen et al. 2007, Knutson et al. 2013). Several field research projects are underway in our program to elucidate field ecology in relation to cotton cultivar, irrigation, and fertility and develop management strategies against cotton fleahopper. These efforts require large numbers of stage-specific cotton fleahoppers to release in field plots. In addition, studies of behavioral response to resistant hosts in a laboratory or greenhouse also require a consistent supply of cotton fleahoppers. Large-scale field collection of cotton fleahoppers for controlled studies is rarely feasible. However, cotton fleahoppers can be reared with predictable success in a laboratory by collecting dead croton plants from areas with abundant fleahoppers in the winter. To rear cotton fleahoppers successfully, host substrate such as croton twigs that contain overwintering eggs needs to be soaked to activate hatching of the eggs, and these requirements might vary with regional ambient humidity. Currently, no data are available on rearing methods for drier regions such as the Texas High Plains. A rearing study in a laboratory at Texas A&M AgriLife Research and Extension Center Cotton Entomology Program at Lubbock characterized cotton fleahopper emergence from overwintered eggs. The objective of the study was to evaluate the effects of soaking duration of woolly croton on the emergence of cotton fleahopper nymphs from overwintered eggs.

Materials and Methods

Standing, but dead twigs of woolly croton, *C. capitatus* (Figs. 1A,B), were collected from the Brazos Valley (College Station, TX area), in January 2013 (Gaylor and Sterling 1975). Croton twigs were stored at 4ºC in a walk-in cooler (Fig. 1C). The twigs (110 g) were cut into ≈27-cm lengths and placed in cylindrical 3.79-liter tin metal containers (approximately 27.5 cm tall and 16.51 cm in diameter). Both ends of the rearing container were covered with coarse-mesh aluminum window screen to hold the croton in place yet allow newly emerged cotton fleahopper nymphs to exit from the rearing substrate. Another layer of muslin cloth was placed on top of the first screen and secured by a rubber band to enclose newly emerged fleahopper nymphs until shaking to dislodge them from the substrate (Figs. 1D,E). Five moisture treatments with four replications (tin cans) were: 1) 24-hour initial soaking and no further moistening of the substrate for the remainder of emergence duration (T_1); 2) 2-hour initial soaking followed by daily mist spraying the substrate (T_2); 3) 2-hour initial soaking followed by 30-minute soaking for the next 7 days and thereafter mist spraying daily (T_3); 4) 2-hour initial soaking followed by 30-minute soaking for the next 7 days and thereafter dipping the substrate in water daily (T_4); and 5) soaking for 15 minutes every other day (T_5). The last treatment (T_5) adapted from Breene et al. (1989) was used as a check (Fig. 1D). An experimental check would have been a treatment with no moisture, but such treatment is unrealistic because cotton fleahoppers do not emerge from diapausing eggs without moisture activation. Woolly croton was soaked in water to provide moisture to eggs deposited under the bark by fleahopper adults (Fig. 1F). Nymphs (Fig. 1G) that emerged from eggs were fed fresh green beans, *Phaseolus*

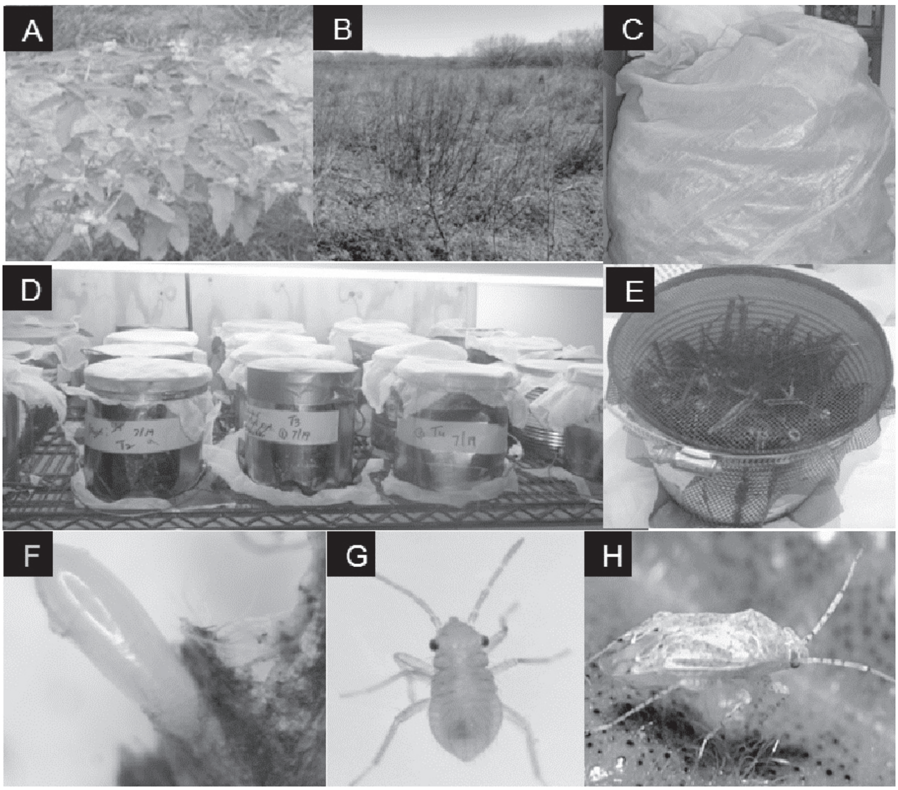

Fig. 1. A) Actively growing woolly croton plant, B) dry woolly croton with diapausing cotton fleahopper eggs *in situ*, C) woolly croton twigs collected and stored in a walk-in cooler, D) arrangement of the diapause termination and rearing experiment, E) rearing container (3.79-liter tin metal can) with 110 g croton twigs per experimental unit, F) cotton fleahopper egg under croton bark (exposed), G) cotton fleahopper nymph, and H) cotton fleahopper adult.

vulgaris L., until adulthood (Fig. 1H) for other behavioral experiments. Incubation was initiated on 19 July 2013. The rearing room was maintained between average daily cool and warm temperatures of 25 and 34ºC and a photoperiod of 12:12 light:dark hours (Fig. 2). Temporarily removing the muslin cloth and shaking the rearing cans to dislodge emerging nymphs from the substrate began on the 5th day of the experiment and continued for the next 4 weeks. Rearing cans were shaken daily by beating the sides of the cans 12 times at 2-second intervals to dislodge nymphs onto a white poster board. Dislodged nymphs were counted and transferred into small plastic containers and fed green beans.

Results

A total of 6,344 cotton fleahopper nymphs emerged from the total of 2,200 g croton substrate. Significantly more ($P < 0.05$) nymphs emerged per 110 g of croton substrate from the T_4 treatment [2-hour initial soaking followed by 30-minute soaking for the next 7 days and then daily dipping the substrate in water thereafter (n = 425 ± 22)] and T_3 [2-hour initial soaking followed by 30-minute soaking for the next 7 days and mist spraying daily (n = 404 ± 6)], followed by T_2 [2-hour initial soaking followed by daily mist spraying the substrate (n = 290 ± 12)] and T_5 [soaking for 15 minutes every other day (n = 294 ± 35)], and the fewest nymphs emerged from the T_1 treatment [24-hour initial soaking and no further moistening of the substrate for the remainder of emergence (n = 173 ± 36)] (Fig. 3). Cotton fleahopper emergence began 6 days after initial soaking at 24-36ºC in T_3 while the last cotton fleahopper nymph emerged from the host (croton) 32 days after the initiation of incubation in T_2. The temporal pattern of nymphal emergence was similar across the five moisture treatments, with peak emergence occurring 11 or 12 days after initiation of diapause termination (Fig. 4). Nevertheless, the size of the emergence peak and duration of emergence varied across treatments. The largest 1-day emergence (153 nymphs per 110 g croton) occurred 12 days after incubation in T_4 (2 hours initial soak, then 30-minute soak for 7 days, thereafter dipping).

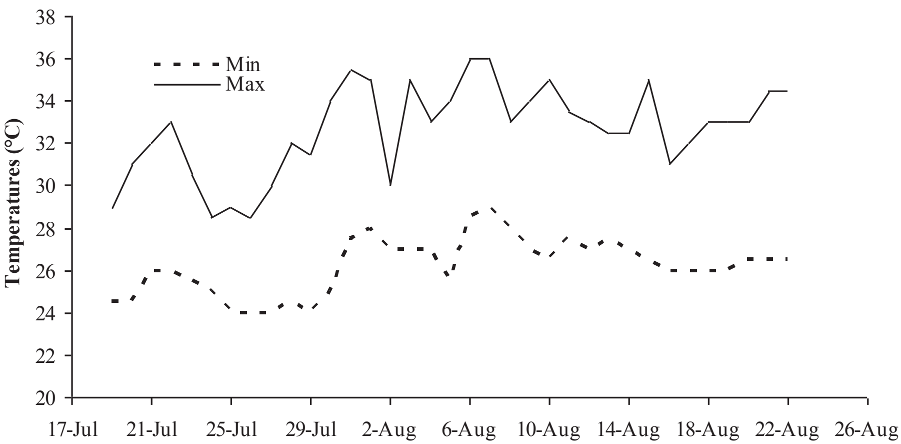

Fig. 2. Daily temperatures recorded in the laboratory when cotton fleahoppers were reared from overwintering eggs, Lubbock, TX, 2013.

