COTTON ENTOMOLOGY
RESEARCH REPORT 2016

COTTON ENTOMOLOGY PROGRAM

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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 boll weevil winter survival survey of High Plains overwintering habitats and grid-trapping of weevils across our vast High Plains cotton producing areas. 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 and is managed as a single zone called West Texas Maintenance Area (WTMA). 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 transgenic *Bt* technology (now >70%), the cotton insect research and extension program focus has changed considerably during the last 16 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 and economics. Our Program has successfully leveraged research funds based on the funding provided by PCIC to support our research effort. 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, 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 1900GLT, was planted at a targeted rate of 54,000 seeds/acre on May 27, 2016. 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 14. Pre-treatment soil samples (consisting of three soil cores; 0 to 24-inch depth), were collected from each of the 25 experiment plots on July 1. Three 3-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. Two weeks into cotton squaring (July 17), the most critical phenological stage of cotton for cotton fleahopper management in the Texas High Plains, three cotton fleahopper augmentation treatments (5 cotton fleahopper nymphs per plant, manual removal of 100% squares pre-flower, and no fleahopper augmentation as control) were deployed in these designated row sections to simulate an acute infestation of fleahoppers. With 20% field survivorship, this density is equivalent to 3-4 times current cotton fleahopper threshold (25-30 fleahoppers per 100 plants) for the High Plains.

Results: Cotton fleahoppers induced ~20% square drop across all N plots. Varying rates of N augmentation resulted in phenotypic expression of N deficiency in cotton across treatment plots, more pronouncedly between zero N plots and N augmented plots, which were reflected on temporal chlorophyll and leaf N contents of the fifth leaf (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 1,209 lb/acre in the control to 976 lb/acre in fleahopper plots. Lint yield was not significantly affected by ~20% fleahopper-induced square loss at zero N and the two highest N plots, either via pruning of undesirable fruit load (zero N) or compensation (high N). On the other hand, lint yield was lower in fleahopper augmented 50 and 100 lb/acre plots compared to that in control plots, suggesting that the plant response to cotton fleahopper injury is greatly influenced by the availably of N fertility. Manual removal of 100% squares pre-flower did not impact the lint yield.
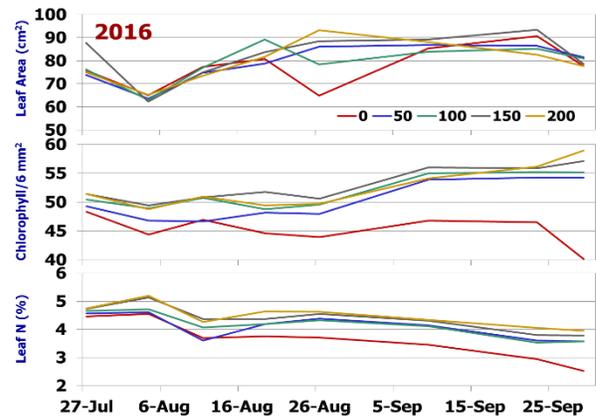

Fig. 1. Temporal dynamics of leaf area, chlorophyll concentration, and leaf N content on 5th mainstem leaf as influenced by varying N rates, 2016.
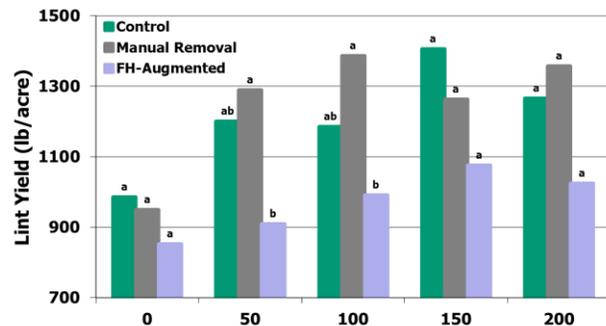

Fig. 1. Effect of N rates on lint yield following a single acute infestation of cotton fleahopper versus uninfested control and manual removal of 100% squares prior to the initiation of flowering, 2016.

TITLE:

Cotton yield response to cotton fleahopper acute infestations as influenced by irrigation level treatments, Lamesa, TX, 2016.

AUTHORS:

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

MATERIALS AND METHODS:

Plot Size:	4 rows by 300 feet, 3 replications
Planting date:	May 24, 2016
Fertilizer:	10-34-0
Treatments:	

Cultivar:	FiberMax 2011 GT
Irrigation:	*Low*: Pre-plant = 3.5 inches; In-season = 3.1 inches *High*: Pre-plant = 3.5 inches; In-season = 6.2 inches
Cotton fleahopper:	Three insect release treatments [*Control* (zero cotton fleahoppers), *Low fleahopper density* (2 bugs per plant), *High fleahopper density* (5 bugs per plant),

Herbicides:	2,4-D 1 qt/A – March 14 Roundup PowerMax 1 qt/A – March 14 Prowl 3 pt/A – April 27 Roundup PowerMax 1 qt/A – June 22 Roundup PowerMax 1qt/A – July 28 Warrant 3 pt/A – July 28

Insect release date:	July 9, 2016 at fleahopper susceptible stage
Plant mapping date:	August 3, 2016 (in-season); October 25, 2016 (pre-harvest)
Harvest date:	October 25, 2016 (hand-harvested)

Cotton fleahopper feeding injury and resulting cotton lint yield were evaluated on cotton variety FM 2011 GT, as affected by irrigation level and infestation densities. Two seasonal irrigation levels, *High* (9.7 inches) and *Low* (6.6 inches) were evaluated under a center pivot irrigation system. Laboratory-reared and/or field collected cotton fleahopper adults were released onto cotton terminals in 3-ft. (L) x 2-ft. (W) x 3 ft. (H) multi-plant cages (Fig. 1). Each cage contained seven plants. Experimental design consisted of three insect release treatments (*high*, *low*, and *control*) and two water levels (*high* versus *low*), replicated three times and deployed in a randomized complete block design (total 18 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 9, 2016 (Fig. 1), and then allowed to feed for one week in order to mimic a natural early-season acute infestation. No natural infestation of cotton fleahoppers were observed at the experimental farm, therefore, insecticides were not applied on the control plots. A single release of cotton fleahoppers was timed to simulate an acute infestation of cotton fleahoppers while cotton was highly vulnerable to fleahopper injury, which was approximately 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 25. 2016.

Figure 1. Examination of cotton squares (left), and multi-plant cages (right) deployed in the field to release cotton fleahopper densities to examine damage potential on cotton yield, Lamesa, TX.

RESULTS AND DISCUSSION:

Percentage square loss tended to be higher on cotton fleahopper infested plots compared to that in control plots under the *high* irrigation level (Fig. 2). Fleahopper crop damage, as measured by cotton square loss, did not significantly vary between the two water levels. Artificial augmentation of cotton fleahoppers caused 36.13% and 37.98% square loss following *low* and *high* levels of infestations, respectively and such pre-flower cotton square loss is considered a moderate level of insect-induced early fruit loss for Texas High Plains cotton.

A significantly higher lint yield was recorded from *control* plots compared to that from *high* fleahopper density plots. In *low* irrigation plots, a significantly higher lint yield was recorded from *control* plots than both *low* and *high* fleahopper densities; however, significantly higher lint yield was recorded from *low* fleahopper densities compared to *high* fleahopper densities in *high* irrigation plots (Fig. 3). These data suggest that the deficit-irrigated cotton is more sensitive to fleahopper-induced square losses than the crop that receives sufficient irrigation even at low cotton fleahopper densities. At high cotton fleahopper densities, cotton fleahopper-induced square loss resulted in significantly lower lint yield regardless of the irrigation water level.
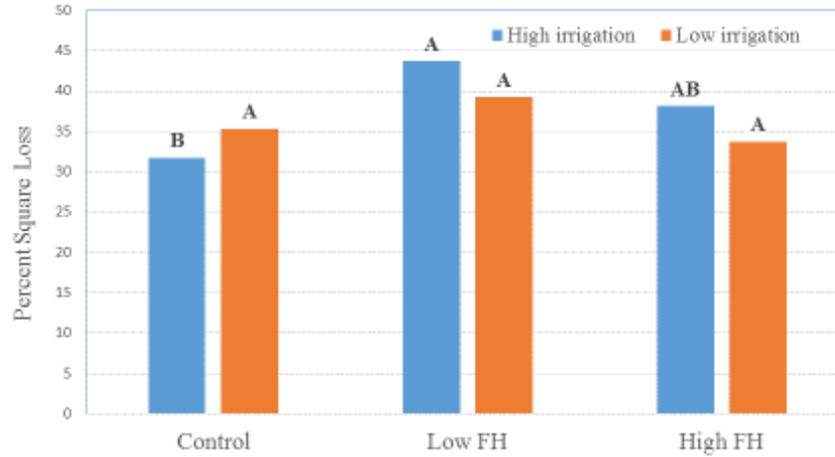
Figure 2. Average percentage square loss following a simulated acute infestation of cotton fleahoppers, achieved by augmenting 2 (low) and 5 (high) bugs per plant during the second week of squaring, under low and high irrigation regimes on cotton, Lamesa, Texas, 2016.
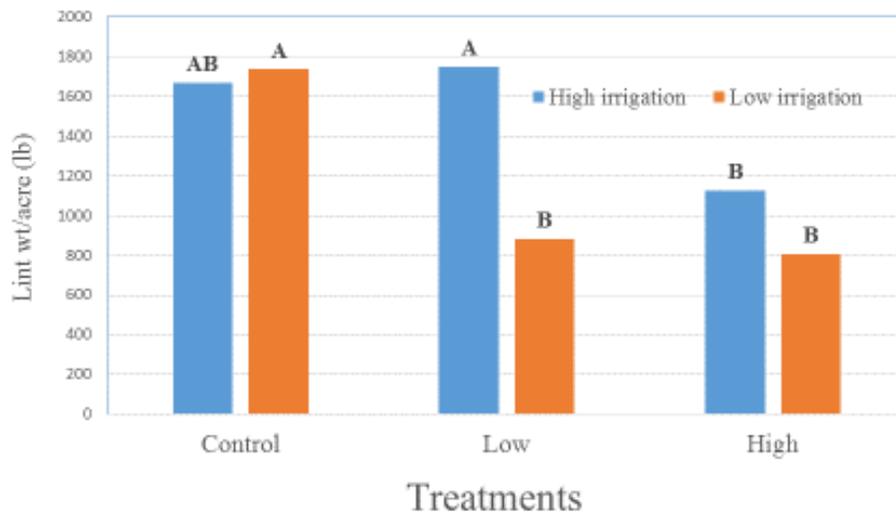
Figure 3. Average lint yield following a simulated acute infestation of cotton fleahoppers under high and low irrigation regimes, Lamesa, Texas, 2016.

FINAL REPORT
2014-2016

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, 16 July 2015, and 1 July 2016. The sub-plot treatment included two cotton fleahopper augmentation treatments [5 cotton fleahopper nymphs (2014 and 2016) 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%, 24-26%, and ~20% square loss in 201, 2015, and 2016, 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 were no maturity delay penalties in 2015 and 2016 due to warm, extended growing seasons. As expected, lint yield varied with N level regardless of the cotton fleahopper infestation in all three years. In uninfested control plots, lint yield displayed a characteristic staircase effect of nitrogen rate, with lowest lint yield in zero N and highest lint yield in 200 N treatments, with numerical increase in lint yield for each incremental nitrogen application of 50 lb/acre. Combined over all N treatments, the acute infestation of cotton fleahoppers rendered the lint yield reduction from 975, 910, and 1209 lb/acre in the uninfested control to 846, 877, and 976 lb/acre in fleahopper augmented treatments in 2014, 2015, and 2016, respectively. In all three 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 insect-induced pruning of undesirable fruit load (zero N) or compensation (200 lb N), whereas lint yield was significantly lower in fleahopper augmented 50 to 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. In addition, plants fully compensated for manually pruned 100% square removal at the onset of cotton flowering.

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, 12 January, 2015, and 4 March 2016. 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 and 2016 tests were planted to Fibermax 9180 B2F and FM1900GLT, respectively, 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 2015 and 16 May 2016. The 2016 test was replanted on May 27 due to poor crop stand. The post-emergence herbicides applications were made on 30 June (RoundUp® @ 32 oz/acre) and 29 July (Warrant® 3 pt/acre) in 2015 and 10 June and 7 July (Warrant® 3 pt/acre) in 2016 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. 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, 26 June 2015, and 1 July 2016 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, 16 July 2015, and 14 July 2016. Two (2014 and 2015) or three (2016) 10-ft. sections of uniform cotton were flagged in the middle two rows of each 16-row main-plot that served as insect treatment sub-plots. The sub-plot treatments included two cotton fleahopper augmentation treatments (5 cotton fleahopper nymphs per plant uncaged [2014 and 2016] 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. In 2016, manual removal of 100% squares around the onset of cotton flowering stage (28 July 2016) constituted the third sub-plot treatment. 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 2016; 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 20-25% 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, 21 July 2015 and 18 July 2016 by aspirating third-instar fleahopper nymphs (2014 and 2016) or adults (2015) 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 and 2016. Therefore, the control sections within each of the 25 plots did not receive supplemental insecticidal interventions until an Orthene® 97UP insecticide application was applied 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 (7 August 2014, 28 July 2015, and 28 July 2016). Plant mapping was conducted in fleahopper-augmented and control sections to estimate the level of fruit loss inflicted by the fleahopper augmentation treatments.

In all three 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 plant-specific fruit loss on 14 August 2014 and 6 August 2015. Complete, plant-specific square loss mapping in 2016 was compromised due to rainfall events, so an estimated fruit loss percentage was derived.

Additional data collected included monitoring of plant height, leaf chlorophyll content, leaf nitrogen content, and squaring patterns in all uninfested control plots to determine the N effect on plant growth and reproductive parameters (5 N rates x 5 replications), starting from the first week of squaring 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. For 2016, samples dates for these parameters were 27 July; 4, 11, 19, and 26 August; and 9, 23, and 29 September 2016. In-season plant mapping and plant height data from five randomly selected plants per plot were collected on 26 August 2014, 30 July 2015, and 27 July 2016. 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, 20 August 2015, and 6 October 2016, 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; 10, 13, 20, and 27 August; and 4 and 11 September 2015; and 19 and 26 August; and 9 September 2016. 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. The 2016 test was terminated by spraying a boll opener (Boll Buster® ½ quart per acre) and a defoliant [ET® (pyraflufen) 1.25 oz per acre] in a tank mix on 24 October, followed by an application of a desiccant (Bonedry® 3SL 1.3 quart per acre) to terminate the plants on 1 November 2016. Final plant mapping and harvesting of test sections were performed on 20 November 2014, 2 November 2015, and 17 November 2016 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 and 2016 (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 leaf N content, 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. 3). 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. 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. 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. In 2015 and 2016, 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. 3).

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, indicating a high degree of physiological stress on plants receiving zero pounds of augmented nitrogen (Fig. 4). 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 (Fig. 5). Leaf dry-weight values were generally increased with increase N augmentation rates, but the two lower N augmented treatments (50 and 100 lb/acre) had numerically (2015, 2016) or statistically 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 in 2014, but no treatment differences were detected in 2015 and 2016 (Fig. 6). 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 and 2016 eliminated the moisture stress in zero N plots during the boll development phase, resulting in no significant penetrability differences in bolls across all N treatments (Fig. 6).

Variation in soil residual N levels (Fig. 2), 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 expressions of N deficiency in zero or low N level treatments were reduced in 2015 and 2016.

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 and 2016) squares per plant across all N treatments. Total square density did not vary with N treatments prior to cotton fleahopper infestation (Figs. 7-8). 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. 7-8). 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. 7). In 2015, square abscission rates were similar at ~25% across all N treatments (Fig. 8). No biological or physiological reasons are speculated for reduced square abscission observed in the two highest N rate plots in 2014. The 2016 abscission rate was about 20% across all treatments (data not shown).

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. 9). 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. Both 2015 and 2016 crop seasons were 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. 10-11). 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 and 2016, 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, 910 lb/acre, and 1209 lb/acre in the uninfested control to 846 lb/acre, 877 lb/acre, and 976 lb/acre in fleahopper augmented treatments in 2014, 2015, and 2016, respectively. In all years, cotton lint yield was not significantly affected by ~25% fleahopper-induced square loss three weeks into squaring at both zero N and 150-200 lb/acre plots, either via pruning of undesirable fruit load (zero N) or compensation (150 and 200 lb N). On the other hand, lint yields were significantly lower in fleahopper augmented 50 and 100 lb/acre plots (2014), 100 lb/acre plots (2015), and 50 lb/acre plots (2016), 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.
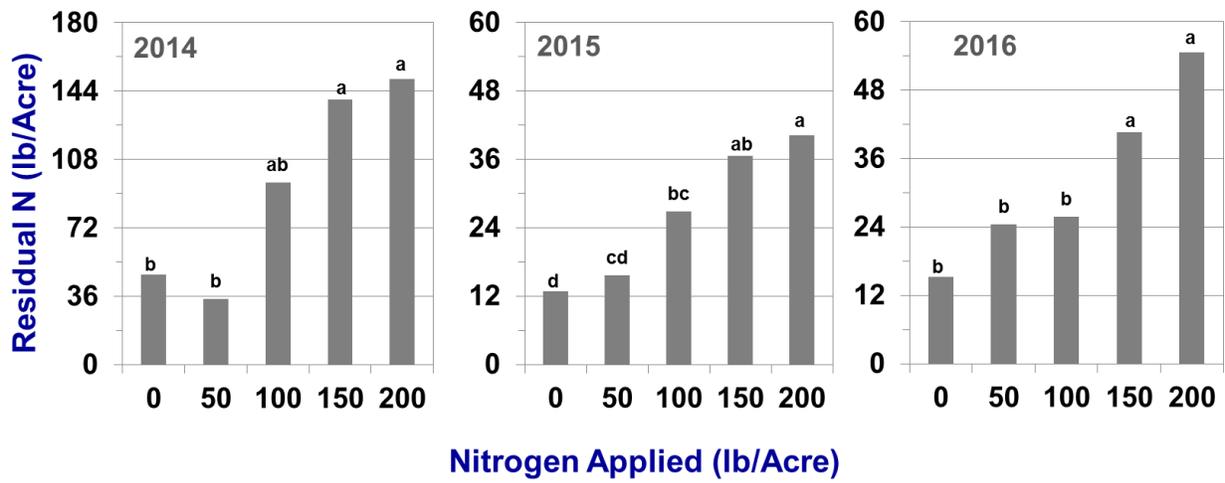
Figure 2. Effect of prior years' N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulation in the soil for the current crop year, 2014-2015.
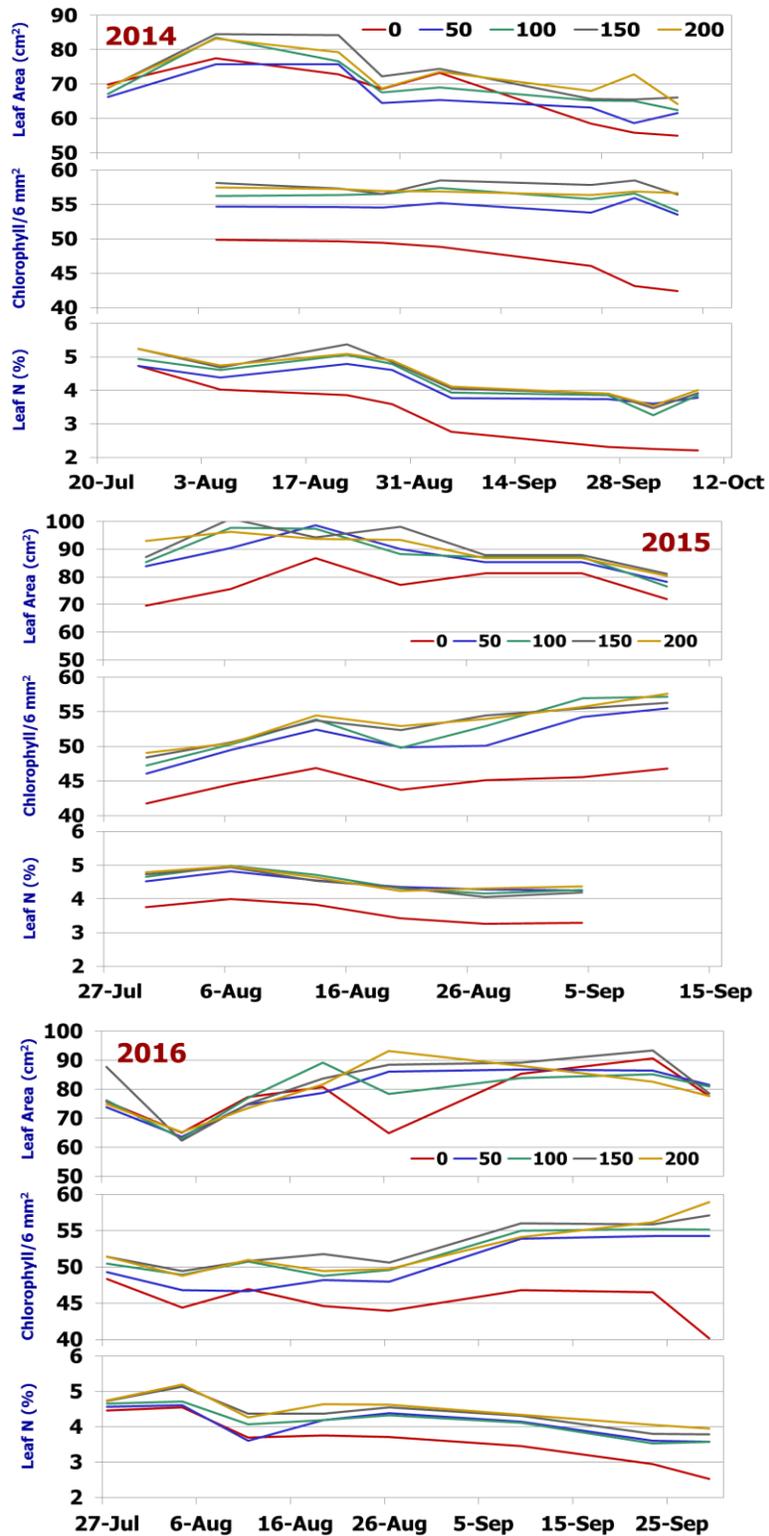
Figure 3. Temporal dynamics of leaf growth (leaf area), chlorophyll concentration, and percentage leaf nitrogen content measured on fifth mainstem leaf as influenced by the variable rates of augmented nitrogen (lb N/acre), 2014-2016.
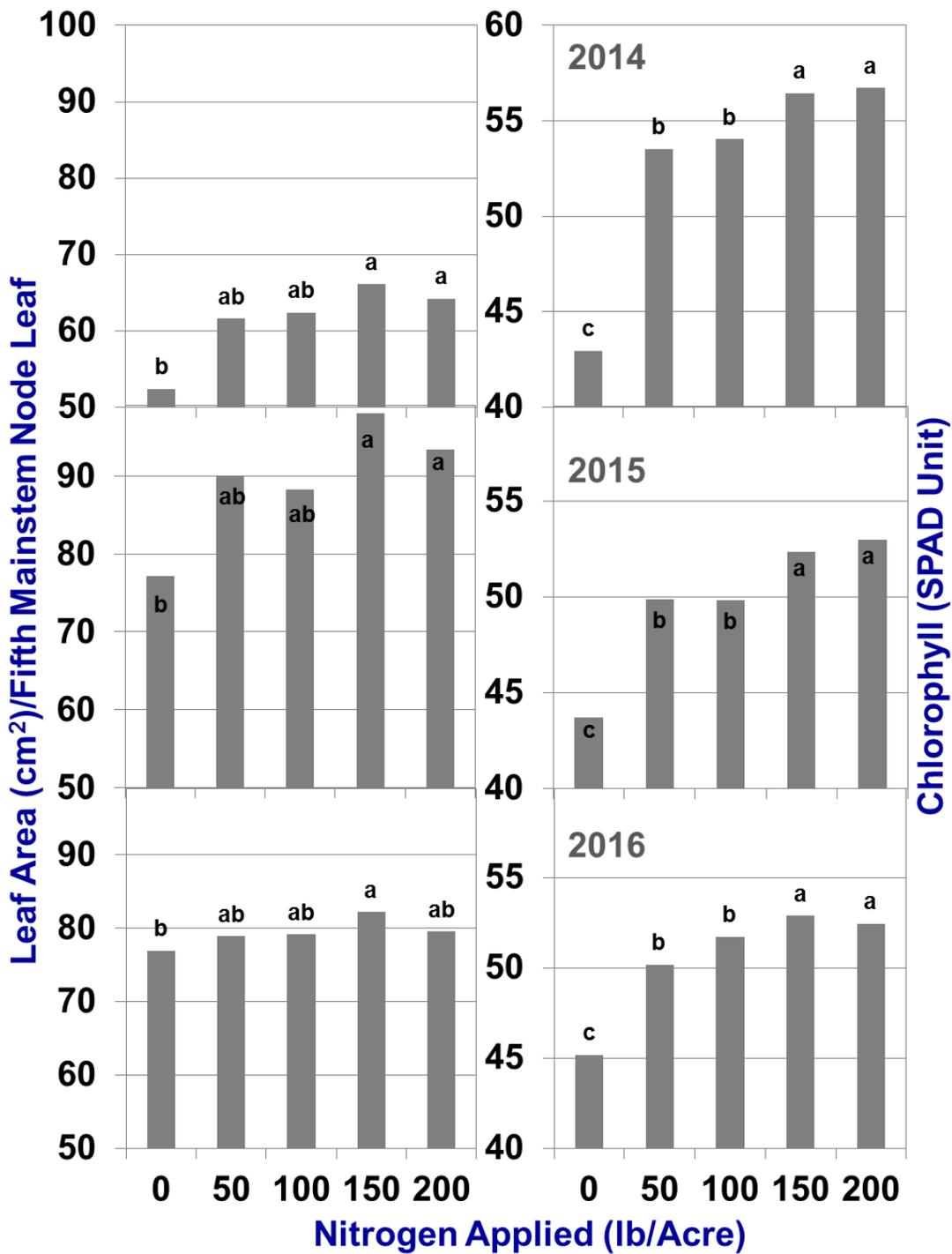
Figure 4. Average leaf surface area (left) and chlorophyll concentration or SPAD values (right) of the fifth mainstem node leaf, averaged over 6-8 weeks, 2014-2016.
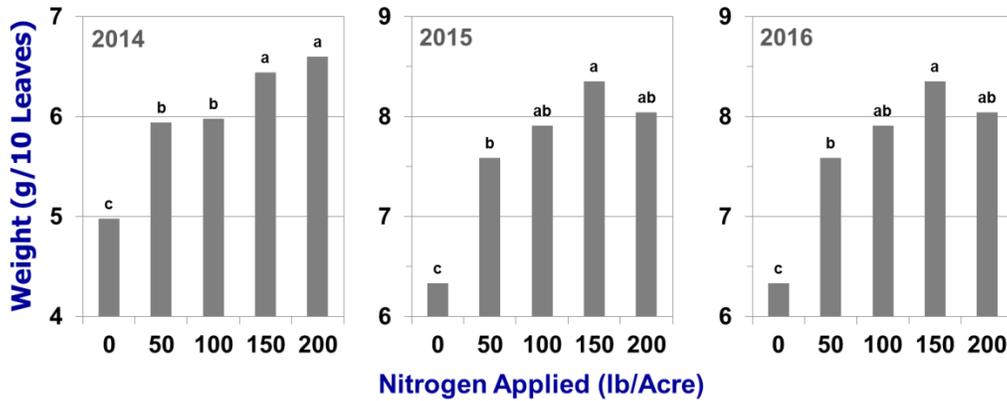
Figure 5. Effect of variable nitrogen on fifth mainstem leaf dry weight, averaged over 6-8 sample weeks during the cotton growing season, Hale Co., Texas, 2014-2016.
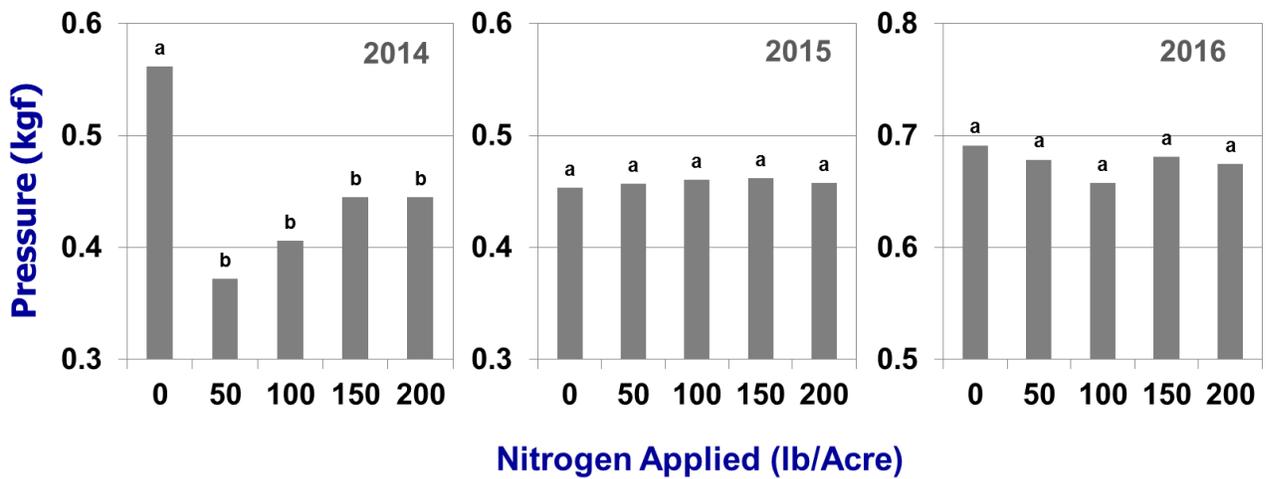
Figure 6. Effect of variable nitrogen on boll maturity as measured by the pressure required to puncture the carpel wall of the fifth mainstem node position bolls, September 26 (2014), August 20 (2015), and October 6 (2016).
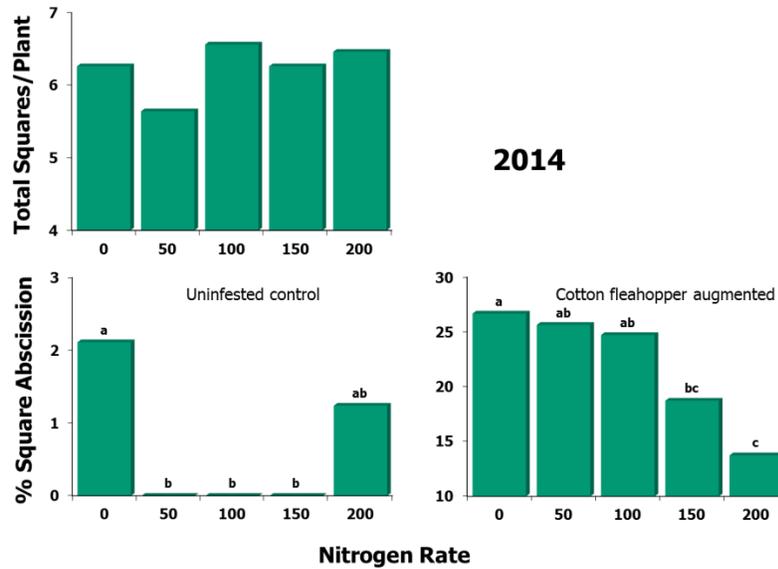
Figure 7. Total square density (number of squares set per plant) at the time of cotton fleahopper augmentation (top panel) and percentage square abscission (bottom panel) in control versus cotton fleahopper augmented treatments, as influenced by augmented variable rates of nitrogen application (0, 50, 100, 150, and 200 lb per acre), 2014, Hale County, TX.
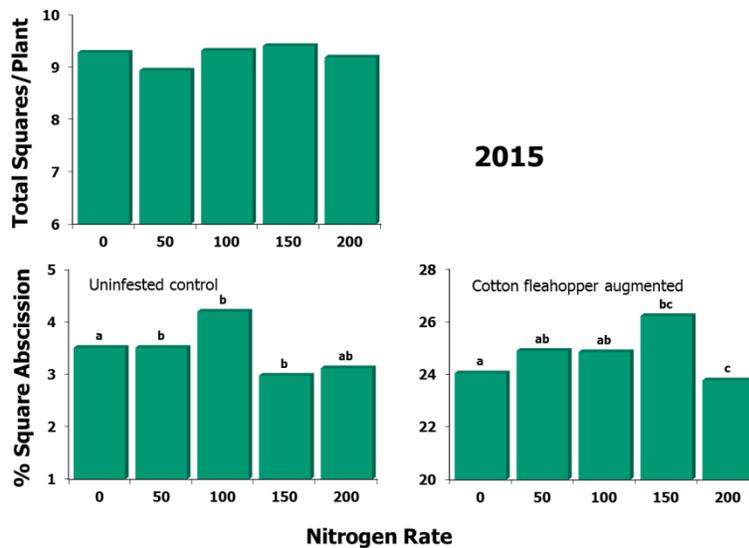

Figure 8. Total square density (number of squares set per plant) at the time of cotton fleahopper augmentation (top panel) and percentage square abscission (bottom panel) in control versus cotton fleahopper augmented treatments, as influenced by augmented variable rates of nitrogen application (0, 50, 100, 150, and 200 lb per acre), 2015, Hale County, TX.
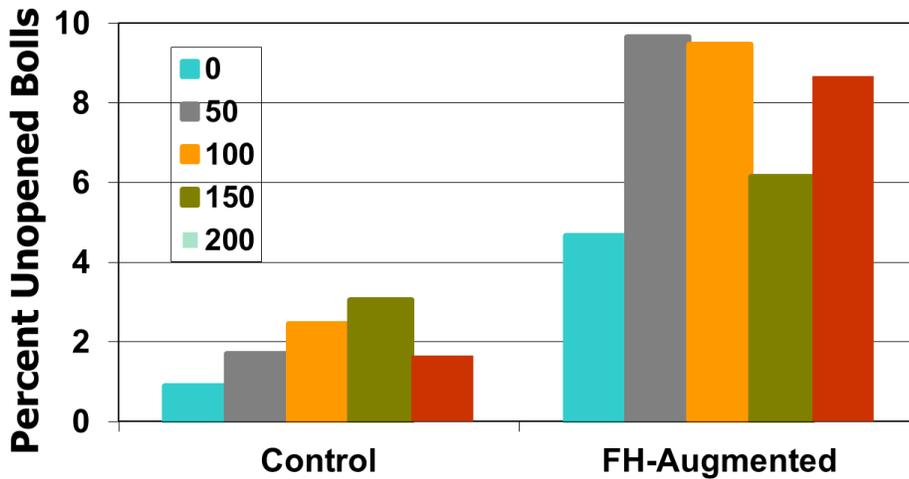
Figure 9. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on cotton maturity as measured by number of unopened (non-harvestable) bolls at harvest, November 20, 2014, Hale County, TX.
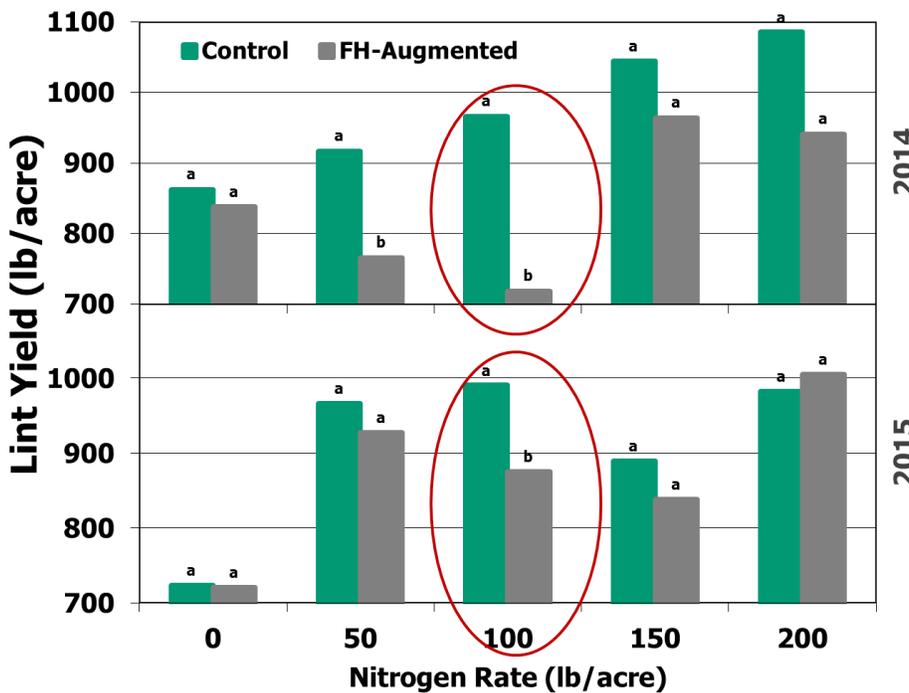
Figure 10. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on cotton lint yield following a single acute infestation of cotton fleahopper versus uninfested control, 2014-2015, Hale County, TX.
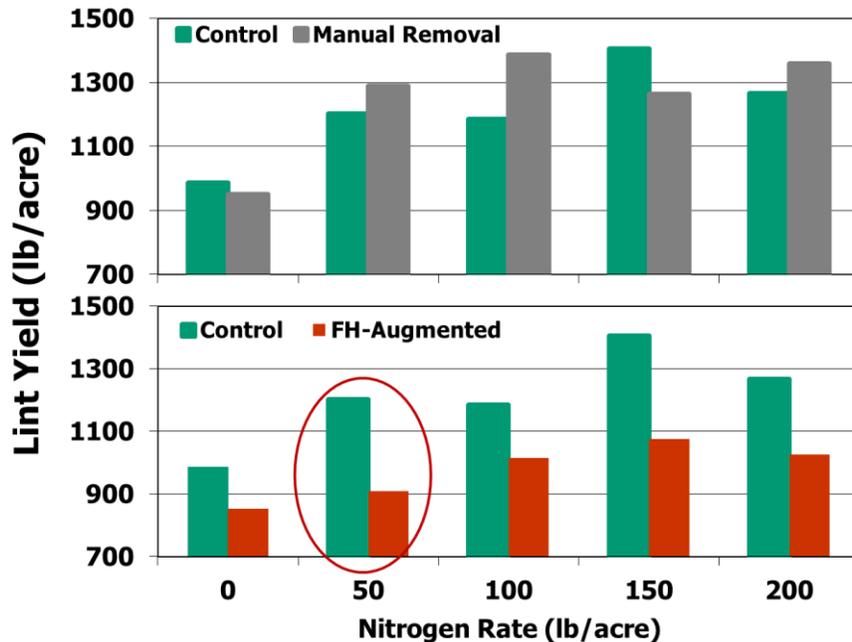
Figure 11. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on cotton lint yield following a manual removal of 100% of the squares up to the pre-flower stage of cotton versus control plants (above) and single acute infestation of cotton fleahopper versus uninfested control (below), 2016, Hale County, TX.