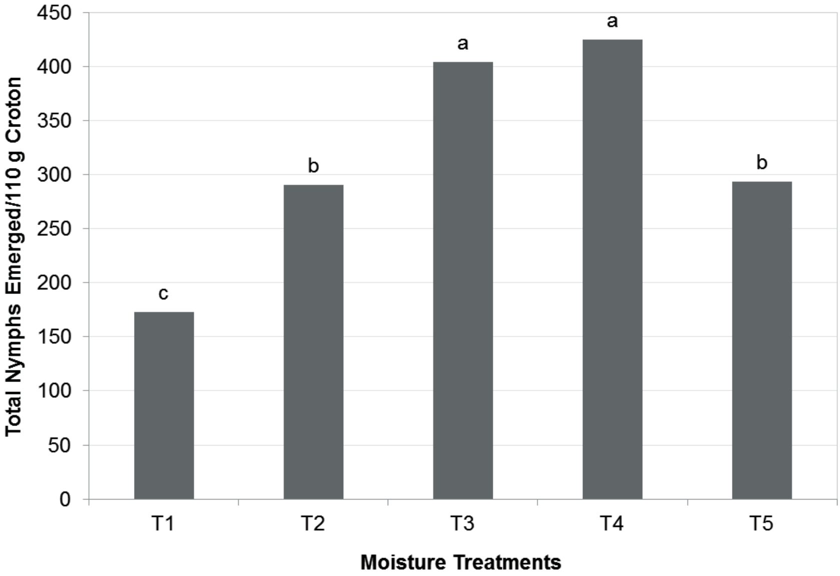

Fig. 3. Total number of cotton fleahopper nymphs emerged per experimental unit (110 g croton per unit) under five moisture regimes, Lubbock, TX.

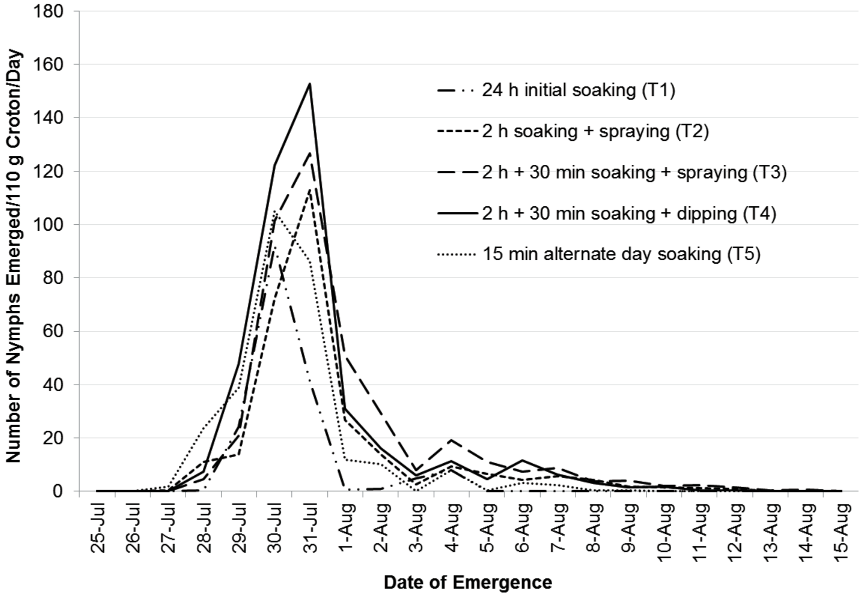

Fig. 4. Average daily emergence of cotton fleahopper nymphs per 110 g of croton substrate under five moisture regimes, Lubbock, TX 2013.

Discussion

Many insects lay eggs inside or on the tissue, fruit, root, or flower of a host plant. Eggs of some insects hatch a few minutes to hours after they are laid but some overwinter and hatch in the spring or early summer when temperature and moisture are favorable. Cotton fleahopper eggs laid in late summer or early fall overwinter and hatch in the spring when temperatures are favorable and rainfall events trigger diapause termination. Greater amounts of spring rain coupled with more eggs in the overwintering host could increase the severity of cotton fleahopper in cotton the following summer.

Soaking the host substrate for 15-30 minutes every other day provided adequate moisture for diapause termination and eggs of cotton fleahopper to hatch in a more humid environment such as the Brazos Valley of Texas (Breene et al. 1989). However, insufficient moisture in a semi-arid region such as the Texas High Plains might limit overwintering survival. In our study at Lubbock, 15-30 minutes soaking every other day for 7 days (T_5) resulted in significantly less emergence of nymphs compared with that in T_3 and T_4 (Fig. 3). A daily mist spray or dipping of the substrate in water for an additional 7 days following the first 7-day exposure of substrate to 30-minute daily soaking (T_3 and T_4) enabled most nymphs to emerge, indicating the need for humidity for better spring emergence of cotton fleahopper from overwintering habitats. A single significant rainfall event might sufficiently activate overwintered eggs and terminate diapause, especially if the temperature was also favorable for emergence, but diapause-terminated eggs might not survive if the substrate was not exposed to moisture for additional days. A single heavy rainfall event was simulated with 24-hour soaking of the substrate, which resulted in least nymphal emergence compared with four other treatments exposed to moisture for an additional 7-14 days. Cotton fleahopper eggs are activated and diapause terminated after a significant rainfall event when temperatures are favorable in the spring. Thus, frequent rainfall events in the spring might result in greater survival of eggs and uniform emergence of nymphs compared to that of a single heavy rainfall.

Amount and frequency of moisture have been demonstrated to affect overwintering survival and spring emergence of many insects. The spring emergence of the adult sorghum midge, *Stenodiplosis sorghicola* (Coquillett), from spikelets of sorghum, *Sorghum bicolor* (L.) Moench, was significantly greater at 90% relative humidity compared to that at <50% (Fisher and Teetes 1982). Parajulee et al. (1996) documented that increased rainfall during winter months contributed to greater overwintering survival of boll weevil, *Anthonomus grandis* Boheman. While supplemental moisture 7 days after initiation of emergence increased nymphal emergence from overwintering eggs of cotton fleahopper (T_3 and T_4 compared with T_2 and T_5), rainfall occurring before the initiation of overwintering emergence favored winter survival to a greater degree than did rain that occurred during emergence of boll weevil. Cotton fleahopper emergence from overwintered eggs was enhanced by intermittently soaking the overwintering substrate for at least 7 days and keeping it moist by mist spraying or dipping for the next 7 days. Because the emergence peak occurred 11-12 days from the time of exposure to diapause termination and emergence decreased rapidly across all treatments after 2 weeks of moisture exposure, moistening the overwintering substrate beyond 2 weeks might not improve emergence, although the current study did not verify this assertion. While T_3 and T_4 resulted in more emergence compared to that in T_5 (Breene et al. 1989) in our Texas High Plains study, it might be possible that moistening the substrate for additional days during the early emergence period might enhance emergence even in humid areas. However, this assertion needs further examination because Breene et al. (1989) terminated moisture treatment when emergence began.

Results of this study should be useful for management of cotton fleahoppers in cotton fields on the Texas High Plains. As shown in this simulated study, rainfall (amount and frequency) could be important in estimating the impact of cotton fleahoppers on the current cotton crop because severity in late spring/early summer depends on winter/spring rain. The pattern of spring rainfall events could indicate potential severity of cotton fleahoppers in historically fleahopper-prone areas.

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Crop dominance exerts specific effects on foliage-dwelling arthropods in *Bacillus thuringiensis* cotton

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Abstract

1 Shifts in crop composition in an agro-ecosystem may have profound effects on plant-associated arthropods.

2 The widespread adoption of transgenic insect-resistant cotton expressing insecticidal toxins *Cry1A* from *Bacillus thuringiensis* (*Bt*) has caused a dramatic shift in agricultural landscapes over the last 15 years, thus raising concerns about the potential impacts of *Bt* crops on nontarget organisms worldwide.

3 The potential effect of crop composition shift as a result of the increasing acreage of *Bt* cotton on an arthropod community was assessed at three levels (0%, 75% and 100%) of *Bt* dominance in a 2-year field trial in northern China.

4 Three findings confirmed that the arthropod assemblage of a certain field was influenced by crop composition and its developmental stage: (i) the proportion of *Bt* cotton in the crop mixture consistently affected the abundance of pests, natural enemies and total arthropods; (ii) bootstrap methods demonstrated significant effects of *Bt* cotton on species richness, diversity and evenness; and (iii) non-metric multidimensional scaling analysis showed that the arthropod community of a certain agricultural ecosystem varied with crop composition and plant developmental stage.

5 The findings of the present study may have significant implications for cotton pest management with respect to transgenic *Bt* cotton, highlighting the potential of conservation biological control via the combination of an appropriate crop genetic ratio and timely chemical control.