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ANNUAL REPORT
2016

Cotton Incorporated Core Program

Project Number: 16-354

Effect of *Lygus* on Drought-Stressed Cotton

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Effect of *Lygus* on Drought-Stressed Cotton

PROJECT SUMMARY

Western tarnished plant bug, *Lygus hesperus,* is the primary *Lygus* species inhabiting cotton and several other hosts in the Texas High Plains. Our previous studies have documented that several non-cotton hosts including alfalfa, sunflower, corn, grain sorghum, as well as weedy habitats along roadside bar-ditches and turnrows could impact *Lygus* severity in adjacent cotton. Our previous projects, supported by the Cotton Incorporated State Support Program, have generated significant information on the damage potential of adult and immature *Lygus* on maturing cotton bolls. A three-year field study has quantified the boll age (measured in terms of heat units from flowering) that is safe from *Lygus* damage. 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 4-year TSSC project (2012-2015) developed economic threshold-based management recommendations for *Lygus* in Texas High Plains cotton, which is expected to recommend a boll management threshold for early versus late season *Lygus* infestations.

While the Texas High Plains is fortunate to experience insignificant *Lygus* pressure in cotton during the recent years, the research on *Lygus* feeding behavior as it relates to low-input production systems in the Texas High Plains needs to continue. In particular, the characteristic low annual rainfall and decreasing irrigation water availability in the region has resulted in increased dryland cotton acreage. This project examined the feeding behavior and plant response to *Lygus* injury in relation to drought conditions. Drought-stress treatments included two irrigation levels (full irrigation versus dryland), each nested with two cotton cultivars (early maturing DP 1518 versus full-season DP 1044). Each irrigation x cotton maturity combination received two *Lygus* infestation levels [untreated control versus 2X threshold (high infestation)], each with four replications, resulting in a total of 32 plots.

Effect of drought-stress on *Lygus*-induced injury was more pronounced in DP 1518 (38.8% lint loss) compared to that in DP 1044 (28.2%), suggesting that DP 1518 may be more susceptible to *Lygus* injury under dryland or water-stressed conditions. Irrigated plots had significantly lower lint loss in both cotton cultivars due to *Lygus* feeding compared with that in dryland plots. Our preliminary results indicated that DP 1044 appeared to show lower sensitivity to *Lygus* injury under both dryland and irrigated conditions, but the impact was more pronounced under dryland conditions. This study is planned to be replicated in 2017.

Effect of *Lygus* on Drought-Stressed Cotton

INTRODUCTION

Western tarnished plant bug (WTPB), *Lygus hesperus,* is the primary *Lygus* species inhabiting cotton and several other crop and weed hosts in the Texas High Plains. Previous research indicates that WTPB is a pest of late-season cotton in the Texas High Plains. Regional survey work suggests that WTPB generally do not move from roadside weed habitats to cotton until late during the season as bolls mature, at which time roadside weeds decrease in prevalence or suitability. However, WTPB can be a significant economic pest of squaring and/or flowering cotton if they are forced to move into cotton in the absence of roadside weed habitats due to drought.

Due to utilization of underground water in excess of its recharge capacity and characteristic low rainfall in this semi-arid region, the Texas Southern High Plains has been facing some significant drought conditions in recent years. This has resulted in many of our cotton acreages going to dryland or limited-irrigation production. The shift in cotton production system from 60:40% irrigated:dryland to 40:60% in just the last 10-15 years has altered our input resources, cultivars, and management practices. It is generally expected that the drought-stressed plants would be significantly more impacted by insect injury than fully irrigated crops, but the drought-stressed plants would also likely have lower fruit load thresholds. However, a plant's ability to compensate for *Lygus*-induced crop damage may be significantly impacted by the drought-stress conditions, with potentially a low infestation rendering proportionately higher damage to the crop.

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 *Lygus* injury under a range of irrigation regimes remain uninvestigated. Plant bugs have a general inclination to attack the stressed plants and cause significant damage. The greater damage on stressed plants compared to healthy plants is partly due to the inability of plants to physiologically react to the injury. Thus, it is expected that the drought-stressed plants would be more vulnerable to *Lygus* injury than unstressed plants. However, the fruit-load threshold of a cotton plant is also dependent on soil moisture availability, among several other input and management factors. There is no information on how *Lygus* feeding behavior will be impacted under various irrigation regimes and how the plants would respond to varying levels of *Lygus*-induced injury under drought conditions. Similarly, cotton cultivars respond differently to various moisture stress conditions and the interactive effect of *Lygus* injury, phenological attributes of cotton cultivar, and drought conditions are unknown. The overall goal of this study was to characterize the effect of drought conditions on *Lygus* infestation/feeding behavior and plant response to *Lygus* injury.

METHODOLOGY

The study was conducted in a multi-factor split-plot randomized block design with four replications (blocks). Drought-stress parameters included two irrigation levels (full irrigation versus dryland) that served as main plot factors, whereas two cotton cultivars (early maturing versus full-season) were used as subplot factors to create an interaction of cultivar maturity and drought-stress situations to mimic the Texas High Plains (THP) scenario during dry summers. The full irrigation water level was created via 100% replenishment of evapotranspiration (ET)

requirement for THP, whereas the dryland treatment received no supplemental irrigation. Two cotton cultivars included in the study were DP 1518 (short-season) and DP 1044 (full-season), planted on May 25, 2016. Each irrigation treatment (2) x cotton maturity (cultivar type) treatment (2) received two *Lygus* infestation levels [untreated control, 2X threshold (high infestation)], each with four replications, resulting in a total of 32 plots.

Lygus density treatments were applied on one 3-ft cotton row section per plot on August 11. For insect release plots, a single release of *Lygus* adults (5 adult *Lygus* per plant, resulting in 1 bug per plant after 80% field mortality) was timed to simulate the acute infestation of *Lygus* while cotton was at peak flowering/boll development stage. Multi-plant (7 plants) cages were used to contain the released adults (Fig. 1). The control plots were flagged and sprayed with insecticides. Two weeks after the deployment of insect release treatments, all experimental plots were sprayed with insecticide Orthene to ensure that the released insects were removed. Two plants from each treatment were removed on August 30 and processed for *Lygus* damage assessment. Variables including number of fruits aborted and internal/external damage to developing bolls were measured. Pre-harvest plant mapping was conducted on October 29 and crop was hand-harvested on November 5 and ginned on a tabletop gin. Hand-harvested yield samples will be sent to Cotton Incorporated for fiber quality analysis.

Figure 1. A and B) Multi-plant cages used to release *Lygus* on cotton, C) Examination and data collection from the test site.

RESULTS

As expected, higher numbers of internal warts were observed in bolls collected from *Lygus*-infested plants compared to that in control plots (Fig. 2). Lygus appeared to cause greater damage to dryland-grown plants compared to that in full irrigation plots. It is somewhat interesting to note that the dryland plots received greater boll injury while the bolls in dryland plots are expected to possess tougher carpel wall. It is possible that the water-stressed bolls are more sensitive to *Lygus* feeding injury.

Averaged across the water level and cultivar treatments, total boll density on *Lygus*-infested plants was lower (2.27 bolls per plant) compared to that on uninfested control plants (3.2 bolls per plant) two weeks after *Lygus* infestation (Fig 3), suggesting possible abortion of small bolls due to *Lygus* feeding. Within varieties, DP 1518 had slightly more bolls compared to DP 1544 (Fig. 4), but this difference was not statistically significant.
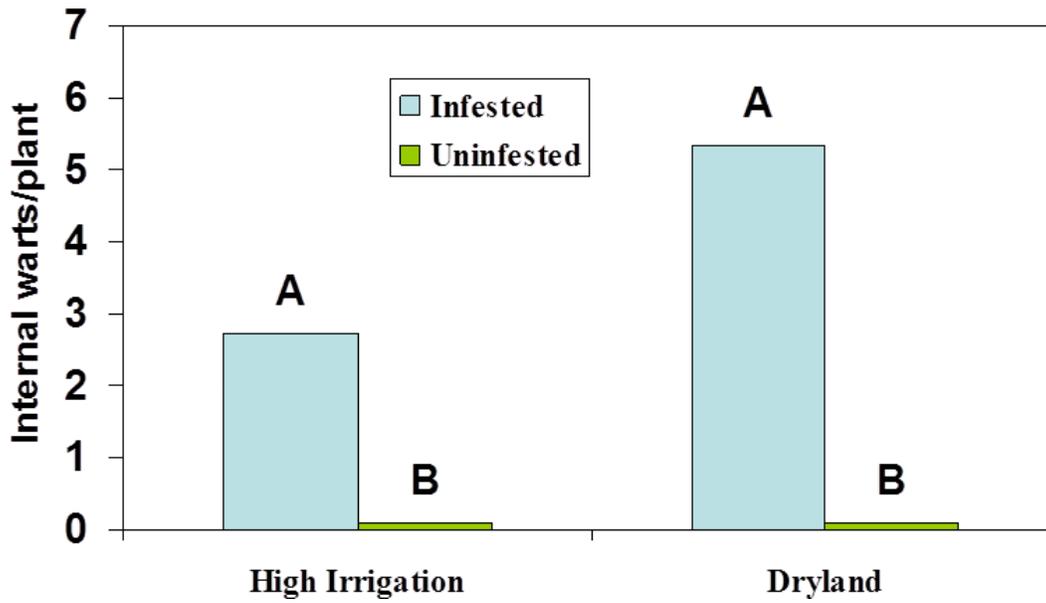
Figure 2. Internal injury warts in developing bolls caused by *Lygus* feeding on plants grown under full irrigation versus dryland, Lubbock TX, 2016.
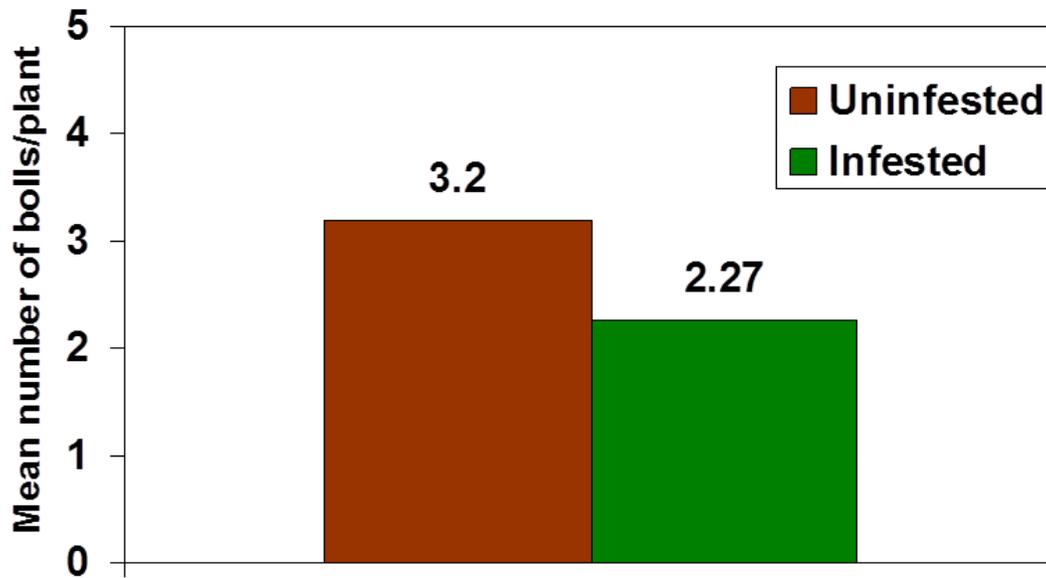
Figure 3. Mean number of bolls per plant following *Lygus* infestation treatment, averaged across water level treatments and cultivars, Lubbock, Texas.
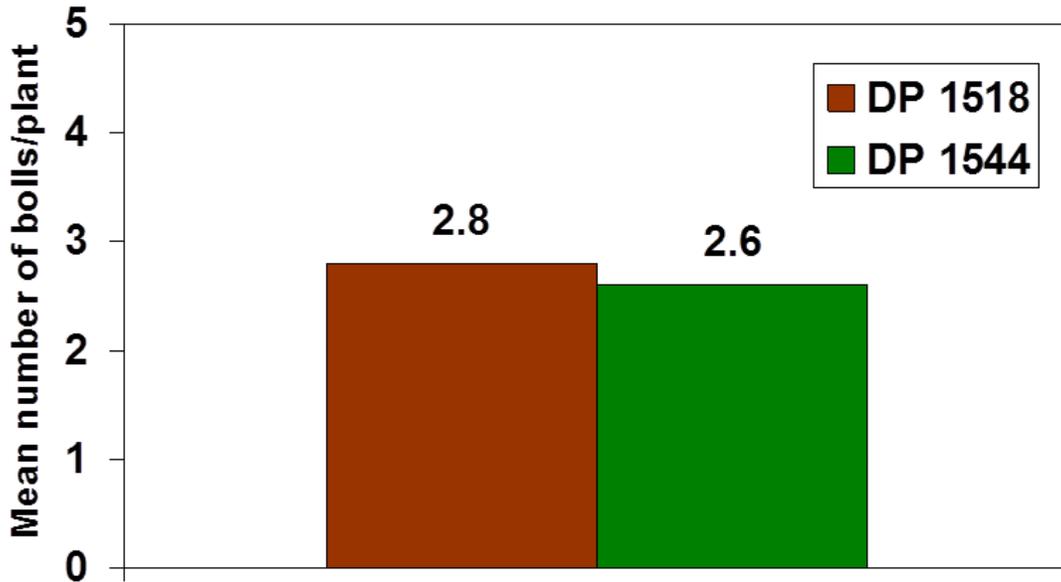
Figure 4. Boll density in early-season versus full-season cotton varieties, Lubbock, Texas.

Averaged across cultivars and irrigation treatments, no significant difference in lint yield was observed between *Lygus*-release treatments and non-release control treatments. However, drought-stress induced significantly greater impact of *Lygus* injury on cotton lint yield. *Lygus* injury caused 34.83% lint yield loss in dryland cotton compared to only 11.3% loss in irrigated cotton (Fig. 5), suggesting a reduced *Lygus* injury sensitivity on full irrigated cotton compared to that in water-stressed production situation.
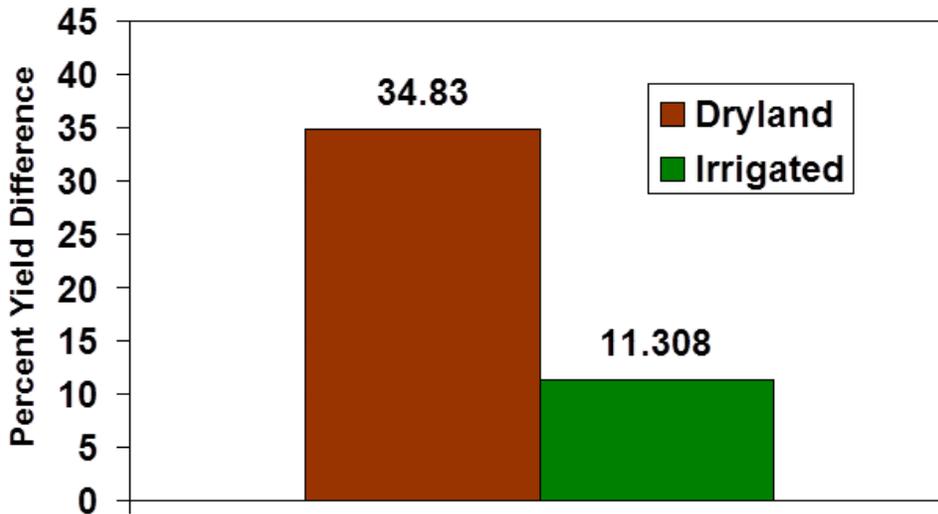
Figure 5. Effect of *Lygus* bugs on lint yield of cotton under dryland and irrigated production conditions, Lubbock, TX, 2016.

Lygus injury sensitivity varied between cultivars. While no significant difference in total lint yield was observed between the two cotton cultivars evaluated, *Lygus*-induced lint yield reduction was significantly greater (28.8%) in DP 1518 compared to 17.3% in DP 1044 (Fig. 6). Additional investigations will be conducted in upcoming years to characterize the detailed behavior of this phenomenon.
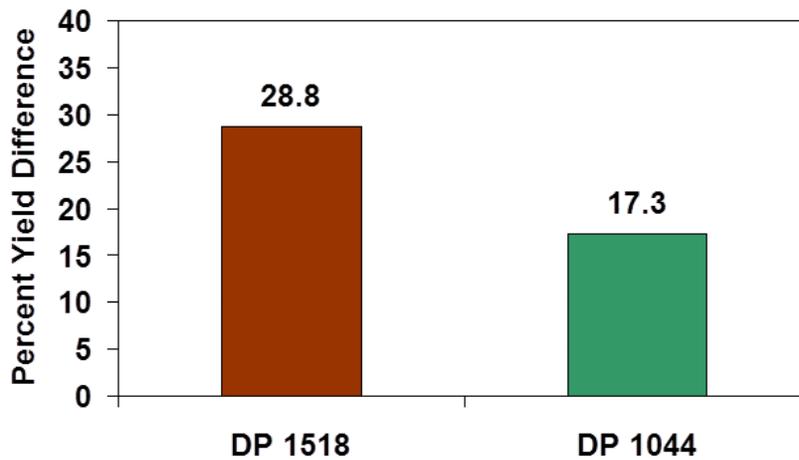
Figure 6. Cotton varietal response to *Lygus* infestation in Texas High Plains.