Keywords Arthropod abundance, bootstrap method, crop composition, diversity, non-metric multidimensional scaling.

Introduction

Habitat management under the auspices of conservation biological control is a widely used approach to ensure a diversity of predator species, which in turn persist spatially and temporally within agricultural landscapes to suppress pests (Schmitz & Barton, 2013). Unfortunately, how to conserve and improve biological control in a certain region is challenged by the complicated relationship between predator diversity and pest suppression. Numerous studies demonstrate that diverse predator assemblages can be more effective at controlling prey populations (Aquilino *et al.*, 2005; Snyder *et al.*, 2006, 2008; Tylianakis *et al.*, 2006; Finke & Snyder, 2008; Straub & Snyder, 2008; Veddeler *et al.*, 2010), whereas other studies show neutral effects of predator diversity on prey mortality, or even negative effects as a result of intraguild predation or interference (Finke & Denno, 2004, 2005; Vance-Chalcraft *et al.*, 2007; Wilby *et al.*, 2013). Just as for the complex interaction among guilds, the relationship between predator diversity and pest suppression remains an issue of hot debate in ecology. Nevertheless, shifts in arthropod assemblages are expected to exert significant influences on predator–prey interactions.

Cotton, one of the most important commercial crops in China, is plagued by various arthropod pests. To efficiently control cotton bollworm (*Helicoverpa armigera* Hübner), pink bollworm (*Pectinophora gassypiella*) and other lepidopteran pests,

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the transgenic insect-resistant cotton expressing insecticidal toxins *Cry1A* from the bacterium *Bacillus thuringiensis* (*Bt*) was officially approved for commercial use in China in 1997. Subsequently, *Bt* cotton has steadily been adopted by the majority of Chinese cotton growers. Presently, *Bt* cotton comprises approximately 95% of the total cotton acreage in Northern China (Lu *et al.*, 2010). The widescale planting of *Bt* cotton has led to significant reduction in insecticide use (Wu *et al.*, 2008) and the apparent enhancement of pest suppression in cotton ecological systems (Lu *et al.*, 2012). However, populations of mirid bugs (Heteroptera: Miridae) have increased dramatically in recent years as a result of a reduction in the use of chemical control as the widescale adoption of *Bt* cotton increased (Wu *et al.*, 2002; Lu *et al.*, 2010). At the same time, the B- and Q-biotypes of sweet-potato whitefly (*Bemisia tabaci*), which has invaded all cotton production regions in China, are becoming a critical limiting factor for cotton yield and quality. To address the aforementioned complexity in cotton production, adopting a more sustainable management practice in *Bt* cotton is crucial.

It is well known that pest densities and pest status in a cotton production system are determined by soil fertility, microclimate, pest management strategies (Head *et al.*, 2005), cropping patterns (Sisterson *et al.*, 2004) and cultivar (Johnson & Agrawal, 2005; Wimp *et al.*, 2005, 2007; Whitham *et al.*, 2006; Robinson *et al.*, 2012). At the same time, a large number of natural enemies, including predators and parasitoids, usually exert a considerable degree of biological pest suppression in cotton. The increased adoption of *Bt* cultivars has been largely attributed to an altered microhabitat of arthropod assemblages, significantly affecting species interactions. However, to our knowledge, no empirical study has addressed the interaction between arthropod pests and natural enemies in *Bt* cotton, particularly in northern China. Previous studies have demonstrated that habitat modification influenced the outcome of species interactions as a result of the differential responses of herbivores and predators (Thies *et al.*, 2003; Tscharntke & Brandl, 2004). Similarly, studies in natural ecosystems have shown that the phenotype or genotype of plant may affect the composition of the dependent community (Johnson & Agrawal, 2005; Wimp *et al.*, 2005, 2007; Whitham *et al.*, 2006; Robinson *et al.*, 2012). However, the influence of intraspecific plant genetic composition on the interactions among arthropods of the same guild and/or different guilds is poorly understood in cotton agroecosystems. Considerable attention on this issue is vital for cotton production sustainability because an unravelling of the intricate interactions among plant, pest and natural enemy repesents the first step toward improving pest suppression.

The present study aimed to describe the ecosystem variables influencing selected features of arthropod assemblage (abundance, species richness, diversity index and community composition) among cotton fields of different plant compositions. The study aimed to investigate: (i) are shifts in abundance, richness, diversity index and community composition of arthropods correlated with the dominance of *Bt* cotton in the crop mix at a local scale; (ii) does the dominance of *Bt* cotton render equivalent effects for pests and natural enemies; and (iii) is the effect of crop dominance on arthropod assemblages circumstance-dependent or biologically coupled?

Materials and methods

Plant background

The transgenic insect-resistant cotton hybrid SiZhuang NC20B (a *Bt* cotton expressing *CrylAc* gene from Shiyuan14 developed by Monsanto, St Louis, Missouri) and the nontransgenic counterpart 93Fu56 were used in the present study. They were selected based on three principles: (i) commonly adapted cotton cultivars in Hebei Province in northern China; (ii) similar phenotypes and lifespan for crop mixing and/or interplanting (both early-maturing crops with a lifespan of approximately 130 days); and (iii) complementary inherent resistance to insect pests and diseases (NC20B is susceptible to Fusarium wilt and verticillium wilt but shows a high resistance to cotton bollworm complex, whereas 93Fu56 is susceptible to cotton bollworm complex but is highly resistant to Fusarium wilt).

Experimental set-up

Field experiments were conducted during 2008 and 2009 at the Langfang Experimental Station of the Chinese Academy of Agricultural Sciences in Hebei Province, China. A randomized complete block design with four replications was used. Experiments consisted of three treatments: (i) a monoculture field cultivated with non-*Bt* cotton (0 *Bt*); (ii) an intercropped field cultivated with 75% genetically modified (GM) *Bt* cotton and 25% non-*Bt* cotton (75% *Bt*); and (iii) a monoculture field cultivated with *Bt* cotton (100% *Bt*). Two monoculture treatment fields received 100% of either *Bt* or non-*Bt*, whereas the 75% *Bt* field was set-up according to the current adoption rate of *Bt* cotton in Hebei Province and the recommended refuge size for the target pest resistance management of *Bt* cotton (Vacher *et al.*, 2003; Sisterson *et al.*, 2004). The total acreage of each experimental block consisted of 0.33 ha, which is the average field size of most cotton growers in Hebei Province. The experimental plots were 16 rows of 20 m in width in each block. The seedling rate was expected to produce 40 000 plants per ha. A 3-m fallow alley was left between blocks to decrease insect dispersion among treatments. Normal agronomic practices in northern China were followed for raising the cotton crops (basal fertilizer N : P : K = 100 : 40 : 60 kg/ha). No pesticide was applied in the experimental blocks during the developmental period of the plants.

Data collection

Arthropod sampling was conducted at intervals of 10 days starting from early June (4 weeks after cotton emergence) until the middle of September (crop defoliation), throughout the growing seasons of 2008 and 2009, with a total of 10 sampling dates per year. Arthropod species were sampled by visually inspecting 100 cotton plants *in situ* per block during fair weather (no precipitation, lightening or wind). For each block, five sites distributed at the two diagonals of the block were randomly chosen and 20 plants were selected from each site. During the sampling period, the number and types of arthropods, irrespective of larva, nymph or adult, inhabiting the cotton plant were quantified. Because of practical concerns as a result of high densities of cotton aphids and whiteflies for some stages, their abundance was quantified from nine leaves for each sampled plant (three leaves from upper, middle and lower main stem portions, respectively) and the total abundance was estimated as the product of mean aphid or whitefly numbers per leaf by the total number of leaves. For other arthropods, such as Pentatomidae, Cicadellidae, Coccinellidae, Chrysopidae, Mantidae and foliage-dwelling spiders, entire plants were visually inspected in the morning (08.00–10.00 h) or afternoon (16.00–18.00 h), with particular attention paid to flowers and squares, which are the likely hiding places for feeding insects.

Statistical analysis

The overall effects of *Bt* cotton's dominance on the seasonal abundance and taxonomical richness of arthropod was tested separately with a generalized linear mixed model (PROC MIXED, SAS, version 9.1; SAS Institute Inc., Cary, North Carolina), with *Bt* cotton's dominance, developmental stage and their interaction as fixed factors and block as random factor. The effect of *Bt* cotton's dominance on abundance and taxonomical richness of arthropods at each sampling date was tested by one-way analysis of variance, with a least significant difference method for multiple comparisons.

The effects of *Bt* cotton's dominance on the diversity of arthropod assemblage were examined by calculating the Shannon–Weaver diversity index ($H' = \sum_{i=1}^{s} P_i \ln P_i$), Simpson's index ($D = 1 - \sum_{i=1}^{s} (P_i)^2$) and Pielou's evenness index ($J' = H'/H_{max}$, $H_{max} = \ln S$), where P_i is the proportion of individuals in taxonomic group *i* and S is the total number of taxonomic groups (i.e. taxanomic richness). The Shannon–Weaver index gives the distribution of species abundance and also reveals rare species, with a higher index indicating higher diversity. Simpson's index shows the distribution of species abundance, with more weight given to common species, and thus a higher index indicating higher dominance, whereas Pielou's evenness index gives information on the distribution of species abundance, with a higher index indicates higher diversity. Values for the evenness index range from 0 to 1, with 0 representing an entirely skewed dominance by one group and 1 representing a perfectly even relative abundance among groups. The seasonal fluctuations of diversity indices as a result of crop dominance and developmental stage were tested with generalized linear mixed model (PROC MIXED, SAS, version 9.1), with *Bt* cotton's dominance, developmental stage and their interaction as fixed factors and block as random factor. Simultaneously, within-season shifts in diversity indices induced *Bt* cotton's dominance at each sampling date were analyzed for temporal comparison of biodiversity indices (Scherer *et al.*, 2013).

The shift in community composition of arthropods was assessed via non-metric multidimensional scaling (NMDS) analysis, using the coefficient of similarity of Bray–Curtis. NMDS avoids the assumption of linear relationships among variables, and relieves the 'zero-truncation problem' using ranked distance to linearize the relationship between distances measured in species space and distances in environmental space. Therefore, NMDS is the most generally effective ordination method for ecological community data and should be the method of choice, unless a specific analytical goal demands another method (McCune *et al.*, 2002). The effects of crop dominance and developmental stage on arthropod assemblage were tested with PER-MANOV in PC-ORD, version 5 (MjM Software, Gleneden Beach, Oregon).

Results

Influence of the dominance of Bt cotton in cotton field on the abundance of arthropod assemblage

In 2008, the dominance of *Bt* cotton in cotton field exerted significant effects on arthropod community size for total arthropod group (Fig. 1A and Table 1), pests (Fig. 1B and Table 1) and natural enemies (Fig. 1C and Table 1). Simultaneously, the densities of each trophic group showed great fluctuations across the developmental stages of cotton. The effect of *Bt* dominance on the abundance of arthropods varied with crop developmental stage and arthropod guild (Fig. 1 and Table 1). Similar trends were observed for 2009 (Fig. 1D–F and Table 1). Overall, the community size of pests, natural enemies or total arthropods was negatively correlated with the proportion size of *Bt* cotton in the crop mixture, although no apparent differences in arthropod densities between 75% and 100% *Bt* cotton fields were observed (Fig. 1).

Change in arthropod biodiversity as a result of the elevated proportion size of Bt cotton in cotton field

On the whole, effects of *Bt* cotton ratio in the crop mixture on arthropod diversity indices were taxonomic-specific and time-dependent. In 2008, the effects of acreage ratio of *Bt* cotton, developmental stage and their interactions on taxonomic group richness, Shannon–Weaver index (except for natural enemies), Simpson's index and Pielow's index were all significant (Figs 2–5; see also Supporting information, Table S1). In 2009, the effects of acreage ratio of *Bt* cotton were apparent for pests and total arthropods but not for natural enemies (see Supporting information, Table S1). However, the effects of developmental stage of cotton and interaction between acreage ratio of *Bt* cotton and developmental stage on taxonomic group richness and diversity indices were significant. On the whole, the responses of the taxonomic group richness and diversity indices to shifts in dominance of *Bt* cotton showed great fluctuations across the developmental stages, and no clear tendency was observed (Figs 2–5; see also Supporting information, Table S1).

Linkage between the proportion size regimes of Bt cotton and the community composition of arthropods in cotton fields

The NMDS analysis based on the arthropod community of different *Bt* cotton acreage size regimes showed how treatments clustered in each of the assessed developmental stages (Fig. 6), indicating that the similarity of the arthropod community in cotton fields changed as a result of the proportion size of *Bt* cotton in the local agricultural landscape. In the growing season

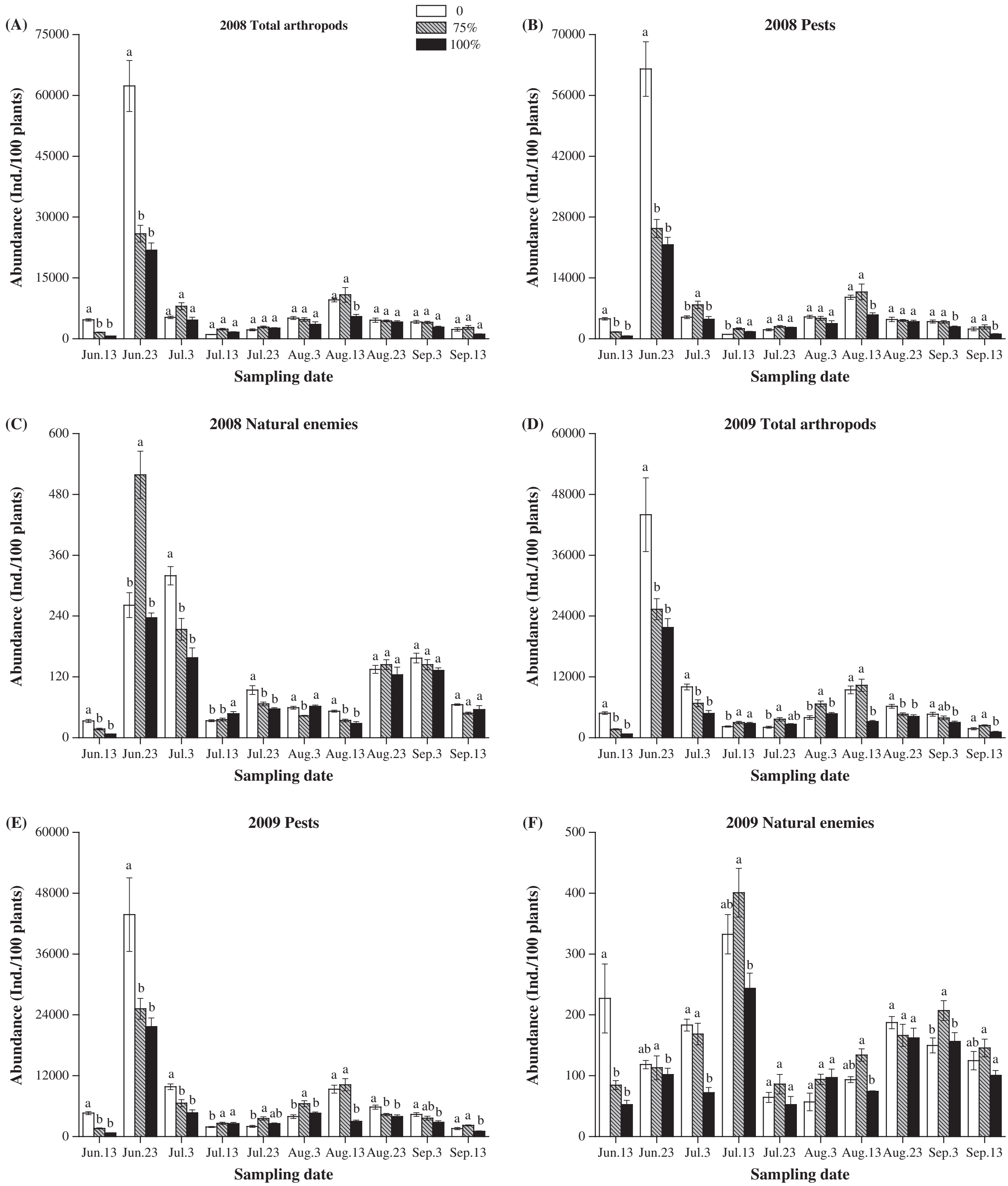

Figure 1 Average abundance ($n = 4$) of total arthropods, pests and natural enemies in cotton fields of different *Bacillus thuringiensis* dominances in the 2008 (A–C) and 2009 (D–F) growing seasons. Error bars represent the SEM. Different lowercase letters indicate significant differences ($P < 0.05$) between treatments at the same sampling date.

Table 1 *F*- and *P*-statistics of repeated-measures analysis of variance of the effects of *Bt* proportion (B) and date (D) on the abundance of arthropod complex, herbivores and natural enemies in a cotton field in 2008 and in 2009

			Arthropod		Herbivores		Natural enemies	
Year	Factor	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
2008	B	2,6	129.3	< 0.001	122.3	< 0.001	47.4	< 0.001
	D	9,81	220.0	< 0.001	213.2	< 0.001	386.3	< 0.001
	B × D	18,81	13.0	< 0.001	12.8	< 0.001	14.1	< 0.001
2009	B	2,6	100.7	< 0.001	92.6	< 0.001	22.8	0.002
	D	9,81	200.3	< 0.001	268.4	< 0.001	37.2	< 0.001
	B × D	18,81	18.8	< 0.001	18.2	< 0.001	5.2	< 0.001

of 2008, arthropod assemblage on June 23, July 3, September 3 and September 13 showed clear separation among treatments, indicating that each treatment resulted in a specific arthropod community. However, at other developmental stages, the separation of blocks as a result of treatment was not clear, with several species and blocks overlapping, especially for *Bt* dominance at 75% and 100%, suggesting a certain similarity among the structures of their arthropod communities. NMDS analysis based on dataset of 10 sampling dates indicated that arthropod assemblage in *Bt* fields under different dominance regimes was distinct (Final stress = 8.38465, Final instability = 0.00031, $P = 0.0476$; Fig. 6A). PER-MANOV analysis indicated that the components of variation in arthropod assemblages were well interpreted by crop dominance ($F = 12.806$, d.f. = 2, $P = 0.0002$), crop developmental stage ($F = 118.63$, d.f. = 9, $P = 0.0002$) and their interaction ($F = 10.205$, d.f. = 18, $P = 0.0002$).