Effect of drought-stress was more pronounced in DP 1518 (38.8% lint loss) compared to that in DP 1044 (28.2%) (Fig. 7), suggesting that DP 1518 may be more susceptible to *Lygus* injury under dryland or water-stressed conditions. Irrigated plots had significantly lower lint loss in both cotton cultivars due to *Lygus* feeding compared with that in dryland plots (Fig. 7). Our preliminary results indicated that DP 1044 appeared to show lower sensitivity to *Lygus* injury under both dryland and irrigated conditions, but the impact was more pronounced under dryland condition. This study is expected to be replicated in 2017.
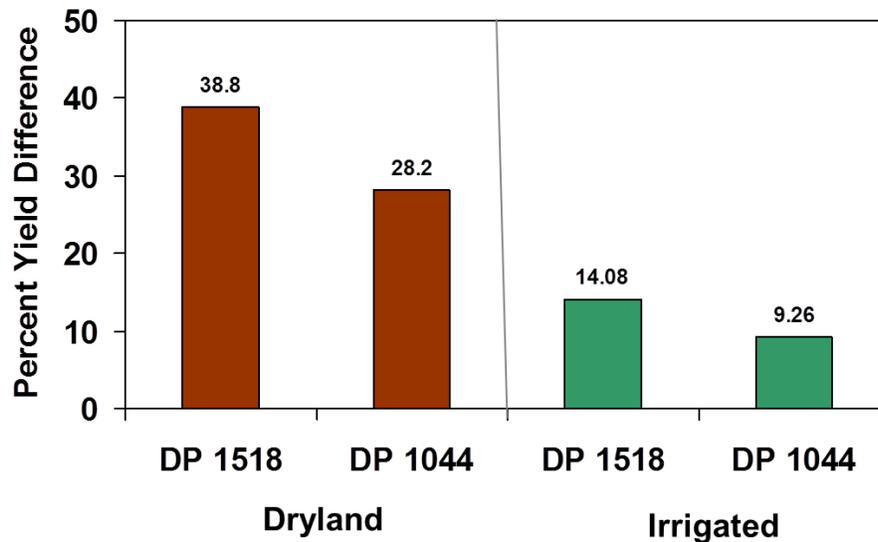
Figure 7. Percentage yield losses due to *Lygus* infestation under dryland versus irrigated production conditions, Lubbock, Texas, 2017.

ANNUAL REPORT 2016

Cotton Incorporated Core Program

Project Number: 16-272TX

Monitoring the Old World Bollworm, *Helicoverpa armigera*, in Texas toward Developing Potential Management Strategies

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Monitoring the Old World Bollworm, *Helicoverpa armigera*, in Texas toward Developing Potential Management Strategies

Project Summary

An ongoing study has been conducted in the Texas High Plains to investigate the seasonal moth flight activity patterns of *Helicoverpa* spp. and to possibly detect the presence of the 'Old World' bollworm (OWB, *H. armigera*), if it has already been introduced into the Texas bollworm population. The primary objectives of the 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. Trap type x pheromone lure combination treatments were deployed in mid- to late July each year, followed by all traps being monitored and the captured moths counted approximately weekly through mid-November. All traps were re-baited with fresh lures approximately every two weeks. Sub-samples of up to 25 moths per trap per sample date are currently in the process of being dissected to determine if the Texas High Plains moth populations contained any *H. armigera*. Trap x lure efficiencies in capturing *Helicoverpa* spp. are discussed. Our current hypothesis is that *H. armigera*, Old World bollworm, invasion has not occurred in Texas. In the absence of *H. armigera*, it is therefore, impossible to determine which lure type and/or lure vendor has the best pheromone lure formulation for attracting *H. armigera*. Among the five selected experimental treatments, the Texas Traps baited with Trécé™ *H. armigera* lure captured the highest number of *Helicoverpa* spp. moths during both 2015 and 2016. During 2016, the *H. armigera* baited traps with the USDA Cooperative Agricultural Pest Survey (CAPS) lures captured very few *H. zea* moths, but caught an equal or greater number of tobacco budworm moths [*Heliothis virescens* (F.)]. The Trécé™ (*H. zea* and *H. armigera*) lure baited traps did not attract tobacco budworm moths, yet both Trécé™ species-specific lures captured numerous *H. zea* specimens. A total of 1,252 moths from Trécé™ and USDA CAPS *H. armigera* lure baited traps have been dissected to date. Based upon these initial dissections, we do not believe that the *H. armigera* has been introduced to the Texas High Plains. All dissected male moths appeared to be *H. zea* specimens.

Introduction

The Old World bollworm (OWB), *Helicoverpa armigera*, is a polyphagous pest, feeding on a wide range of crop and non-crop plant hosts. Its global distribution spans Europe, Asia, Africa, Oceania, and South America. During 2014, *H. armigera* was detected in Puerto Rico and Costa Rica, and then on 17 June 2015, one male moth was collected in a pheromone trap in Bradenton, FL. It is anticipated that this pest will invade the southern U.S. in the very near term and some entomologists have speculated that the invasion has already occurred. Ecological niche modeling indicates that the majority of the U.S. is a suitable habitat for the permanent establishment of reproductive OWB populations. Therefore, the current OWB issue in Texas is a rigorous anticipatory survey.

This continuing Texas High Plains study is being conducted to investigate the seasonal moth flight activity patterns of *Helicoverpa* spp. captured on two different trap designs (Fig. 1) and pheromone lures, obtained from two sources, specifically designed to trap *H. zea* or *H. armigera*. It should be noted that *H. zea* moths commonly respond to *H. armigera* pheromone baited traps and the two species are difficult to distinguish from each other without genetic testing or dissecting the adult males.

The study objectives were to: 1) Investigate the effectiveness of *H. armiger*a and *H. zea* pheromone lures obtained from two sources [Trécé™, Inc. (both species); USDA CAPS (*H. armigera* lures only)], 2) Determine the efficiency of two different trap designs ('Texas Trap' vs. green 'Bucket Trap') in capturing *Helicoverpa* spp. moths, and 3) Perform dissections of seasonal male adult sub-samples of *Helicoverpa* spp. captured on *H. armigera* pheromone baited traps in order to possibly detect Old World bollworm sightings in Texas bollworm moth populations.

Materials and Methods

Survey area for the study included four trapping sites situated in a west-to-east orientation along Texas FM1294 in northern Lubbock County, TX (Fig. 2). Five selected experimental treatments included: 1) 'Texas Trap' baited with Trécé™ *H. zea* lure, 2) 'Texas Trap' with Trécé™ *H. armigera* lure, 3) 'Bucket Trap' (green) with Trécé™ *H. zea* lure, 4) 'Bucket Trap' (green) with Trécé™ *H. armigera* lure, and 5) 'Bucket Trap' (green) with USDA CAPS *H. armigera* pheromone lure. Each treatment was represented at each trapping site, including five treatments and four sites (replications) deployed in a randomized block design.

Figure 2 also displays the yearly trapping periods for 2015 and 2016, typically deploying the traps during mid- to late July with monitoring extending until mid-November annually. Plans include an identical test to continue in 2017. Traps were inspected weekly and re-baited at two-week intervals. All captured moths were counted, placed into Zip-Loc™ bags, and then samples were placed into a freezer for species identification dissections at a later date.
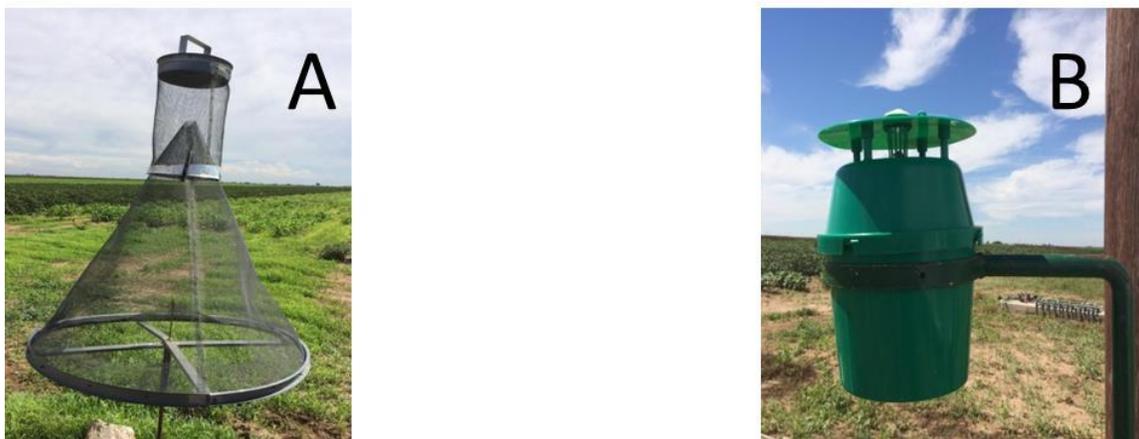
Figure 1. Two trap designs, 'Texas Trap' (A) and green 'Bucket Trap' (B), deployed at four Lubbock County sites, 2015-2016.

Lubbock County, TX trapping sites (4 total)

Texas High Plains: World's most concentrated cotton production region

Three-year study
2015 – Late July to early November
2016 – Mid-July to early November
2017 – To be deployed in mid-July 2017
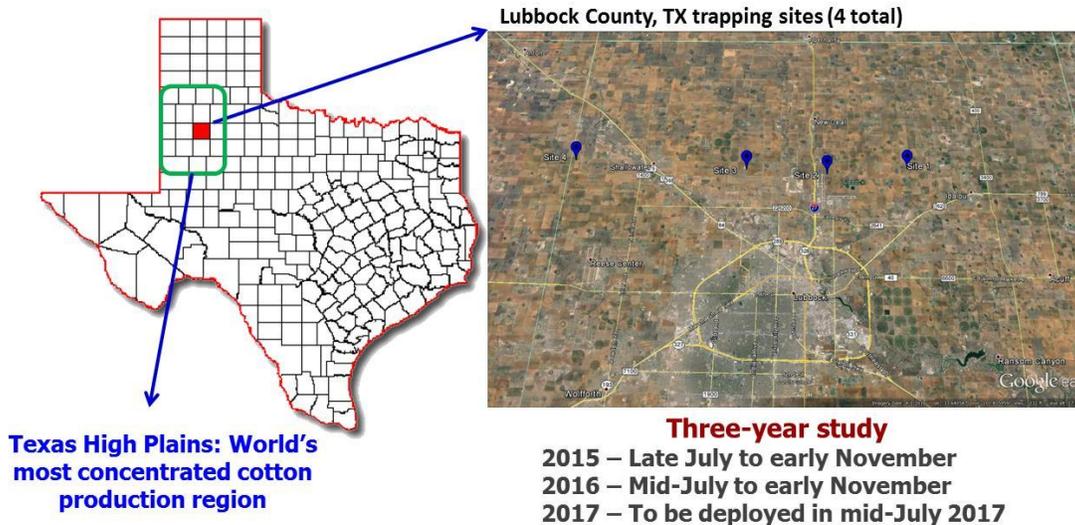
Figure 2. Trapping study sites utilizing two trap designs and two species-specific pheromone lures from two sources. Five traps (one per treatment as listed in section above) were deployed at each of the four Lubbock County sites.

Results and Discussion

'Texas Trap' with Two Associated Pheromone Lure Treatments

The Trécé™ *H. armigera* and Trécé™ *H. zea* lure baited Texas traps yielded 2015 seasonal weekly captures of 119 and 83 bollworm moths per trap, respectively; while during 2016, similar seasonal weekly moth capture averages of 110 and 80 were observed (Figs. 3 and 4). Overall, it should be noted that among the five study treatments, the Texas Traps baited with Trécé™ *H. armigera* lure captured the highest number of *Helicoverpa* spp. moths during both 2015 and 2016 (Figs. 3, 4 and 5). Because *H. zea* cross-responds to *H. armigera* lure, it appears that the Trécé™ lure that is designed for *H. armigera* is as much or more attractive to *H. zea* (Figs. 3, 4, and 5).
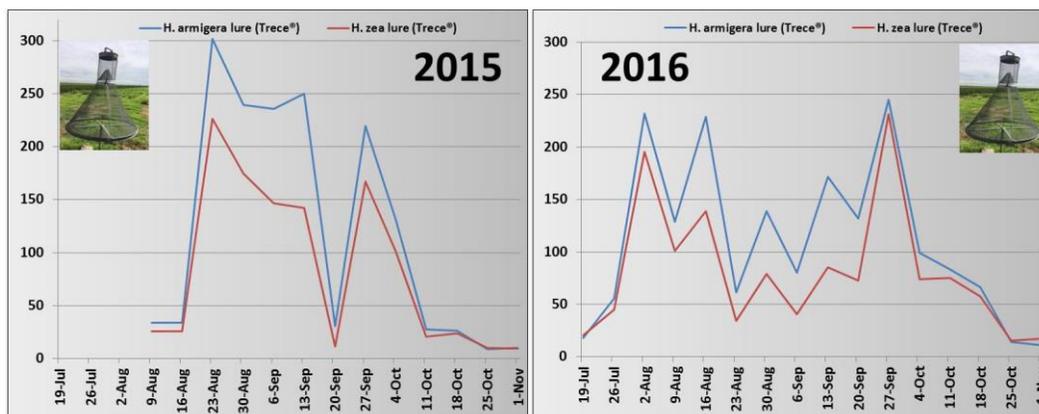
Figure 3. Texas Traps [a.k.a., Texas Pheromone Trap, TP Trap or Hartstack Trap (Hartstack et al. 1979)]: Weekly *Helicoverpa* spp. male moth captures during 2015 (left) and 2016 (right) on 'Texas Traps' baited with *H. zea* or *H. armigera* Trécé™ pheromone lures.
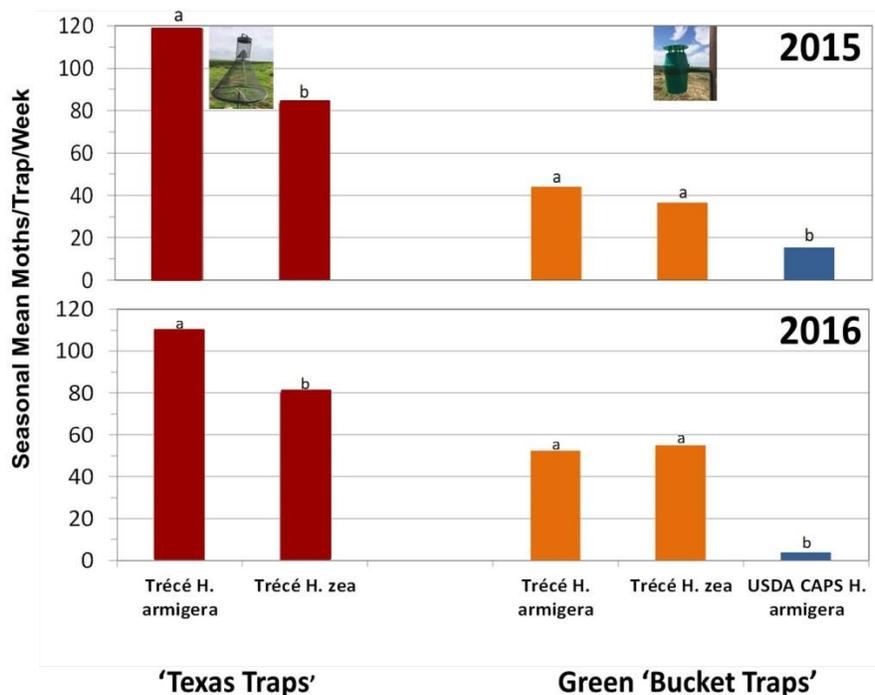
Figure 4. Seasonal mean number of *Helicoverpa* spp. male moths captured per week per trap on Texas Traps (red bars) baited with Trécé™ *H. armigera* and *H. zea* pheromone lures. Likewise, the two orange bars indicate weekly means for green Bucket Traps baited with Trécé™ *H. armigera* and *H. zea* lures. The blue bar illustrates the seasonal weekly means for green Bucket Traps baited with the USDA CAPS *H. armigera* lures. Seasonal means within each trap type indicated by different lowercase letters indicate statistical difference between these means.

Green 'Bucket Traps' with Three Associated Pheromone Lure Treatments
Overall, green bucket traps baited with the Trécé™ *H. armigera* and *H. zea* lures yielded lower numbers of bollworm moths than the Texas Traps, yet overall peak trap response periods were observed on both trap designs similarly (Figs. 3, 4 and 5). The Trécé™ *H. armigera* and Trécé™ *H. zea* lure baited green bucket traps yielded 2015 seasonal weekly moth captures of 44 and 36 bollworm moths per trap, respectively, reflecting the same general moth activity trend as observed from the Texas traps (Figs. 3 and 4).

During 2016, a slightly different numerical trend was observed in which the Trécé™ *H. zea* lure baited traps captured a seasonal mean of 55 moths per trap, whereas the Trécé™ *H. armigera* lure captured slightly lower moth numbers (although not statistically different) at 52 moths per trap (Fig. 4).

What should be noted is that the moth captures on the USDA CAPS baited green bucket traps did not reflect the same moth trap response activity patterns of the other four treatments which utilized lures obtained from Trécé™, Inc. Figures 4 and 5 clearly illustrate that the moth numbers were much lower and only the early season peak trap responses were slightly reflected by USDA CAPS lure as compared to the other pheromone lure treatments. While *H. armigera* lure is expected to cross-capture *H. zea*, USDA CAPS lures were designed to be more sensitive toward

H. armigera compared to commercially available *H. armigera* lure. At the present time, *H. armigera* does not appear to be in the Texas High Plains bollworm population (see below in *Identification* section), therefore it is impossible to determine which lure type and/or lure vendor has the best pheromone lure formulation for attracting *H. armigera*.