Similar patterns were found for the 2009 season, with greater variation for some developmental stages. On June 13, June 23, August 23, September 3 and September 13, each treatment resulted in a different arthropod community, whereas, at other developmental stages, the arthropod community showed some similarity among the treatments. NMDS analysis based on dataset of 10 sampling dates in 2009 indicated that arthropod assemblage in *Bt* fields under different dominances regime was distinct (Final stress = 7.46363, Final instability = 0.00032, $P = 0.0476$; Fig. 6B–D). PER-MANOV analysis indicated that the variance components of discrepancy in arthropod assemblages were well interpreted by crop dominance ($F = 8.936$, d.f. = 2, $P = 0.0002$), developmental stage ($F = 61.102$, d.f. = 9, $P = 0.0002$) and their interaction ($F = 8.960$, d.f. = 18, $P = 0.0002$).

Discussion

In general, the current literature provides three distinct types of controls with respect to the potential effects of GM crops (e.g. *Bt* cotton) on its nontarget fauna: (i) controls entailing non-GM varieties grown under identical conditions but treated with insecticides; (ii) controls entailing non-GM varieties grown under identical conditions and with no insecticide applied; and (iii) combination *Bt* and control plants both treated with insecticides. In the present study, non-*Bt* cotton with a phenotype and lifespan similar to *Bt* cotton, grown under identical condition and free of pesticide application, was assigned as a control. The effects of crop composition as a result of the widescale adoption of *Bt* cotton on arthropod assemblage were explored with a simulation experiment using 75% and 100% *Bt* in the experimental cropping landscape. We hypothesized that the replacement of conventional non-*Bt* cotton by transgenic *Bt* cotton was expected to alter the seasonal dynamics and composition of arthropod assemblage in cotton. In the present study, the impacts of the acreage size of *Bt* cotton in total cotton acreage landscape, otherwise termed as the crop genetic mix ratio, on arthropod community were derived from three ecological aspects.

The proportion size of Bt cotton in cotton cropping landscape modulates the abundance of arthropod assemblage

In the present study, the impacts exerted by proportion size of *Bt* cotton on community size were significant for arthropods, irrespective of the trophic guild. In general, the abundance of pests in cotton field was negatively correlated with the proportion size of *Bt* cotton, demonstrating that overall pest populations decreased when *Bt* cotton ratio increased in the total cotton cropping landscape. This result is in general agreement with Marvier *et al.* (2007) who reported a significantly reduced mean abundance of all nontarget invertebrate groups in *Cry1Ac* cotton fields compared with that in non-GM, insecticide free fields. However, the proportion size of *Bt* cotton in cotton landscape was not a reliable predictor for the abundance of natural enemies. A 3-year study similar to the present study, conducted at two sites in Arizona, found that the abundance of natural enemies was greater in mixture plots of 75% *Bt* and 25% non-*Bt* than in pure *Bt* plots, although it did not differ significantly between mixture plots and pure non-*Bt* plots (Sisterson *et al.*, 2004). With respect to the predator abundance in *Bt* versus non-*Bt* cotton without application of insecticide, a long-term study conducted in the same region as that of the present study during 2001–2010 indicated a similar predator abundance in *Bt* versus non-*Bt* cotton (Lu *et al.*, 2012).

There may be multiple factors accounting for the distinct responses of herbivores and natural enemies to the proportion size of *Bt* cotton in a cotton cropping landscape. As reported in previous studies, herbivores are influenced primarily by vegetation composition within habitats, whereas predators and parasitoids may be more sensitive to changes in field size and habitat arrangement (Stoner & Joern, 2004; Tscharntke & Brandl, 2004).

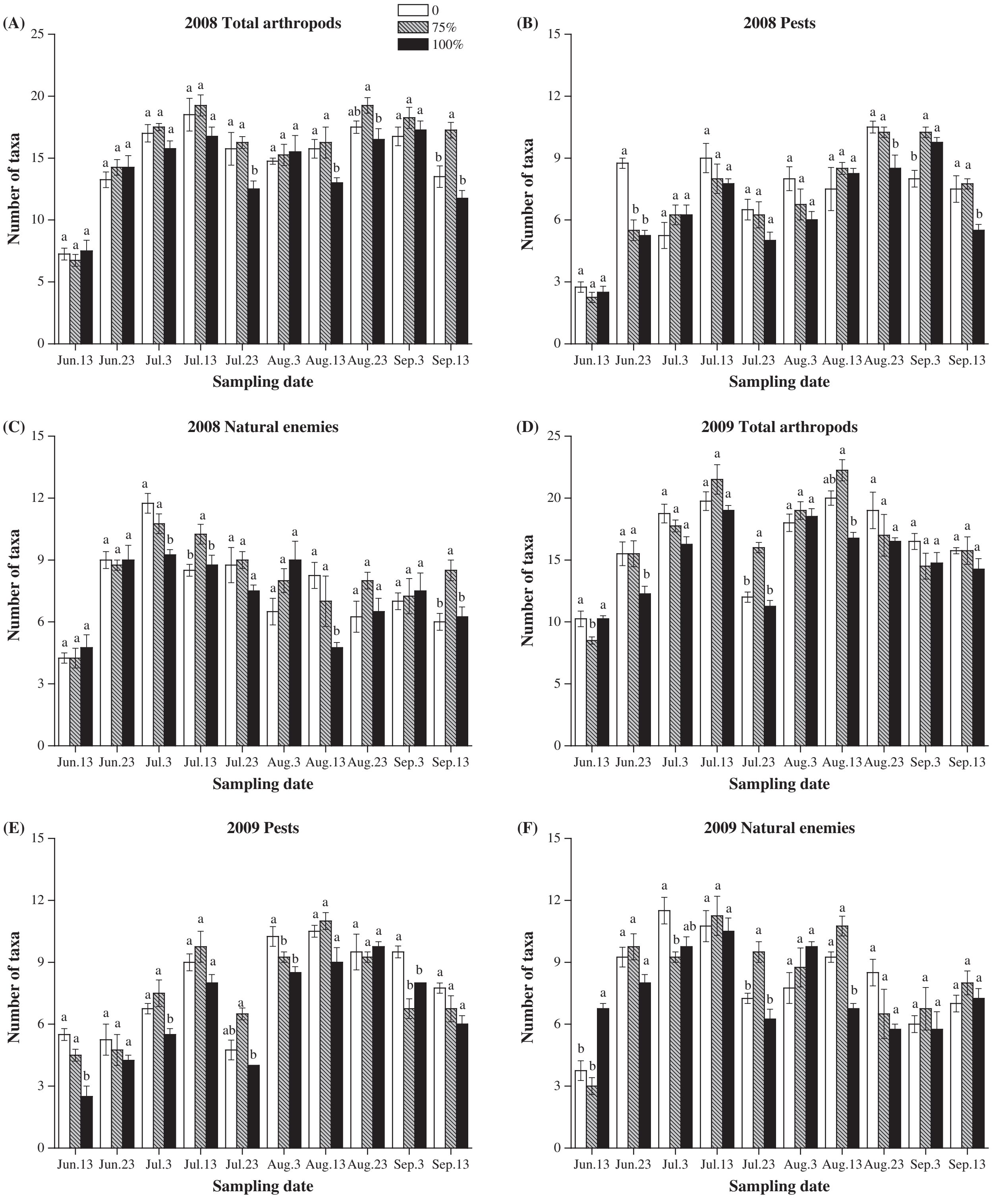

Figure 2 Average richness ($n = 4$) of total arthropods, pests and natural enemies in cotton fields of different *Bacillus thuringiensis* dominances in the 2008 (A–C) and 2009 (D–F) growing seasons. Different lowercase letters indicate significant differences ($P < 0.05$) between treatments at the same sampling date.

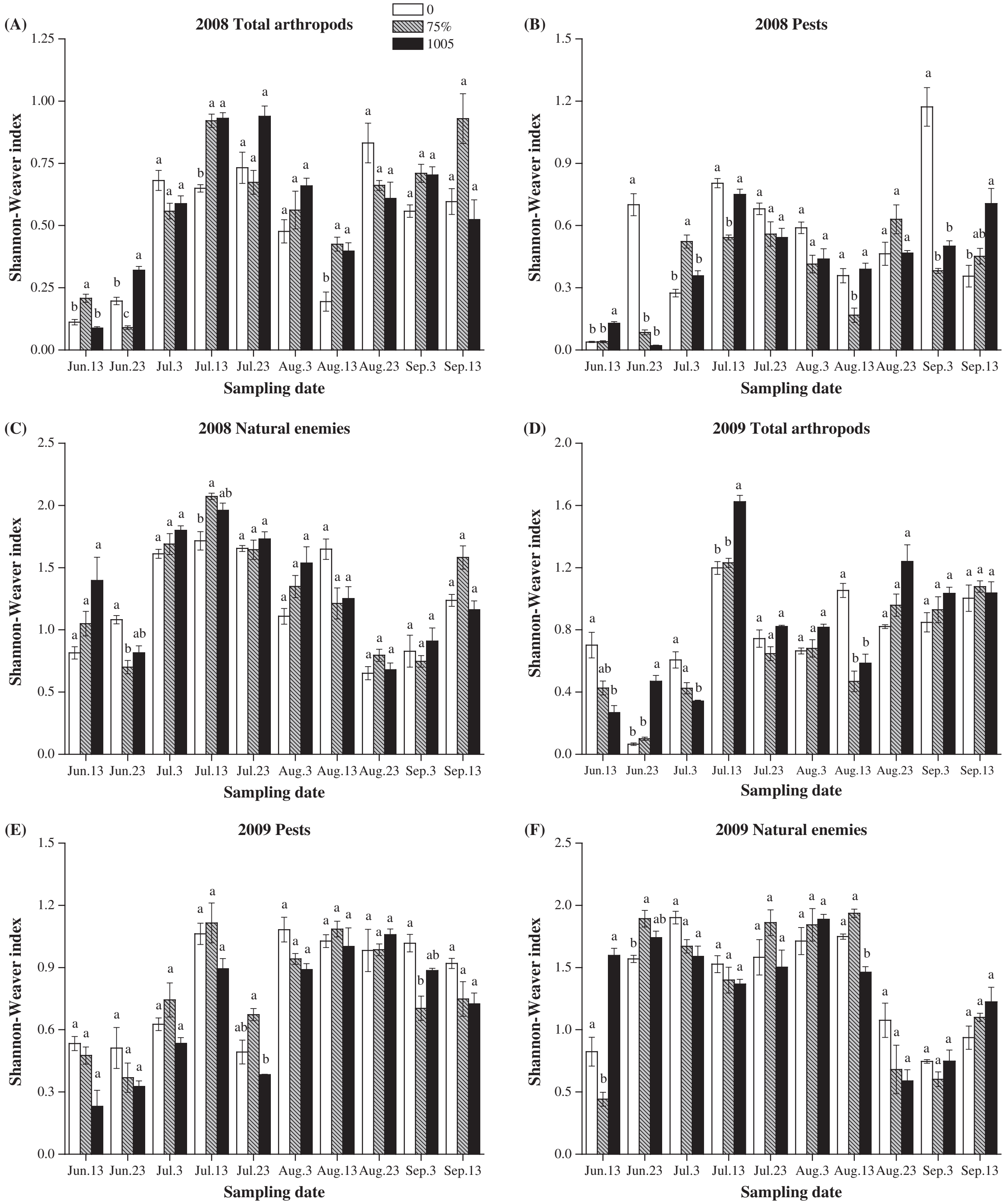

Figure 3 Average Shannon−Weaver indices ($n = 4$) of total arthropods, pests and natural enemies in cotton fields of different *Bacillus thuringiensis* dominances in the 2008 (A−C) and 2009 (D−F) growing seasons. Different lowercase letters indicate significant differences ($P < 0.05$) between treatments at the same sampling date based on the exact permutational min-P method with bootstrap.

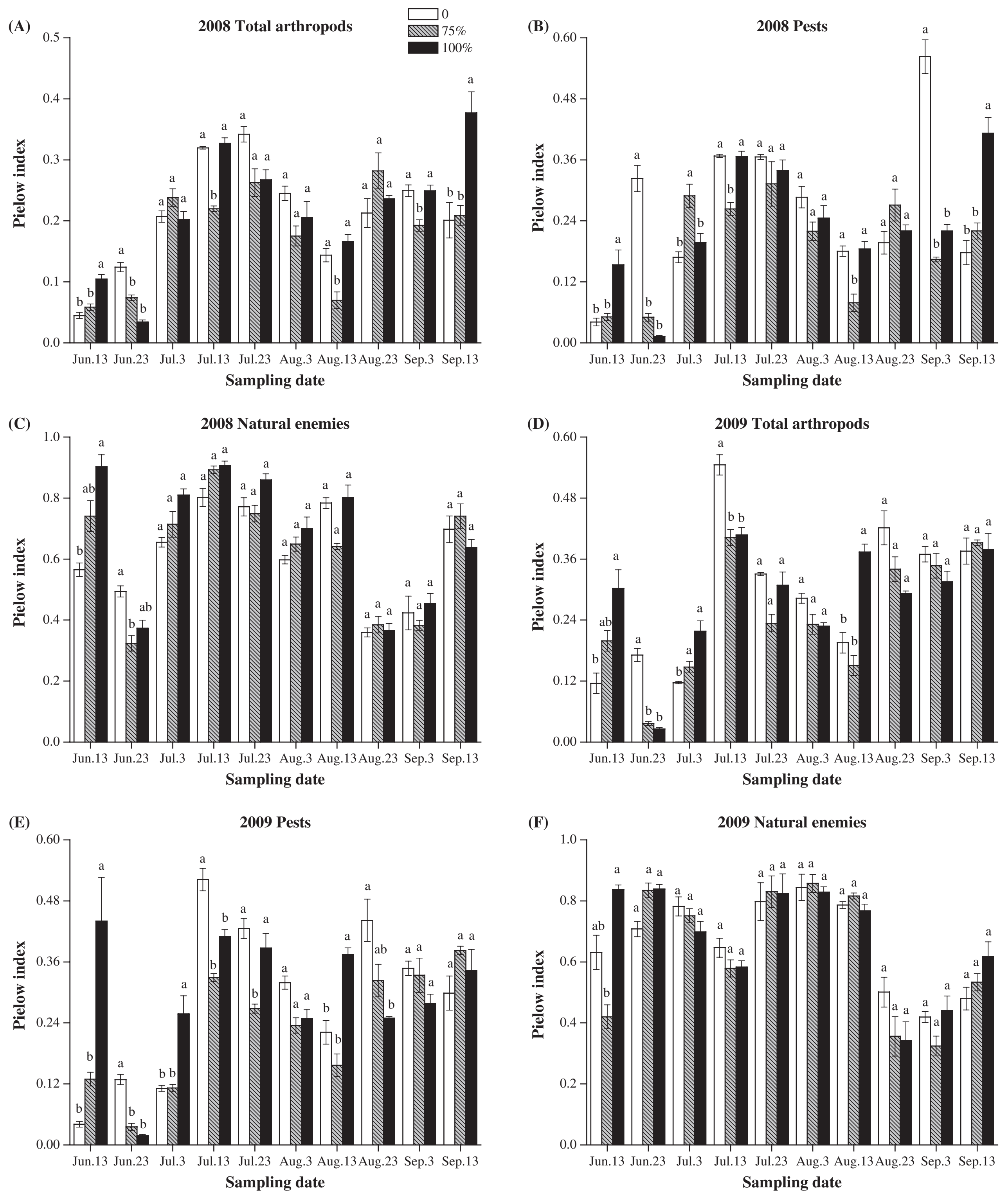

Figure 4 Average Pielow's indices ($n = 4$) of total arthropods, pests and natural enemies in cotton fields of different *Bacillus thuringiensis* dominances in the 2008 (A–C) and 2009 (D–F) growing seasons. Different lowercase letters indicate significant differences ($P < 0.05$) between treatments at the same sampling date based on the exact permutational min-P method with bootstrap.