During 2016, the traps baited with the USDA CAPS lures were observed to also capture tobacco budworm [*Heliothis virescens* (F.)] moths, while the Trécé™ (*H. zea* and *H. armigera*) lure baited traps did not attract tobacco budworm moths. In fact, traps baited with CAPS lure captured significantly greater abundance of tobacco budworm moths than *Helicoverpa* spp. For instance, during the 11-week trapping period of 18 August to 4 November, the four USDA CAPS lure baited traps captured a total of 170 tobacco budworm moths, while during the same time period these traps captured only 58 *Helicoverpa* spp. moths.
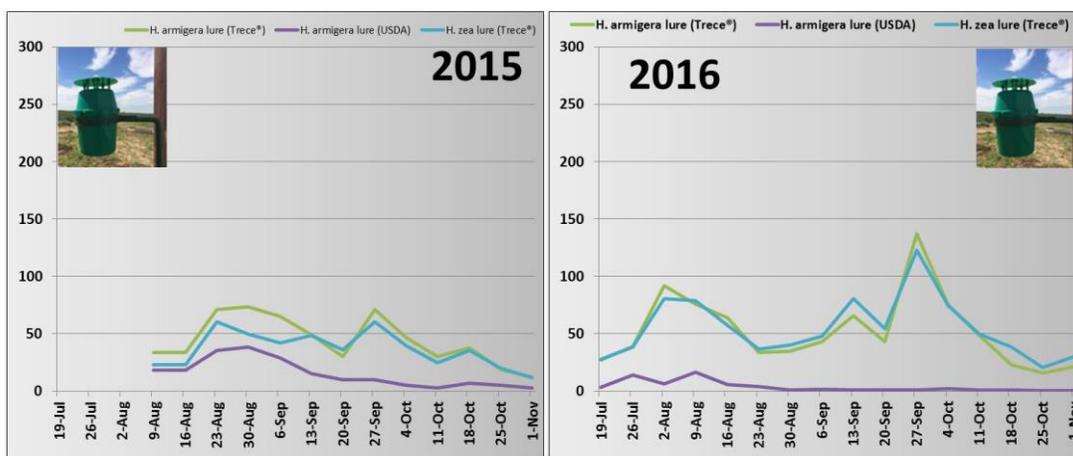
Figure 5. Green 'Bucket Traps': Weekly *Helicoverpa* spp. male moth captures during 2015 (left) and 2016 (right). Traps were baited with *H. zea* or *H. armigera* Trécé™ pheromone lures, and *H. armigera* USDA CAPS lure.

Dissections to Determine *Helicoverpa* spp. Identifications

A total of 1,252 moths from Trécé™ and USDA CAPS *H. armigera* lure baited traps have been dissected to date. Based upon these initial dissections, we do not believe that the 'Old World' bollworm' (*H. armigera*) has been introduced to the Texas High Plains. All dissected male moths appeared to be *H. zea* specimens. More Texas High Plains specimens are yet to be dissected, along with some samples from South Texas (Hidalgo County location). We plan to repeat this survey in 2017 with two locations from South Texas added to the 2016 study.

FINAL REPORT
2013-2016

Cotton Incorporated Texas State Support Committee Program

Project Number: 13-456TX

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.

Both 2015 and 2016 growing seasons were marked by 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 in both years. Lint yield did not vary across treatments, suggesting that the cotton crop is able to compensate for the low level thrips-induced crop injury at the seedling stage. 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.

2015 Study

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, 2015.

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.

2016 Study

Tests were planted May 25, June 7, and June 13 at Halfway, Cotton Center, and Chillicothe, respectively. Thrips populations subjected to various foliar insecticide treatment regimes and thresholds were monitored. The individual experimental plots were 4 rows by 50 feet. 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 leaf stage, 6) foliar treatments based on the current action threshold, and 7) foliar treatments based on 50% of the current action threshold. Orthene 90S at a rate of 3.2 oz/acre was used for all foliar applications. Plots were sampled and treatments were applied on June 16 at the Cotton Center location. Thrips populations were sampled once at all three locations (June 6, 16, and 24 in Halfway, Cotton Center, and Chillicothe, respectively) and treatments were applied on the same day. Thrips were sampled via thrips washing technique. The Cotton Center location was hailed out after the first sampling and the test site was lost. Similarly, the Chillicothe location faced unusual rain events followed by seedling disease and the test was abandoned. Three additional samplings were performed at the Halfway site (June 16, 22, and July 1), followed by treatment applications.

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

2015 Study

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.

2016 Study

The 2016 study was conducted at the two 2015 study locations, Halfway (Texas High Plains) and Chillicothe (Rolling Plains). Cotton cultivar 'FM 1900 GLT' treated with imidacloprid or thiamethoxam (seed treatment) or the 'base' seed (foliar treatment) was planted on May 10 and June 13 at Halfway and Chillicothe, respectively. The Chillicothe site was sampled on June 24 and July 7, but the test was severely compromised due to frequent rain events during the seedling stage. However, the study was continued and harvested the test plots for lint yield. The Halfway location received a significant hailstorm and damaged the plants, but the study was continued and the samples were taken for four weeks post-emergence (May 17, June 6, 16, and 22). Adult and immature thrips were sampled via whole-plant sampling technique (5-plant sample). Collected plant samples were processed to determine the thrips densities. Test plots were harvested November 22 (Halfway) and December 14 (Chillicothe) and samples were ginned to determine lint yield.

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.

As noted previously, the 2016 cotton growing season also received unusual rainfall events during early plant growth period, resulting in insignificant thrips colonization. The entire Halfway test plots had only five thrips specimens collected whereas only two thrips were recorded from the Chilliothe site. Therefore, the study did not allow us to examine the economic value of the various foliar application treatments.

Because the thrips densities were very low (Halfway) to non-existent (Chillicothe) in both years, lint yield did not significantly vary with foliar application treatments that were targeted toward thrips population suppression (Fig. 2). However, lint yield in 2016 varied across foliar application treatments without reasonable explanation (Fig. 3). Lint yield was lower at Halfway compared to that at Chillicothe across all treatments in 2015. Lint yield data from Chillicothe are not reported for 2016 due to failure of the test at this location.

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. 4). 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 in 2015 (Fig. 5). However, in 2016, cotton vigor, measured in terms of total leaf area, was significantly higher in the two neonicotinoid seed treatment applied plots compared to other treatments (Fig. 5). These data suggest that the seed-applied insecticide treatments may have some agronomic benefit to seedling growth even in the absence of thrips injury. Thrips populations did not develop at the Chillicothe site.

Lint yield did not vary across treatments at the Chillicothe site in 2015 where no thrips infestations occurred. Lint yield was significantly lower in imidacloprid treatment plots compared to that in thiamethoxam plots at Halfway in 2015 (Fig. 6), 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. The 2016 yield at Halfway varied numerically with treatments, but there were no significant differences (Fig. 7).
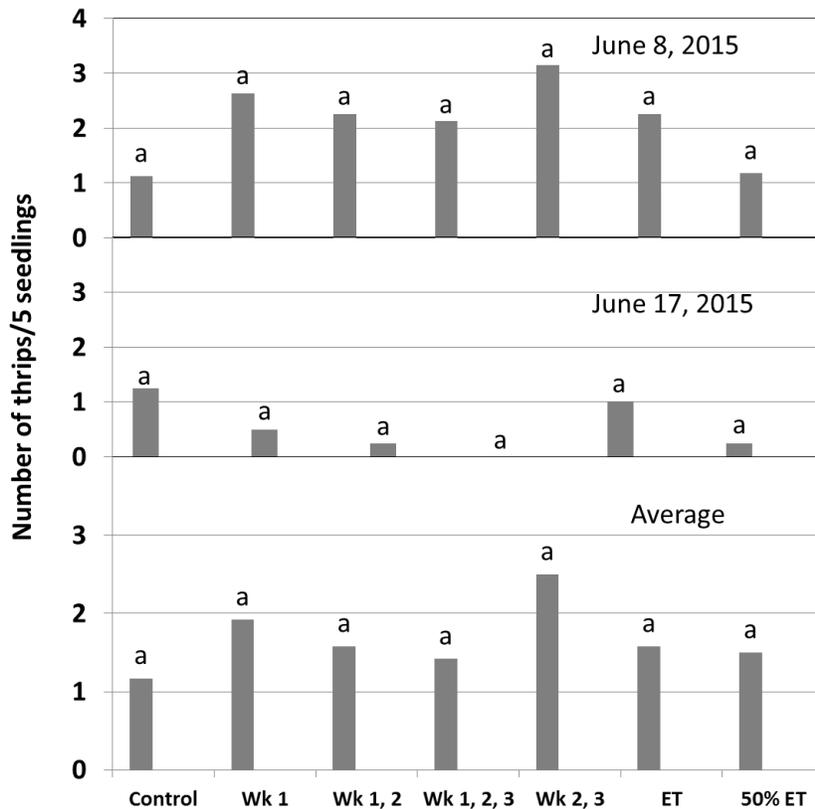
Figure 1. Number of thrips per 5-plant samples at two sampling dates and seasonal average thrips densities at Halfway as affected by foliar application of Orthene® 97UP at different application frequencies in managing thrips in seedling cotton, 2015.
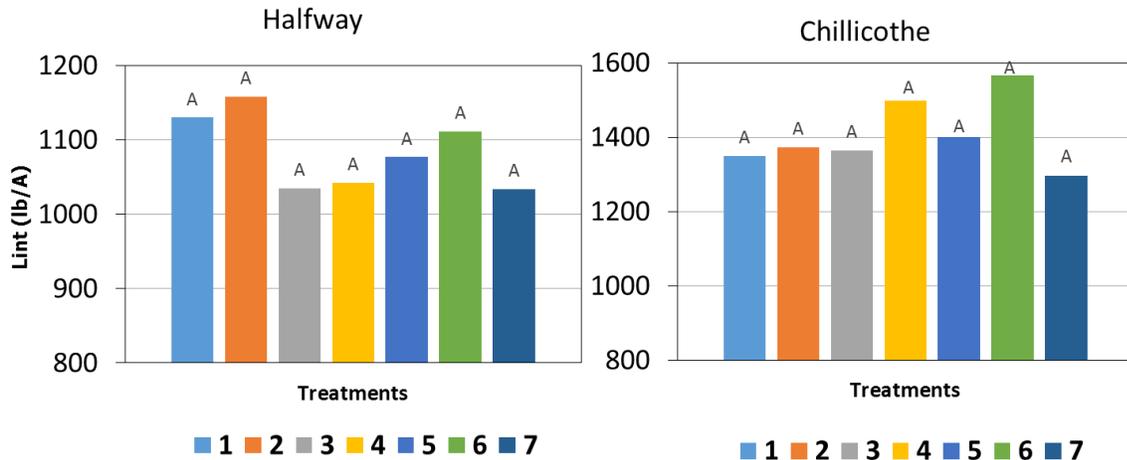
Figure 2. Lint yield (lb/acre) as influenced by foliar applications of Orthene® 97UP at different application frequencies in managing thrips in seedling cotton at two locations. Insecticide treatments numbers are as follows: 1) untreated check, 2) one foliar application at cotyledon stage (100% seedling 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.
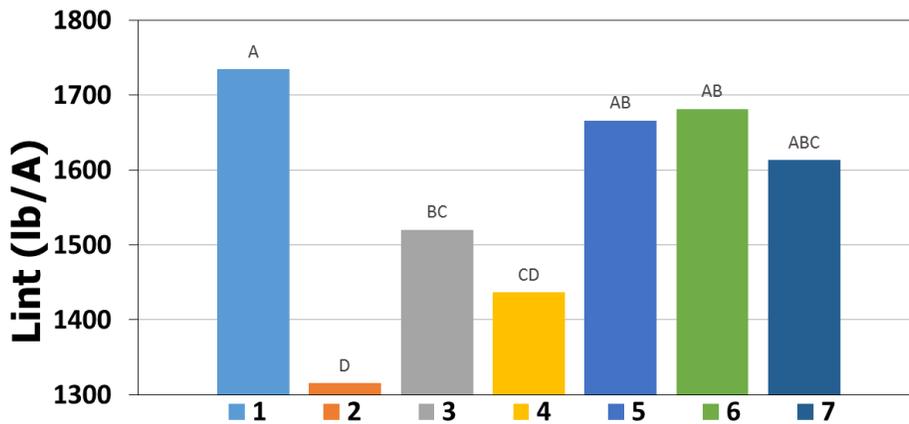
Figure 3. Lint yield (lb/acre) as influenced by foliar applications of Orthene® 97UP at different application frequencies in managing thrips in seedling cotton at Halfway, Texas, 2016. Insecticide treatments numbers are as for Figure 2.
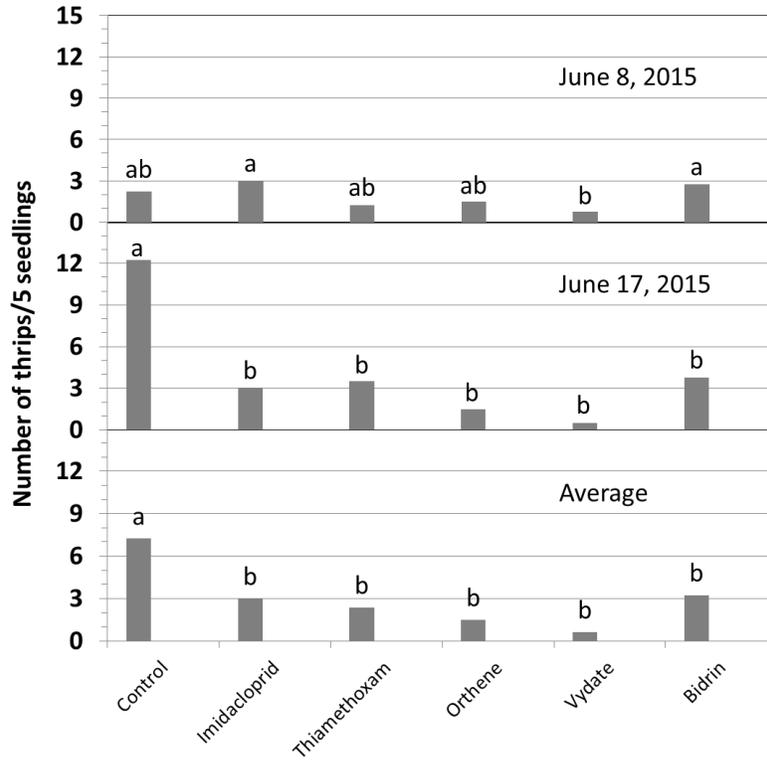
Figure 4. Number of thrips per 5-plant samples at two sampling dates and seasonal average thrips densities at Halfway as affected by seed treatment and foliar applications of selected thrips management products, 2015.
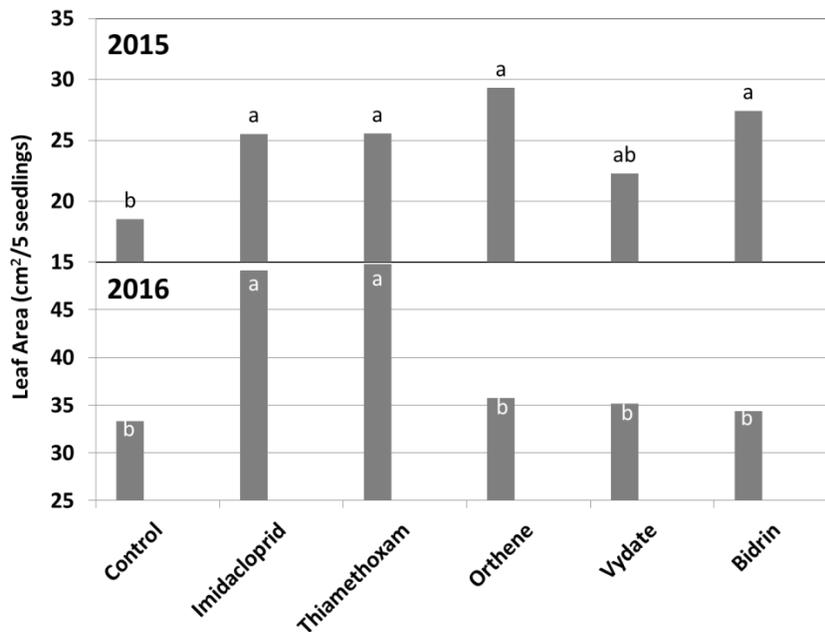
Figure 5. Total leaf area per 5-seedling sample as influenced by seed treatments and foliar applications of selected thrips management products, Halfway, TX, 2015-2016.
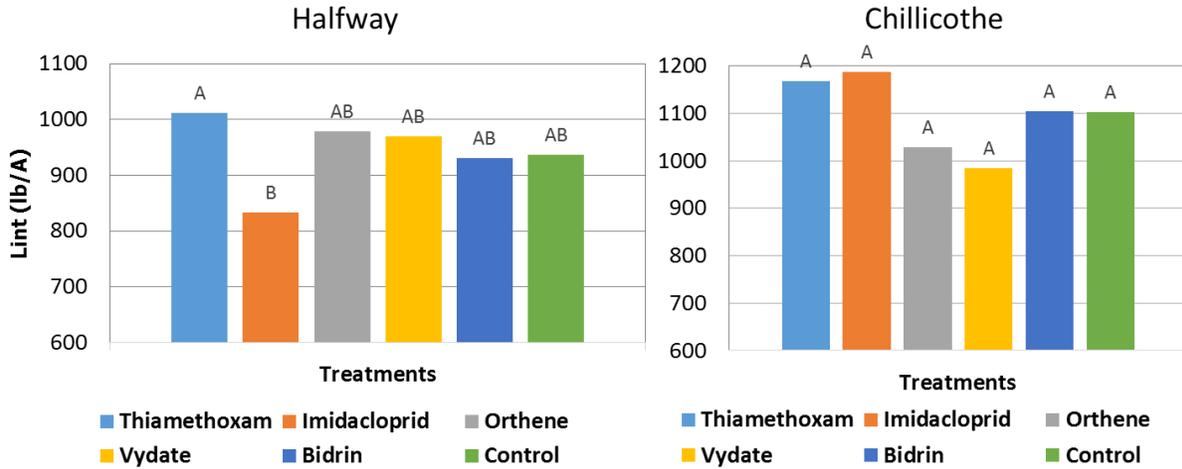
Figure 6. Lint yield (lb/acre) as influenced by seed treatments (thiamethoxam and imidacloprid) and foliar applications (Orthene®, Vydate®, and Bidrin®) of selected thrips management products at two locations, 2015.
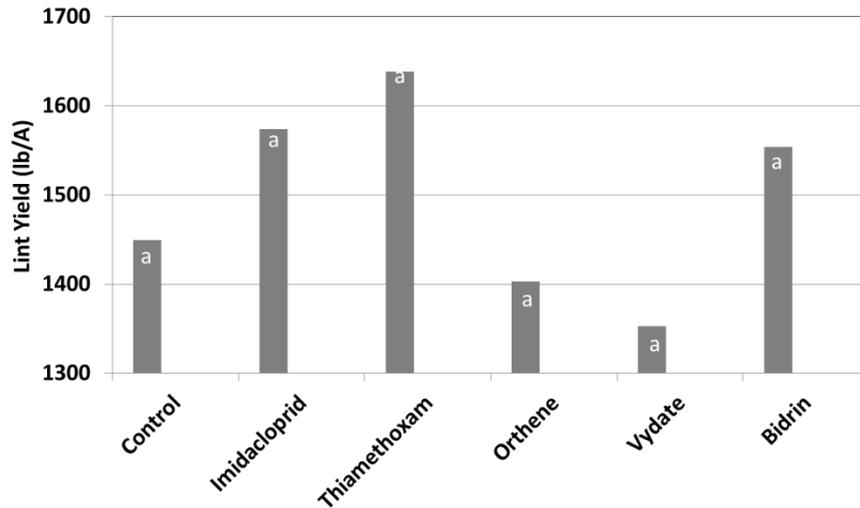
Figure 7. Lint yield (lb/acre) as influenced by seed treatments and foliar applications of selected thrips management products at Halfway, Texas, 2016.

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Efficacy of Insecticide Seed Treatments to Thrips on Cotton and Fleahopper Response to Selected Cotton Varieties in Texas

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In Texas, achieving cotton production goals often is dependent on managing pre-flowering insect pests such as thrips, cotton aphid, whiteflies, and fleahoppers and stink bugs later in the season. Thrips feeding in the terminal bud of cotton cause leaves to have a crinkled, tattered appearance as they expand and heavily damaged foliage often is stunted and curls upward at the margins. Another characteristic of thrips damage is a silvery appearance of leaves at the feeding sites.

Early-season pest management in cotton was primarily achieved with an in-furrow treatment of aldacarb (Temik®). In 2010 the Environmental Protection agency and Bayer CropScience reached an agreement to terminate production and use of aldicarb in the United States (EPA Newsroom, 2010). Consequently, achieving cotton production goals has required adoption of alternative practices for early-season pest management. Neonicotinoid insecticide seed treatments have become the primary solution to managing early-season pests of cotton in Texas. Thiamethoxam and imidacloprid are two common systemic insecticide seed treatments applied to commercial cotton seed. They are relied on to provide protection from early-season cotton pests in Texas. Although the two insecticides belong to the same insecticide group, their physical and chemical properties vary and they may exhibit differential mortality among target pests, especially under extreme variations in Texas cotton production regions.

Introduction of new management strategies, such as new insecticides in a cropping system, may lead to secondary pest outbreaks. The twospotted spider mite, *Tetranychus urticae*, is a sporadic, yet, potentially serious pest to cotton production in Texas. Although it is generally considered a late-season pest of cotton, increasing frequency of mite infestations on seedling cotton has been reported where neonicotinoid seed treatments have replaced furrow applied aldacarb (Sclar et al. 1998, Beers et al. 2005). Troxclair (2007) and Smith et al. (2013) reported cotton with thiamethoxam and imidacloprid seed treatments had a higher percentage of plants with twospotted spider mites than those treated with aldicarb or untreated cotton. Smith et al. (2013) noted that a larger mite density on neonicotinoid treated cotton was the result of deleterious effects to predators with no effect on mites.

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), is another key insect pest of cotton with induced yield loss estimates of 0.4% over the past decade and was the leading cause of yield loss due to insect damage in Texas during 2012-2013 (Williams 2011). The cotton fleahopper can cause excessive loss of cotton squares resulting in reduced yield and

harvest delays (McLoud et al. 2015). Damage to individual fields may vary from none to extremely high square loss when heavy populations develop and are left uncontrolled. The reason for variability in losses caused by the cotton fleahopper is not understood but may, in part, be associated with cultivar differences (Holtzer and Sterling 1980, Barman et al. 2012). Understanding cotton fleahopper response to cotton varieties will allow better management strategies for managing this pest on cotton.

The objectives of the current research were to determine the efficacy of neonicotinoid insecticide seed treatments to manage thrips on seedling cotton to evaluate cotton fleahopper populations on selected cotton varieties to determine if colonization differs among varieties with unique genetic backgrounds and determine if fleahopper damage differs among varieties and if this damage influences yield.

Materials and Methods

Thrips study. Cotton seeds treated with two different neonicotinoid insecticides (imidacloprid and thiamethoxam) were used to evaluate their efficacy against thrips on seedling cotton at multiple TX locations. Seeds of FM1900GLT, a widely-adapted cotton variety, was separately treated with imidacloprid and thiamethoxam. An additional seed treatment, including the check with no insecticide seed treatment, consisted of a base fungicide for protection against fungal pathogens. Five cotton producing regions were selected for the placement of seed treatment trials including the Texas High Plains (3), Rolling Plains (1), Blacklands (1), Lower Rio Grande Valley (1), and Coastal Bend (2) regions. In each region, 1-3 locations were chosen to conduct the seed treatment trials. Site selection were based on the historical early-season population pressure and experience of the local collaborator. A trial consisted of three different treatments (two insecticide seed treatments and one untreated control), replicated four times. Planting dates were adjusted per recommendations for respective production regions. After planting and seedling emergence, thrips counts were made and several plant parameters were recorded. A washing method (Burris et al., 1989) was used to determine thrips populations instead of a visual sampling method to reduce the sampling variability. Plant samples were collected at four different time period/growth periods; cotyledon, 2-leaf, 3-leaf, and 4-leaf stages. For each sampling date and experimental plot, 10 randomly selected cotton seedlings of each respective growth stage, were cut above the soil and preserved in a quart size glass jar, half-filled with 75% ethanol. The samples were brought to the laboratory and processed to extract the thrips (both adults and immatures) for each sampling date. Adult and nymph counts were recorded separately for each plot and at each location. Later in the season, delays in plant maturity were assessed by counting nodes above white flower (NAWF). Yield data were obtained from the successful test sites.

Fleahopper Evaluations: Four cotton varieties, Stoneville ST4946 (Bayer), DeltaPine DP1219 (Monsanto), Phytogen Phy333 and Phytogen PHY444 (Dow), were planted at multiple TX locations and one site in New Mexico. Varieties DP1219 and Phy444 are smooth-leaf and

ST4946 and PHY33 are hairy-leaf varieties. Cotton fleahoppers were sampled weekly beginning at pinhead square to 1/3 grown squares using the beat bucket technique. Samples were taken from the middle two rows of four row plots by folding over 2-5 plants per sub-sample into a 5 gallon bucket, beating the plants onto the side of the bucket, and immediately counting the cotton fleahoppers. This procedure was continued moving up the middle of the 2 center rows alternating rows with each sub-sample until a total of 25 plants per plot were sampled. Cotton fleahopper counts were divided into adults and nymphs. Sampling for cotton fleahopper was discontinued after the first or second week of bloom.

Corpus Christi: One week following first bloom, cotton fleahopper injury was evaluated on each of 6 plants in one of the center two rows within each plot using PMAP. Fruiting structures match-head size and larger and abscission sites were counted on each branch and first three positions from each of the 6 plants to determine percent fruit retention. Twenty plants, ten each from rows 1 and 4, were removed from the field and bolls on each branch and first three positions to determine retention at harvest. The center two rows were harvested for yield. Cotton fleahopper counts were analyzed by date (Corpus Christi only) and variety using SAS 9.4 (SAS Institute 2013) (Corpus Christi) or ARM. In-season and harvest retention data for positions 1 and 2 combing branches 7 through 12 and yield were analyzed with SAS9.4.

Results

Thrips study: Thrips densities were low throughout the study locations in 2016. Many thrips study sites were abandoned due to crop stand failure (severe rain or hailstorms) or due to the lack of thrips existence in test plots. Thrips failed to recolonize after the rain events in many locations where thrips densities were beginning to colonize prior to the rain. In general, neonicotinoid seed treatments (imidacloprid and thiamethoxam) are expected to significantly reduce thrips populations compared to that in untreated control plots, but the lack of thrips activity failed to detect that phenomenon in our tests sites (Fig. 1). For example, only Victoria site had any meaningful number of thrips where adult thrips densities were lower in neonicotinoid treatment plots than on control plots on May 24 sampling date, but the numbers declined to below economic thresholds after that date.

Insecticide treatments significantly increased leaf area compared with that in control plots at Halfway (Texas High Plains) location for both 2015 and 2016 (Fig. 2). In 2016, thrips were not detected at the Halfway test location. Nevertheless, cotton vigor, measured in terms of total leaf area, was significantly higher in the two neonicotinoid seed treatment applied plots compared to control plots, suggesting that the seed-applied insecticide treatments may have some agronomic benefit to seedling growth even in the absence of thrips injury. More detailed study is planned for 2017 to examine the effect of these seed treatments on seedling root health. Lint yield did not vary across treatments at any of the test sites in 2016.

Cotton Fleahopper Assessments: Cotton fleahopper nymphs ($F_{3,53}$=0.14; P=0.9358), adults ($F_{3,53}$=0.14; P=0.9358), and the combination of the two stages ($F_{3,53}$=0.14; P=0.9358) showed no preference for smooth-leaf or hairy-leaf varieties at Corpus Christi (Table 1). Results from Victoria and Swisher counties in TX and the location in NM were similar (Fig. 3). Cotton fleahopper nymphs ($F_{1,53}$=33.52; P<0.0001), adults ($F_{1,53}$=13.24; P<0.0001), and the combination of the two stages ($F_{1,53}$=18.97; P<0.0001) differed by assessment date (Table 2).

Boll Retention and Yield: In-season plant mapping revealed differences among cotton varieties in boll retention on branches 7 through 12 for first ($F_{3,9}$=9.86; P=0.0033) and second ($F_{3,9}$=95.87; P=0.0168) position sites (Table 3). The number of first position bolls differed among cotton varieties at harvest (($F_{3,9}$=13.31; P=0.0012) but boll retention differences were not observed second position sites ($F_{3,9}$=1.66; P=0.2434) (Table 3). Yield differences occurred among cotton varieties at the location in Corpus Christi ($F_{3,9}$=18.22; P=0.0004).

Discussion

The lack of thrips activity in 2016 prevented us from conducting the species composition study. Also, we noted that the seed treatments improved the seedling vigor even in the absence of thrips infestation compared to that in plots with no seed treatments. Therefore, we plan to investigate the seedling health in relation to root growth behavior as influenced by seed treatment in 2017. We also expect to characterize the species composition in 2017, provided that the thrips activities will be more pronounced in our study locations. We will also plan to select more thrips-prone locations in 2017.

Cotton fleahoppers showed no preference for hairy-leaf or smooth-leaf cotton varieties in all TX locations. This contrasts with previous research reporting cotton fleahopper was usually more abundant on pubescent cotton varieties (Schuster and Frazier 1977). These results suggest that hairy leaf varieties are not only suitable for adult cotton fleahopper but nymphs are capable of feeding and surviving on cotton with dense pubescence.

In-season and harvest assessments showed that cotton varieties classified as 'hairy leaf' had greater first position boll retention on branches 7 through 12 when compared with smooth leaf varieties at the Corpus Christi location. First position bolls on these branches are most vulnerable to damage by cotton fleahopper. One explanation is that hairy leaf varieties in this trial may have restricted nymphs moving from leaves to terminals where they could feed on pin-head and match-head squares. The smooth-leaf varieties in this trial would allow nymphs easy access to the terminals where they could feed and damage developing squares.

Hairy-leaf cotton varieties ST4946 and PHY333 out-yielded DP1219, one of the smooth-leaf cotton varieties in this trial. Phy333 provided significantly more lint when compared with PHY444, a smooth-leaf variety. Although lint produced by ST4946 was not statistically different

from that produced by PHY444, there was a numerical advantage in lint production by the hairy-leaf cotton. The ability of the two hairy-leaf cotton varieties to retain more first position bolls than the smooth leaf varieties on branches 7–12 corresponds well with the yield advantages offered by these products. Their boll retention advantage over the smooth-leaf varieties on branches 7-12 also suggests they may interfere with cotton fleahopper nymph movement from leaves to developing squares.

Results of the 2016 research are intriguing and warrant further investigation into the possibility of hairy-leaf cotton varieties limiting cotton fleahopper damage to developing squares. In 2017 we propose to increase the number of cotton varieties evaluated to between 8 and 10 with the majority of entries hairy-leaf varieties. We also intend to double plot size from 4 to 8-rows with four rows not-treated and four rows treated with an insecticide to provide a 'cotton fleahopper free' sub-plot for boll retention comparisons. We also will simplify boll retention evaluations by counting only first position sites.

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Table 1: Mean number of cotton fleahopper nymphs, adults, and a combination of developmental stages on 100 plants of four cotton varieties with different trichome densities.

Variety	Mean (±SE) number of cotton fleahopper by developmental stage		
	Nymphs	Adults	Total
ST4946	9.0 ± 1.9 a	3.0 ± 0.6 a	9.0 ± 1.1 a
DP1219	7.0 ± 1.5 a	5.0 ± 1.1 a	1.0 ± 0.4 a
PHY333	8.0 ± 1.6 a	5.0 ± 1.0 a	21.0 ± 1.8 a
PHY444	8.0 ± 1.9 a	3.0 ± 0.7 a	15 ± 1.1 a

Means not followed by the same letter are significantly different (Tukey's LSD, $p < 0.05$). Standard error of the mean is represented by SE.

Table 2: Mean number of cotton fleahopper nymphs, adults, and a combination of developmental stages on different sampling dates.

Date	Mean (±SE) number of cotton fleahopper by developmental stage		
	Nymphs	Adults	Total
5/6/2016	6.0 ± 1.0 c	3.0 ± 1.0 b	9.0 ± 1.1 c
6/1/2016	1.0 ± 0.5 d	1.0 ± 0.3 b	1.0 ± 0.4 d
6/8/2016	15.0 ± 1.6 a	6.0 ± 1.1 a	21.0 ± 1.8 a
6/16/2016	10.0 ± 0.8 b	6.0 ± 0.9 a	15.0 ± 1.1 b

Means not followed by the same letter are significantly different (Tukey's LSD, $p < 0.05$). Standard error of the mean is represented by SE.

Table 3: Mean percent boll retention on 24 plants on each of four cotton varieties with different trichome densities. Percent retention is the total number of bolls on branches 7-12 divided by the total nuber of sites for each of positions one and two one week after flowering and at harvest.

| Variety | Mean (±SE) retention (%) | | | |
| | In-Season | | Harvest | |
	Position 1	Position 2	Position 1	Position 2
ST4946	88 ± 4 a	78 ± 4 a	57 ± 3 a	20 ± 1 a
DP1219	68 ± 7 b	63 ± 3 ab	32 ± 6 b	22 ± 2 a
PHY333	87 ± 3 a	72 ± 4 a	63 ± 3 a	27 ± 4 a
PHY444	66 ± 4 b	48 ± 8 b	42 ± 2 b	23 ± 1 a

Means not followed by the same letter are significantly different (Tukey's LSD, P<0.05). Standard error of the mean is represented by SE.

Table 4: Mean yield (lbs/a) of four cotton varieties with different trichome densies.

Variety	Mean (±SE) yield (lbs/a)
ST4946	1008 ± 10 ab
DP1219	744 ± 38 c
PHY333	1173 ± 51 a
PHY444	912 ± 53 b

Means not followed by the same letter are significantly different (Tukey's LSD, P<0.05). Standard error of the mean is represented by SE.
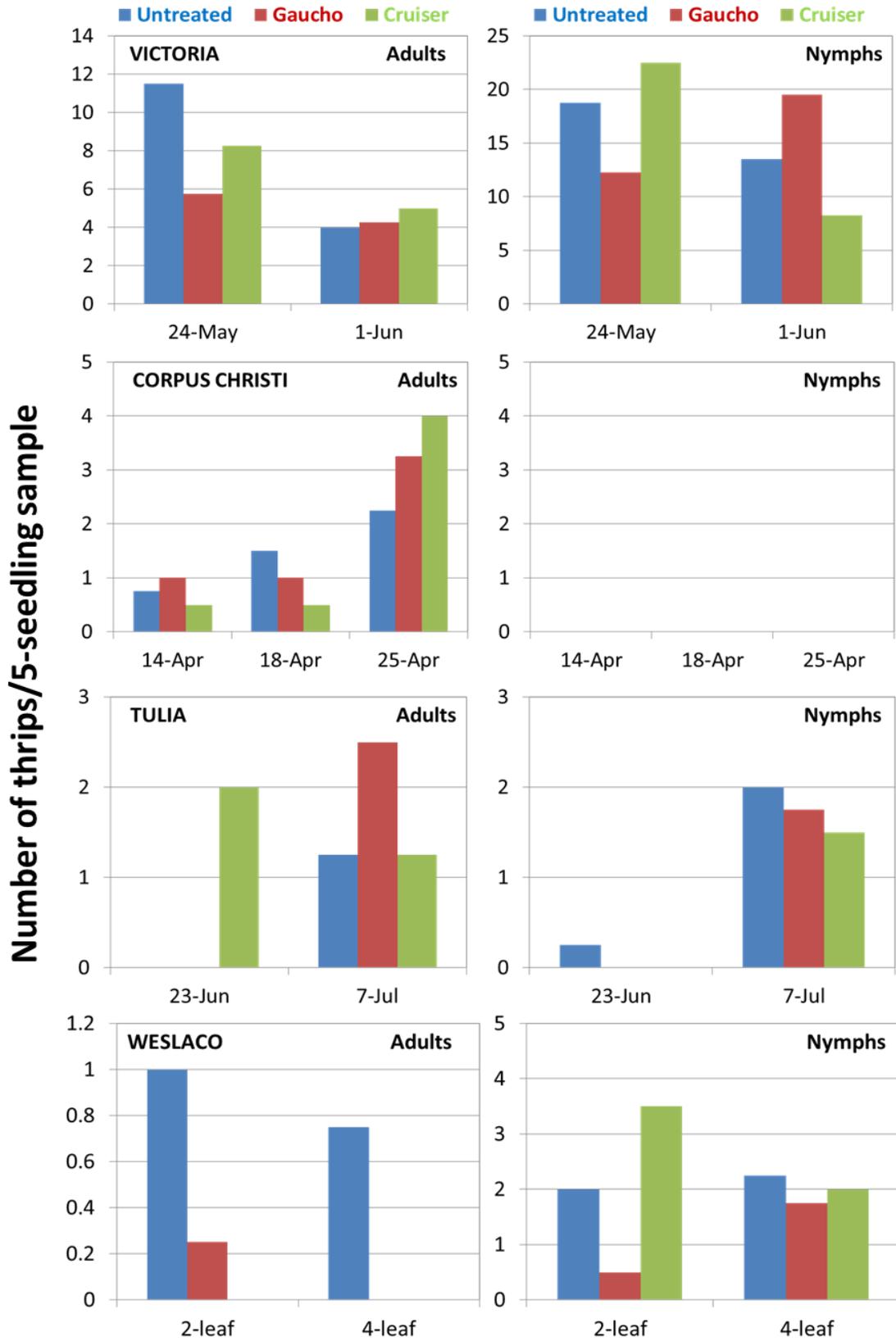
Fig. 1. Number of thrips per 5-seedling sample at various thrips study sites in Texas, 2016.
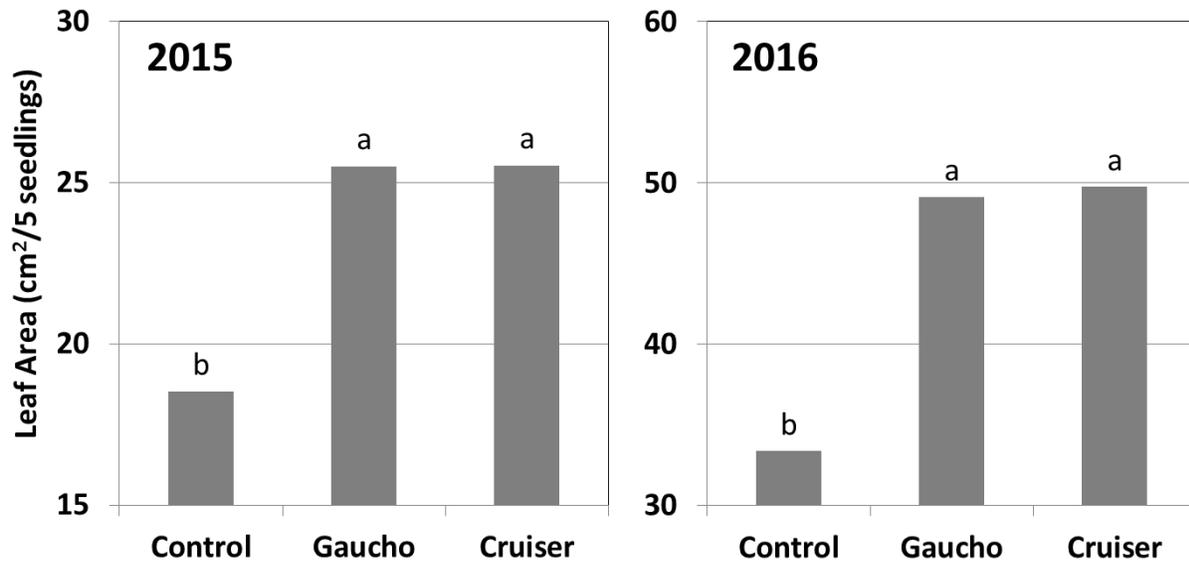
Fig. 2. Seedling vigor, measured in terms of total leaf area per 5-seedling sample, affected by neonicotinoid seed treatments, Halfway (Texas High Plains), Texas, 2015-2016.