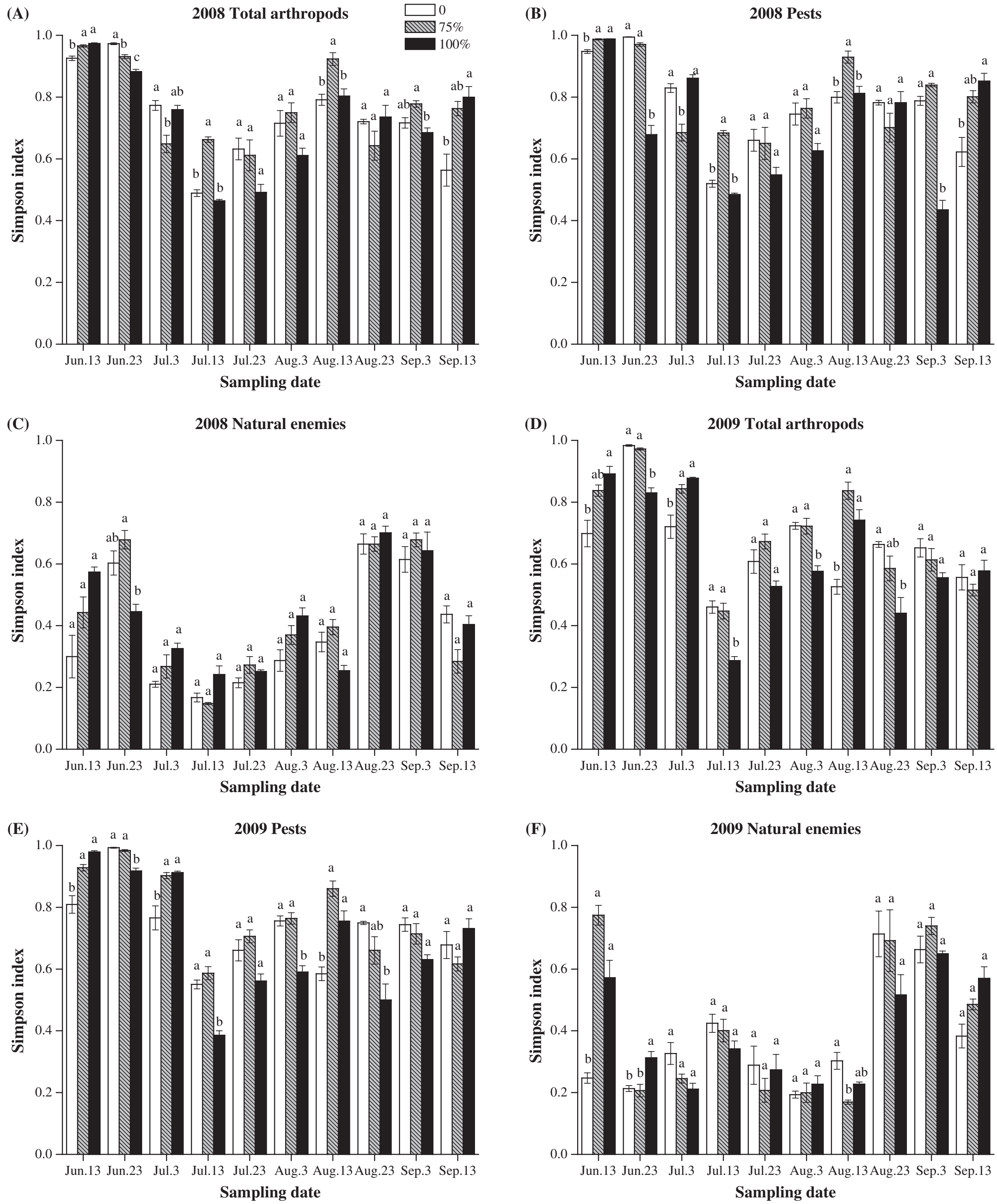

Figure 5 Average Simpson's indices ($n = 4$) total arthropods, pests and natural enemies in cotton fields of different *Bacillus thuringiensis* dominances in the 2008 (A–C) and 2009 (D–F) growing seasons. Different lowercase letters indicate significant differences ($P < 0.05$) between treatments at the same sampling date based on the Westfall–Young bootstrap min-P test.

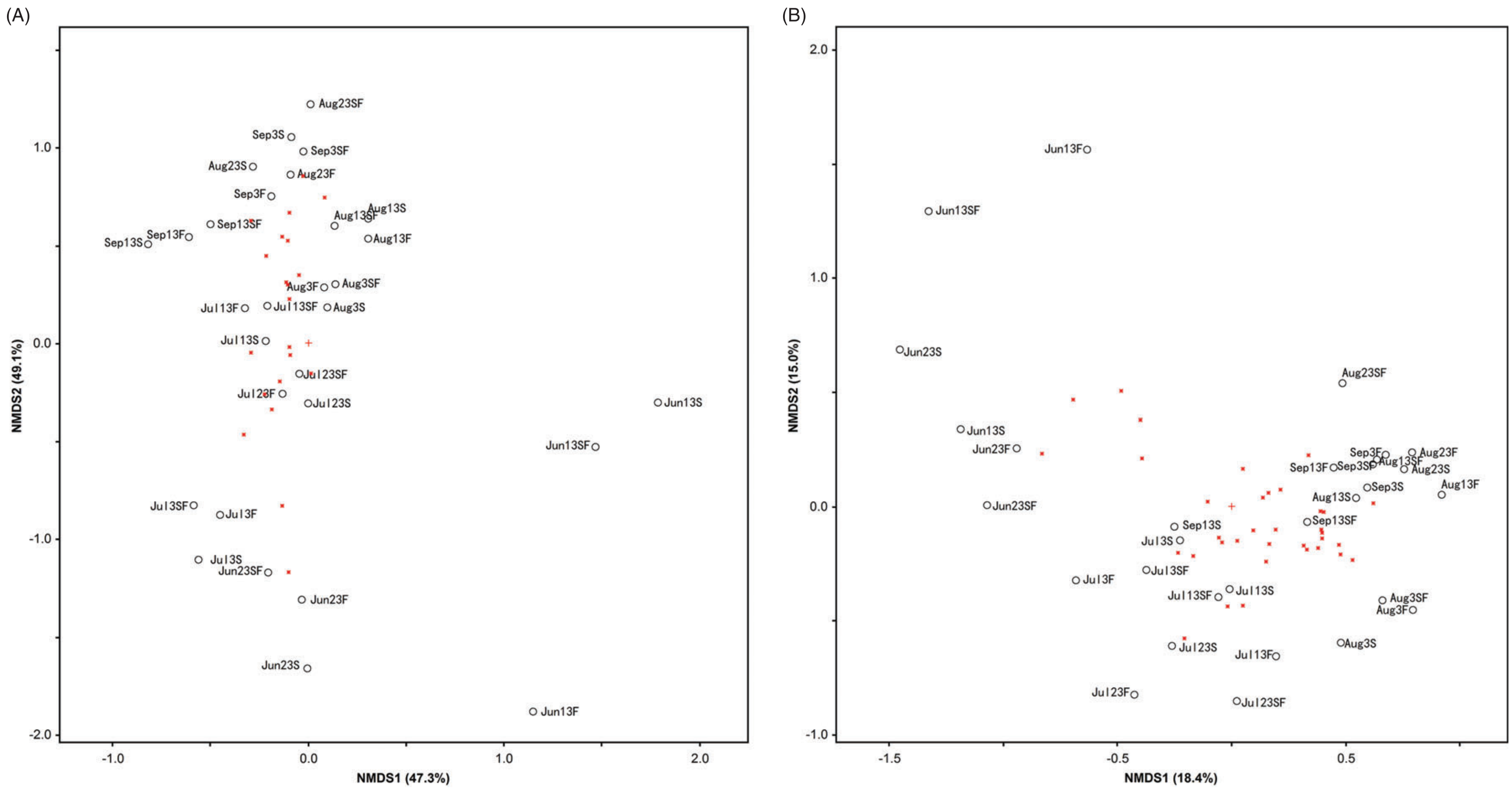

Figure 6 Non-metric multidimensional scaling (NMDS) analysis of arthropod assemblages in cotton fields of different *Bacillus thuringiensis* (*Bt*) dominances in the 2008 (A) and 2009 (B–D) growing seasons. F, 0Bt; SF, 75%Bt; S, 100Bt.

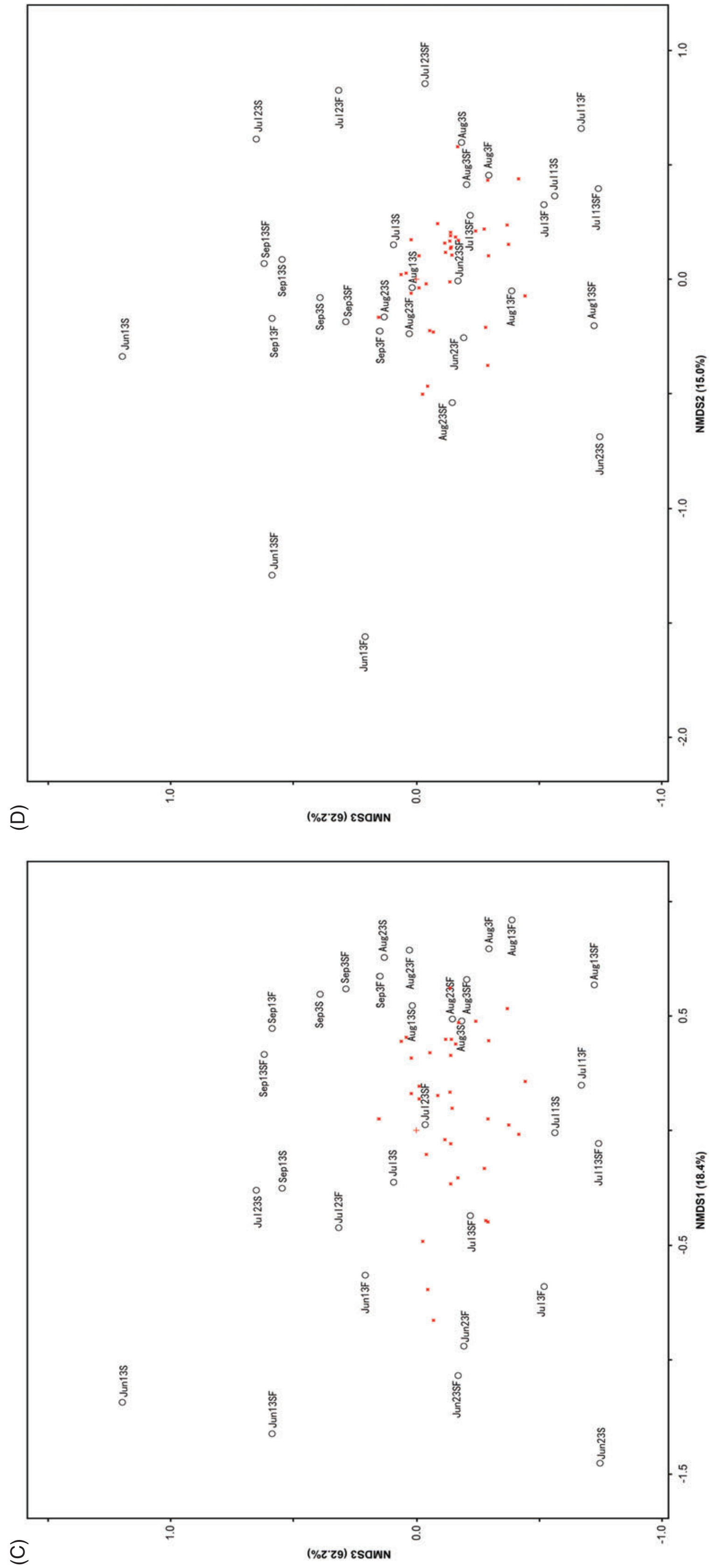

Figure 6 Continued.

In addition, crop species richness significantly affected the pest species richness, although there were no significant effects of the pests' natural enemies on richness (Shi *et al.*, 2014). In general, herbivores are generally dominated by insects with a relatively lower migration capability, whereas most of the predators show higher mobility. On the other hand, the effect of plant composition on pest is direct, whereas that on natural enemies primarily resulted from trophic cascade. Consequently, the variation between genotypes of one host crop species generally renders a weak effect at the third trophic level.

Plant traits showing specific attracting or deterring effects to predators are relatively fewer than those affecting herbivores (Johnson & Agrawal, 2005). Furthermore, the effect of pest density and dispersion capability on foraging behaviour, foraging efficacy and interaction of natural enemies may be additive, and these response variables in turn influence the abundance of natural enemies. Consequently, in contrast to the relatively homogeneous genetic background of monocultures in an agricultural ecosystem, a change in genetic diversity within a crop in an intercropping field, such as in the present study, is expected to trigger a corresponding alteration in trophic interactions. However, we were unable to clarify the underlying mechanism in the present study because the apparent change in environmental factors as a result of altered plant genetic composition (*Bt*) was not quantified concurrently.

The proportion size of Bt cotton affects arthropod composition and biodiversity

The results from community composition and diversity index analyses provided further evidence indicating that arthropod community was characterized by the proportion size of *Bt* cotton. A previous study suggested that, when plants are more genetically similar, their arthropod communities are also similar, whereas plants that are less related have less similar arthropod communities (Wimp *et al.*, 2005). Subsequently, it was found that plant chemistry was an important mechanism by which plant genetics affects the arthropod community composition (Wimp *et al.*, 2007).

Similarities of arthropod communities among cotton fields of distinct dominances occupied by *Bt* cotton in the present study were clearly displayed using NMDS ordination. The results suggested that there are significant variations (discrepancies) in community variables of arthropods for non-*Bt* monoculture, a cotton field mixture of 75% *Bt* plus 25% non-*Bt*, and *Bt* monoculture. Furthermore, the diversity and community composition of arthropod assemblage among cotton fields showed a great fluctuation across crop developmental stages and within growing seasons. In light of a dramatic change in crop physiology, defence strategy, microclimate and other associated parameters, it is difficult to isolate a single factor (or factors) determining the seasonal dynamics of arthropod assemblage. Simultaneously, the proportion size of *Bt* cotton in cotton cropping landscape exerted significant effects on the richness of predator fauna, whereas the direction and strength of effect showed great discrepancies among the diversity indices tested. These findings support the proposal that plant genotypes can have a direct impact at the third trophic level, affecting the abundance and richness of predators in a natural system (Johnson, 2008). In addition, the effect of the *Bt* adoption ratio on diversity indices of arthropod assemblage showed appreciable variability among trophic groups and between the two growing seasons. Numerous other studies have addressed the responses of arthropod community to *Bt* cotton from China and other major cotton producing countries (Men *et al.*, 2003; Lu *et al.*, 2012).

The potential impacts of *Bt* cotton to ecosystem functioning, especially for pest suppression, remain a controversial issue. For example, Li *et al.* (2003) investigated the effects of transgenic cotton carrying *Cry1A* + *CpTI* and *Cry1Ac* genes on diversity of arthropod communities in cotton in northern China and found that the diversity of arthropod communities in *Bt* cotton plots was similar to that in unsprayed conventional cotton fields. Men *et al.* (2003) suggested that *Bt* cotton increased the diversity of arthropod communities and pest subcommunities, although it decreased the diversities of sub-communities of natural enemies. Whitehouse *et al.* (2005) compared the invertebrate community in unsprayed conventional and unsprayed *Bt* cotton fields in Australia over three growing seasons using suction sampling methods. They found that the most consistent differences between unsprayed *Bt* and conventional communities were higher numbers of *H. armigera* (Lepidoptera: Noctuidae) in conventional crops and slightly higher numbers of Chloropidae (Diptera) and Drosopillidae (Diptera), damsel bugs (Hemiptera: Nabidae), and jassids (Hemiptera: Cicadellidae) in conventional crops. Simultaneously, a 6-year study conducted in Arizona reported that arthropod predator abundance (five taxa combined) declined by 19% in unsprayed *Bt* cotton compared with that in non-*Bt* cotton, although the minor reductions in *Bt* cotton have little ecological meaning because most of these reductions were likely associated with reductions in lepidopteran prey (Naranjo, 2005a). By contrast to the currently available literature, in which the control ranges from conventional non-*Bt* of the same variety to a similar variety, in the present study, a non-*Bt* cotton with an identical phenotype and lifespan but complementary resistance to *Bt* cotton was assigned as a control. In addition, we focused on addressing the response of the arthropod community to the proportion size change of *Bt* cotton in the cropping landscape.

It should be noted that the abundance of *H. armigera* in the monoculture non-*Bt* cotton field was much higher than that of the monoculture *Bt* and mixture field of 75% *Bt* plus 25% non-*Bt* because the primary threat to the long-term efficacy of *Bt* toxins is the evolution of resistance by pests (Tabashnik, 1994; Gould, 1998). We did not monitor the resistance frequency of cotton bollworm from fields of different dominance occupied by *Bt* cotton in the present study. According to previous studies, the frequency of cotton bollworm resistance to *Cry1Ac* is higher in northern China, where *Bt* cotton has been grown intensively, than in areas of northwestern China, where *Bt* cotton planting has been limited (Zhang *et al.*, 2011, 2012). Consequently, planting more cotton that produces no *Bt* toxins would increase the abundance of *H. armigera* which are sensitive to *Bt* protein and could help to delay the development of resistance (Tabashnik & Wu, 2012).

Overall, our findings indicate that the arthropod community of a certain agro-ecosystem was shaped by the crop composition and its developmental stage. However, this result was obtained from a case study simulating the effect of regional *Bt* adoption progress on arthropod community at a small scale, where the interplot movements of some actively mobile arthropods were not rigorously controlled. Furthermore, plot size is an important factor for evaluating population level toxicological effects with optimum plot size largely driven by the mobility, phenology and ecological requirements of the species under consideration. Thus, further studies with multiple sites and cultivars are needed to confirm the robustness of this conclusion. Despite these limitations, season-long thorough monitoring (i.e. 10 sampling dates in each growing season) provided detailed information about the temporal change of arthropod community characteristics in fields of differential *Bt* cotton acreage size. In addition, the analytical methods used in the present study, such as bootstrap, NMDS and PER-MANOV, should provide a guide for the risk assessment of new cotton cultivar or other GM crops.

Implications for the pest management of Bt crop in the future

In accordance with most of the associated studies, significant spatial and temporal variations in arthropod assemblages were observed in the present study. Head *et al.* (2005) proposed that management practices such as tillage and irrigation, differences in the surrounding land use patterns, and climatic factors such as temperature and precipitation all determine the arthropod populations of a given region. A comprehensive analysis using variance partitioning analysis or structural equation modeling is warranted to quantify the respective effect of the factors with multiyear, large-scale field trials. An insignificant change in predator abundance (< 20%) may not impact upon the biological control potential of the natural enemy community (Naranjo, 2005b). It is expected that future agricultural ecosystems would maximize the proportion of high-yielding focal crop without hampering the ecologically driven pest control services supported by biodiversity.

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Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference:
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Table S1. *F*- and *P*-statistics of repeated-measures analysis of variance which testing the effects of *Bacillus thuringiensis* proportion and date on richness (S), Shannon–Weaver diversity index (H'), Pielow evenness index (J') and Simpson's index of diversity (D) for arthropod, herbivores and natural enemies in a cotton field in 2008 and 2009.

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