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 (15 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 records were maintained related their GPS coordinates.

THP Cotton & Cottonseed Production Statistics
65% of Texas
20-30% of the U.S.
3-5% of the world!
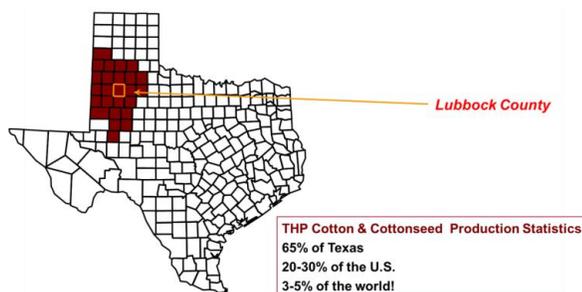
Figure 1. Texas High Plains pheromone trapping study site, Lubbock County, TX, 2002-2016.

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 15-year trapping study has been sub-divided into four successive periods, including: 1) 2002-2005, 2006-2009, 2010-2013, and 2014-2016, representing boll weevil eradicated and beginning of *Bt* cotton adoption in THP, low *Bt* cotton acreage (<50%), majority *Bt* cotton (70%), and the most recent 3-year period, respectively.
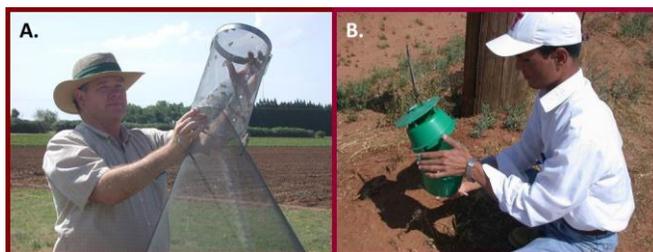
Figure 2. S. Carroll services a Texas pheromone (TP) trap to monitor bollworm and tobacco budworm moths (A). R. Shrestha counts beet armyworm moths in a green bucket trap (B).
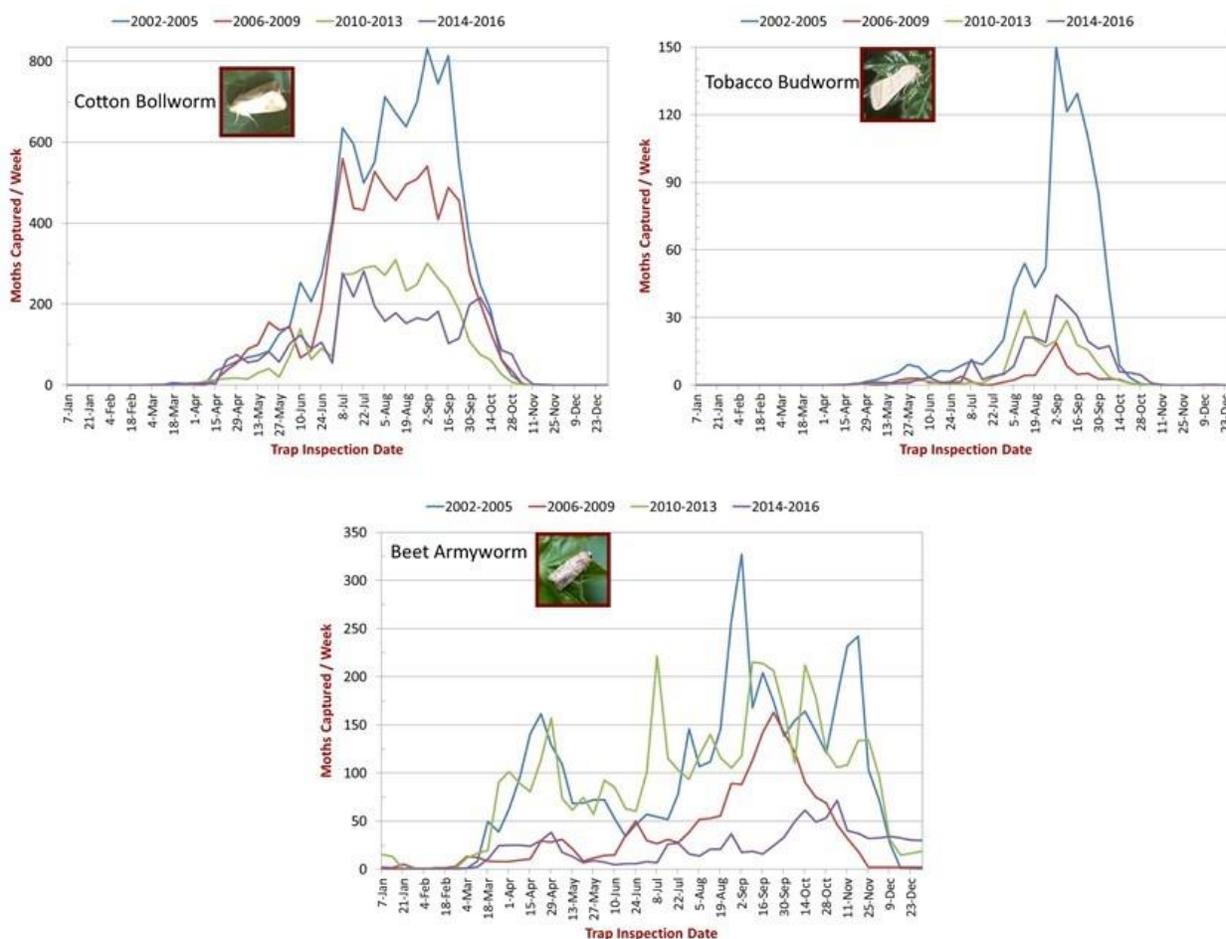
Figure 3. Number of bollworm (top-left), tobacco budworm (top-right), and beet armyworm (bottom-center) moths captured per week, averaged across four selected 3-4 consecutive-year groupings spanning the 15-year study, Lubbock County, TX, 2002-2016.

Cotton Bollworm. The cumulative annual number of bollworm moths captured per trap averaged 10,618, 7,970, 4,071 and 3847 for 2002-2005, 2006-2009, 2010-2013, and 2014-2016, 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 relatively level, overall annual bollworm total captures (per trap) from 2010 to 2016. Interestingly, although bollworm numbers decreased over time, the seasonal 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 284 for 2002-2005, 2006-2009, 2010-2013, and 2014-2016, 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 11 years with the exception periods for peak flight from late August through 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 4,650, 1,790, 4,593, and 1090 for 2002-2005, 2006-2009, 2010-2013, and 2014-2016, 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 most recent years (2014-2016).

Influence of annual rainfall on moth abundance and flight profiles. Within the 15-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 profiles 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.
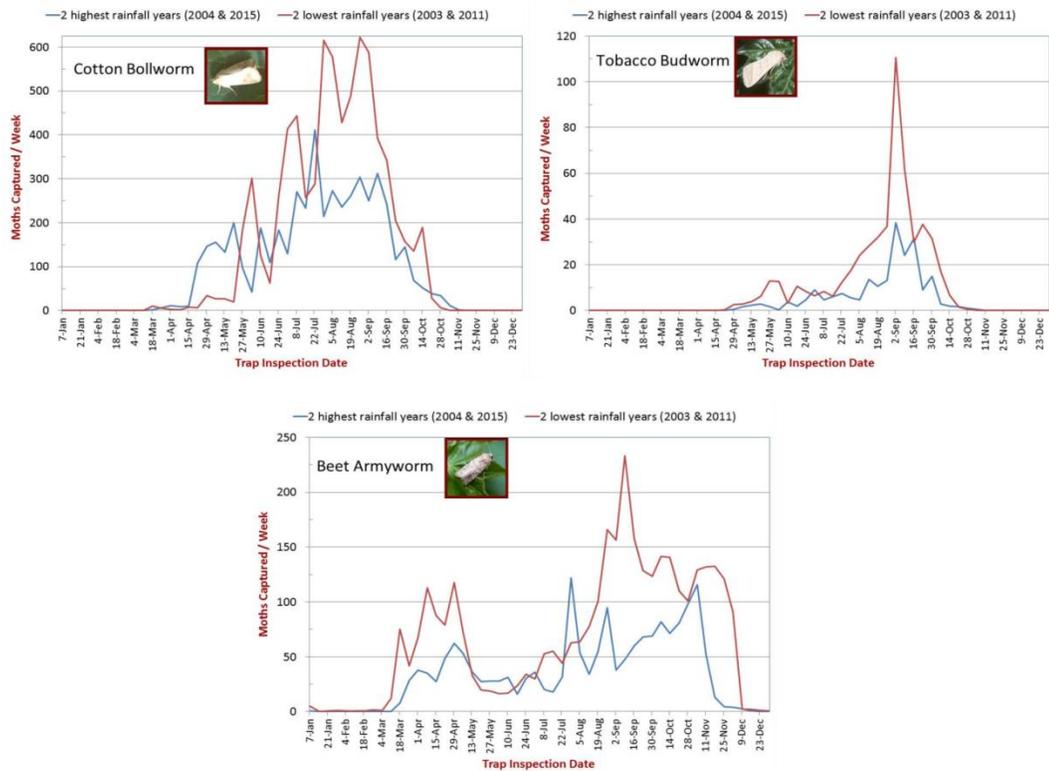
**Figure 4.** Cotton bollworm (top-left), tobacco budworm (top-right), and beet armyworm (bottom-center) moth seasonal flight profiles averaged for: 1) Two study years with the highest rainfall (2004 & 2015), and 2) Two lowest rainfall years (2003 & 2011). Lubbock County, 2002-2016.

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EFFECT OF SOIL NITROGEN RATES ON RESIDUAL SOIL N, PLANT GROWTH PARAMETERS, YIELD, AND QUALITY: AN ECONOMIC ANALYSIS

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Abstract

Supplemental nitrogen and irrigation water are critical agricultural inputs for cotton production in the Texas High Plains region. A multi-year field study was conducted to examine the effect of soil nitrogen (residual nitrogen plus applied nitrogen) on cotton agronomic growth parameters, yield, and fiber quality attributes under a drip irrigation production system. Fixed-rate nitrogen application experimental plots consisted of five annual augmented nitrogen fertility levels (0, 50, 100, 150, and 200 lb/acre) with five replications. Each year, soil to a 24-in. depth in each experimental plot was sampled for residual nitrogen analysis prior to planting or before treatment deployment. Rates of applied N exceeding 100 lb/acre resulted in higher residual nitrogen detection during the following season. Rates of N application exceeding 100 lb/acre resulted in higher lint yield, but micronaire values were significantly reduced. In this study, we examined the effect of augmented soil N and residual N in cotton production in the Texas High Plains. Specifically, we estimated the marginal product value of nitrogen and water inputs in Texas High Plains cotton production.

Introduction

Nitrogen fertility and irrigation water are the primary constraints in cotton production in the Texas High Plains. Because Texas High Plains cotton production is a low-input system, increasing production costs, limited productivity, and low cotton price are becoming increasing challenges for our cotton producers. On the other hand, nitrogen deficiency negatively impacts cotton plant growth and reproduction, causing reduced plant height, fewer fruiting branches, fewer bolls, and ultimately lower yields. However, the use of excess nitrogen fertility beyond the crop demand can have negative impacts on lint yields and fiber quality. The excessive use of nitrogen also tends to produce excessive vegetative growth and harvest delays, increasing pest pressure, and increased concern for groundwater pollution due to nitrate contamination. The objective of this study was to conduct an economic analysis of the impact of nitrogen fertilizer on cotton lint yield and fiber quality.

Materials and Methods

The study was conducted on a 5-acre sub-surface drip irrigated field at the Texas A&M AgriLife Research farm near Plainview, Texas. Five nitrogen application rates (0, 50, 100, 150, 200 lb/acre) had been deployed to the same experimental units consistently for six consecutive years since 2009. Soil residual nitrogen was monitored annually by taking two 24-inch core samples from each plot. The 0-12 inch portions of each core were combined to form a single, composite soil sample, and likewise, the 12-24 inch portions were combined, resulting in two samples per experimental plot. Samples were sent to Ward Laboratories, Kearny, Nebraska for analysis. Regionally well-adapted cultivars were used in this study over the duration of the study: FM960B2R was planted on May 20, 2009 and May 27, 2010, DP104B2RF on June 14, 2011, and FM9063B2RF on May 17, 2012, May 23, 2013, and June 16, 2014. The experiment consisted of a randomized block design with five treatments and five replications (Fig. 1). The five treatments included side-dress applications of nitrogen fertilizer at rates of 0, 50, 100, 150, and 200 lb N/acre. Cotton was planted (56,000 seeds/acre) in 30-inch spaced rows and was irrigated with a subsurface drip irrigation system.

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

Figure 2. A) Annual pre-season soil sampling of 25 sub-surface drip irrigated cotton plots; B) Annually near the time of first bloom, each plot received the same side-dressed nitrogen application treatment rate; C) Differential cotton plant growth responses are often visually apparent between plots receiving high and low N application rates, Hale Co., TX.

Several plant growth parameters including plant height, root length, leaf surface area, and chlorophyll content were measured throughout the growing season annually. In order to determine the level of residual nitrogen, soil samples were taken from the experimental plots prior to the deployment of nitrogen fertility treatments. Fertility treatments were applied when plants began squaring with a soil applicator ground rig. Hand-harvested yield samples were obtained from each plot. Fiber samples were analyzed for lint quality parameters at the Cotton Incorporated Fiber Testing Laboratory.

Economic analysis consisted of developing a lint yield function based upon the numerous variable inputs. The classical production function of a farm producing cotton lint (Y) with the available X inputs, Y= f(X), was used. The input variables included N application, residual soil nitrate N, rainfall, irrigation, and planting date. Rainfall and irrigation were further categorized into two groups, pre-plant and post-plant (during the growing season). The purpose of categorizing the time period was to examine the effect of each of these factors on two different sets of time (pre-plant versus during the growing season). This will allow for an effective management of crop inputs, especially the more economic allocation of the irrigation water.

The cotton lint production function can be further illustrated as, lint yield= f (total N, pre-plant water, post-plant water, planting time). Different parameters were estimated using the following ordinary least squares (OLS) regression: $Y = \alpha_1 + \alpha_2 X_1 + \alpha_3 X_2 + \alpha_4 X_3 + \alpha_5 X_4 + \alpha_6 X_5 + \varepsilon$, where Y = cotton lint yield (lb/acre)

X_1 = Total N available to the crop (lb/acre)
X_2 = Pre-plant water (mm) (irrigation plus rainfall)
X_3 = Post-plant water (mm) (irrigation plus rainfall)

X4 = Total N * Pre-plant water
X5= Total N *Post-plant water
ε is the least square regression residual

Binary logistic model was used to analyze the effect of the variable inputs on fiber quality parameter, micronaire. The probability function for the model is: $P(Y_i = 1) = P_i = \frac{1}{1+exp^{-z}}$

This can be operationalized as,

$$Logit \ P(Y_i^*) = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \mathcal{E}_i \qquad Logit \ (Y_i^* = j) = \gamma' X + \mathcal{E}_i$$

where j = grade, dummy (1 if premium quality, 0 otherwise).
(micronaire value in the range of 3.5 – 4.9 units were considered as premium quality)

The estimated model has the following functional form: $Prob \ (grade = j) = \alpha1 + \alpha2X1 + \alpha3X2 + \alpha4X3 + \alpha5X4 + \alpha6X5 + \alpha7X6 + \varepsilon$
Where,

X1 = Pre-plant water (mm)
X2= Post-plant water (mm)
X3 = Residual N (lb/acre)
X4= Applied N (lb/acre)
X5= Planting time dummy (1 if May planting, 0 otherwise).
ε is the least square regression residual

Results and Discussion

In general, soil residual N levels were significantly higher in plots that received the two highest annual application rates of N fertilizer versus plots receiving lower-rate N applications or no N augmentation (Fig. 3). Averaged over the six-year study period, soil residual N levels were lowest in zero and 50 lb N/acre plots, although the 50 lb N/acre plots had numerically higher residual N than in zero N plots. The highest N augmentation plots (200 lb N/acre) had the significantly highest average residual N; the year-to-year residual N was always the highest amount in this treatment, at least numerically. The two second highest N augmentation plots (100 and 150 lb/acre) resulted in significantly higher amounts of soil residual N compared to that in zero and 50 lb/acre plots.
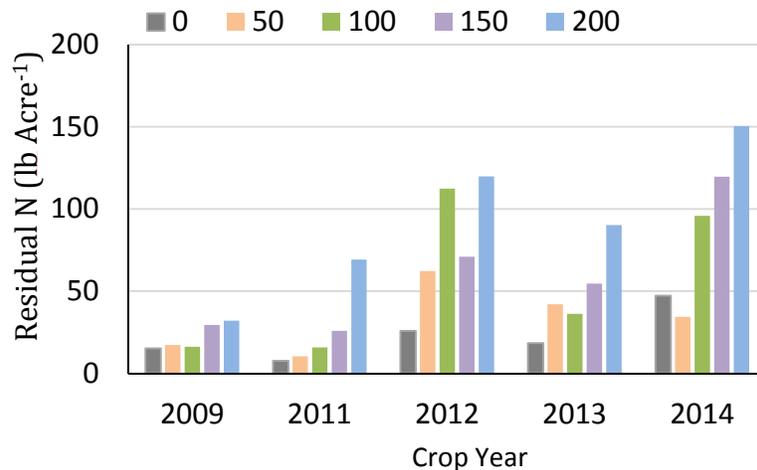
Figure 3. Effect of prior year's N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulations. In some cases, residual N carry-over resulted over more than one season's N augmentation.

As expected, zero N plots consistently produced the lowest lint yield compared to that in N-augmented plots (Fig. 4). Overall, 150 and 200 lb/acre plots produced the highest lint yield, followed by 100, 50, and zero N plots. Yield

increased curvilinearly with each additional 50 lb N added, with the numerically highest average yield occurring in augmented 150 lb N/acre treatment, but the yield numerically decreased beyond 150 lb N/acre with additional N. Consistent numerical decline in yield beyond 150 lb N/acre in most years suggests that N application beyond 150 lb/acre may be unfavorable for cotton yield. Yield advantages because of optimal N application have been attributed to larger bolls at greater number of fruiting sites (Boquet and Breitenbeck, 2000; Moore, 1999).
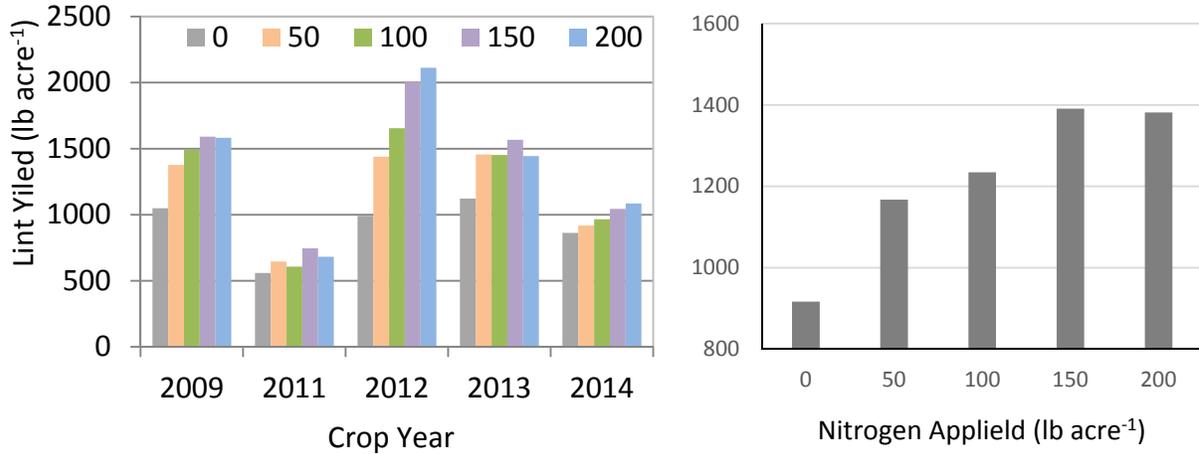
Figure 4. Effect of nitrogen application rates on cotton lint yield, Hale Co., TX, 2009-2014.

Lint maturity, measured in terms of micronaire values, also varied with N treatments (Fig. 5). Averaged over five years, micronaire values were similar and at the base range (3.5-3.6) across the three lower N levels, whereas the two highest N levels resulted in micronaire values in a discount range (<3.5).
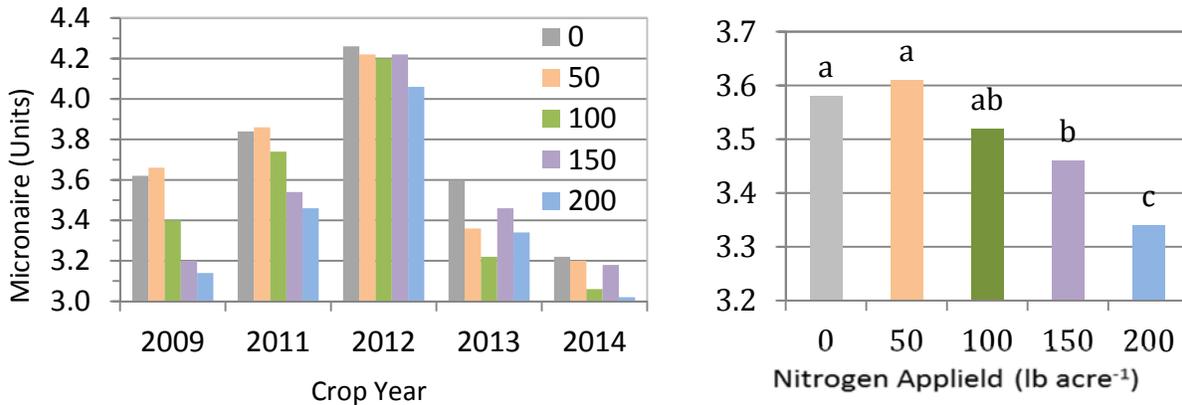
Figure 5. Effect of nitrogen application rates on cotton lint micronaire, Hale Co., TX, 2009-2014.

The yield response estimation results for the OLS with different N and irrigation application are summarized in Table 1. The N was applied on the third week of July. Pre-season irrigation was generally applied 1-3 weeks before planting, whereas post-irrigation was applied throughout the growing season on a regular basis. Pre-plant rainfall was recorded from 3 months prior to planting and post-plant rainfall was recorded during the growing season. Pre-plant water is the

sum of pre-plant irrigation and pre-plant rainfall, whereas post-plant water is the sum of post-plant irrigation and post-plant rainfall. The rainfall after crop cut-out, although included in the post-plant water, likely did not contribute toward fruit maturity and lint.

Table 1: Simple statistics of variables used in the analysis, 209-2014, Hale Co., Texas.

Variable	Description	Mean	Std Error	Minimum	Maximum
Applied N	0, 50, 100, 150, 200 (lbs N acre^{-1})	100.00	71.00	0	200.00
Residual NO$_3$-N	Residual nitrogen (lbs acre^{-1})	64.75	80.73	5.00	481.00
Total nitrogen	Applied nitrogen+ residual nitrogen	164.75	126.92	6.00	631.00
Pre-plant water	Pre-plant rainfall + pre-plant irrigation (inch)	10.40	2.80	6.93	13.41
Post-plant water	Post-plant rainfall + post-plant irrigation (inch)	19.77	8.57	12.60	24.47
Yield)	Lint Yield (lbs acre-1	1218.10	473.33	406.21	2744.59
Micronaire	measure of fiber fineness & maturity (Units)	3.56	0.45	2.56	4.52
Planting date	Dummy (Early planting 1, 0 Otherwise)	0.66	0.13	0	1.00

Parameter estimates for the lint yield model are presented in Table 2. Coefficients total N (applied N plus residual N), pre-plant water, and total N x pre-water interaction showed a positive effect on lint yield, whereas post-plant water, total N x post-plant water, and pre-water x post-water interactions showed negative relationships. Nevertheless, all parameters, except the constant term, were significant in the model. The marginal effect of pre-plant water was found to be higher than post-plant water. Neupane (2010) noted a similar relationship in prior year data from the same study. Total water available to plants (rainfall and irrigation) at different stages showed varied the impact on lint yield. Post-plant water, if irrigated late or rainfall occurs around crop cut-out, may contribute to undesirable vegetative growth at the cost of cotton boll growth and maturity. Neupane (2010) reported the marginal effect of residual NO$_3$ on lint higher than the variable N. Boquet (2005) reported that increasing N from 90 to 157 kg ha^{-1} did not result in increased lint yield in irrigated or rain-fed cotton.

Table 2. Parameter estimates for the lint yield model using OLS.

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	66.17	155.89	0.42	0.672
Total N	1.25	0.18	6.78	<.0001
Pre-water	176.14	18.23	9.66	<.0001
Post water	-40.03	12.38	-3.23	0.0016
Total N* Pre-water	0.52	0.11	4.81	<.0001
Total N* Post water	-0.13	0.07	-1.79	0.0767
Pre-water *Post water	-12.44	2.81	-4.42	<.0001

The model gave a reasonably acceptable fit with R-square of 0.73. Observed values were fitted around 0 between 600 to -600 which indicated that OLS model behaved well with equal variance of error term and reasonable linear relationship (Figure 6).
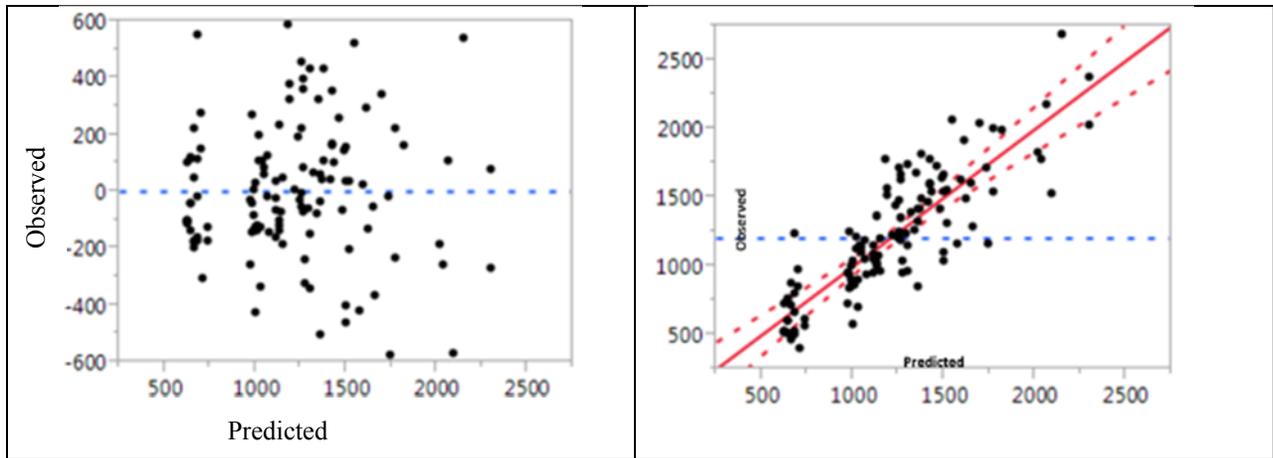
Figure 6. Residual analysis of the lint yield model (left) and observed versus predicted lint yield (right).

Table 3: Parameter estimates for micronaire using binary logistic model

Term	Estimate	Std Error	Wald $\chi2$	Prob>|t|	Odds Ratio
Intercept	3008.80	1253.90	5.76	0.0164	
Pre-plant water	2.15	0.55	15.28	<.0001	0.12
Post-plant water	-1.02	0.21	24.77	<.0001	2.78
Residual N	0.01	0.01	3.50	0.0614	1.00
Applied N	-0.02	0.01	14.53	<.0001	1.02
Planting date	0.15	0.06	5.78	0.0162	0.86

Binary logistic model was used to analyze the effects of variable inputs on micronaire quality. The coefficient was estimated from regression and results were interpreted in terms of change in the odds ratio. The marginal effects of increased inputs on the odds ratio was calculated and compared with the standard value (1.0). If the marginal effect is higher than 1, an increase in the input causes the production of a premium micronaire quality for the lint. Similarly, a value less than 1 will increase the chance of the fiber being in a lower grade. Rainfall and irrigation water received during the different crop stages showed different relationships with micronaire quality. Pre-plant irrigation and pre-plant rainfall showed a positive relationship on premium quality lint production, whereas post-plant water resulted in negative relationships regarding micronaire values. Residual N and early planting showed positive relationships and N application rate had a numerical negative relationship, but they were all significant (Table 3). Neupane (2010) reported a positive linear relationship between micronaire and water/N fertility level.

Acknowledgments

COTTON WATER-DEFICIT STRESS, AGE, AND CULTIVARS AS MODERATING FACTORS OF COTTON FLEAHOPPER ABUNDANCE AND YIELD LOSS

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Abstract

Field experiments were conducted in 2012 and 2013 during drought conditions in South Texas and the Texas High Plains to test whether cotton water-deficit stress, age, and cultivars are moderate factors that affect cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), abundance and yield loss. Irrigation and sequential plantings of several cultivars were used to simulate a range of water-deficit stress, plant ages, and cultivar variability. Cotton grown under these experimental conditions were exposed to cotton fleahopper using natural and artificial infestation. Cotton cultivars had a strong influence on cotton fleahopper abundance, with higher densities on Stoneville cultivar 5458 B2RF, which is relatively pubescent, than densities on the Phytogen cultivar 367 WRF, which is relatively glabrous, in South Texas ($p < 0.04$). But the strong cultivar effects on cotton fleahopper abundance did not correspond to yield reduction. No water regime effects on cotton fleahopper densities were observed in 2012 ($p > 0.05$), whereas cotton fleahopper densities increased on older cotton grown under no water stress in 2013 in South Texas ($p < 0.05$). In contrast, yield response was primarily sensitive to soil moisture conditions (up to 50% yield reduction when grown in dryland mimic conditions below 75% crop ET replacement, $p < 0.0009$). Yield loss attributable to cotton fleahopper activity was relatively lower than that attributable to water-deficit stress. Modest water and cotton fleahopper stress synergies occurred, with enhanced yield loss attributable to cotton fleahopper seen in cotton grown in high water-deficit conditions in the High Plains ($p < 0.05$). These yield trends were consistent across cultivars (no interaction with cultivar), even though cotton fleahopper populations varied significantly across cultivars.

Introduction

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), feeding on squares (i.e., pre-floral buds) of cotton, *Gossypium hirsutum* L. (Malvaceae), has reduced yield by up to 6% and also has delayed harvest in the southwest and mid-south (USA) cotton growing regions. But variability in the relationship of cotton fleahopper-induced square loss to subsequent yield loss under similar cotton fleahopper feeding pressure occurs and presents a challenge to cotton fleahopper management using traditional sampling and economic threshold methods. In practice, field history of cotton fleahopper damage, weather conditions, and IPM practitioner sensitivity to square loss have been used to adjust decision-making locally. In South Texas, one to four foliar sprays for cotton fleahopper control are common across cotton fields that have apparently similar pest risk based on similar cotton fleahopper density estimates generated from pest monitoring (Brewer, pers. obs).

In review of the literature, cotton yield loss variability to cotton fleahopper feeding has been partly associated with cultivar differences (Holtzer and Sterling, 1980), including heritable traits considered for plant resistance (Knutson et al., 2013). Ring et al. (1993) calculated visual-based cotton fleahopper economic injury levels (EIL) of between 0.015 and 0.45 insects per plant. The wide range was attributed to cultivar influences, based on comparison of yield—cotton fleahopper density relationships. Parajulee et al. (2006) partly attributed severity of cotton square loss to susceptibility differences across stages of cotton development and age of the reproductive tissues when cotton fleahopper migrated into fields from overwintering sites. Cotton may also compensate for early square loss (Anon, 2015). Cotton water deficit-induced stress (water stress) also has been associated with square retention rates (Stewart and Sterling, 1989), which may influence plant sensitivity to cotton fleahopper feeding. These factors may be the underpinning of why thresholds in outreach materials vary across cotton growing regions of the southwest (i.e., 0.10 to 0.30 insects per terminal visually inspected during the first three weeks of squaring) (Anon, 2015), and why this insect is a minor pest in other locations. But if management strategies (i.e., planting time and cultivar selection) and weather conditions (i.e., poor rainfall in dryland production areas) influence cotton sensitivity to cotton fleahopper feeding, direct density

estimation of cotton fleahopper for decision-making may give false indication of damage potential and improperly trigger insecticide applications using economic thresholds based on insect population estimates.

Here, we hypothesize that cotton water stress, age, and cultivars affect cotton fleahopper abundance and yield loss. The practical goal of understanding these relationships is to improve our assessment of cotton risk from cotton fleahopper and begin generation of a data base to make objective economic threshold adjustments under variable weather and management practices.

Methods

Drought conditions in Texas, 2012 and 2013, provided opportunity to assess cotton fleahopper activity and cotton response in a high contrast of water stress conditions manipulated by using irrigation in a field setting. Cotton fleahopper abundance and cotton response including yield were evaluated in high to low water-deficit conditions in two widely separated cotton growing regions: the coastal region of South Texas and the Texas High Plains. Standard agronomic practices were used. Cultivars, planting dates, and natural and artificial infestations of cotton fleahopper were used to optimize contrast in cotton fleahoppper pressure and cotton response. Experimental manipulation varied between South Texas and the Texas High Plains per opportunities and constraints outlined below.

South Texas location
A natural cotton fleahopper population was followed across time at a Corpus Christi, TX, location. A split plot design was used to expose a natural population of cotton fleahopper to a soil moisture gradient of three (2012) and two (2013) water regimes (main plot), to two different plant ages by using two planting dates (sub-plot), and to two cotton cultivars (sub-sub-plot). An insecticide treatment was added as a final split plot in the design to directly test for cotton fleahopper-induced yield loss. Water regimes were established by using an above-ground drip irrigation system. Square injury from cotton fleahopper feeding was also confirmed by visual observation. The specific plot site was moved yearly so that the previous year crop was either sorghum or corn. There were five replications, and individual plot size was four 15.24 m rows on 96.5 cm centers.

In 2012, the water regimes used were a substantial water-deficit dryland mimic using minimal irrigation during drought (2.90 cm of irrigation), a moderate water-deficit dryland mimic using irrigation targeting 75% crop evapotranspiration replacement (crop ET) (6.245 cm of irrigation), and a non-water-deficit mimic using irrigation targeting 90% crop ET (10.85 cm of irrigation). Cumulative rainfall from planting to harvest was 15.5 cm for both plantings. The surface irrigation drip tubes were 17 mm (dia.) and emitted 3.4 liters per h. The two planting dates were April 12 and 30. The two cultivars were Phytogen 367 WRF (Dow AgroSciences) and Stoneville 5458 B2RF (Bayer CropScience). The Stoneville cultivar was relatively pubescent, a trait which has been associated with high cotton fleahopper populations (Knutson et al. 2013), while the Phytogen cultivar was more glabrous with a lower density of trichomes on the leaves. The last split was a foliar insecticide treatment: no insecticide and acephate applied four times at a rate of 560.4 g per ha weekly beginning at first week of squaring. In 2013, two water regimes were used: a substantial water-deficit dryland mimic which required irrigation due to the continuing drought (15.49 cm of irrigation for an earlier planting and 20.07 cm of irrigation for a later planting) and the non-water-deficit mimic using irrigation targeting 90% crop ET replacement (26.42 cm of irrigation for an earlier planting and 35.05 cm or irrigation for a later planting). Cumulative rainfall was 31.0 cm and 27.9 cm for the earlier and later planting, respectively, measured from planting to harvest. The two planting dates in 2013 were April 22 and May 6, moved later this year to further encourage cotton fleahopper movement into the crop during the ongoing drought. The same cultivars were used as in 2012. The insecticide treatment was changed to thiamethoxam (Centric 40 WG, Syngenta) applied three times at a rate of 87.6 g per ha weekly beginning at first week of squaring.

High Plains location
The Lamesa, TX, location experienced barely detectable cotton fleahopper populations in 2013 likely due to the extended drought; therefore we focused on boll retention and subsequent yield using an augmented population of cotton fleahopper. Water stress and cotton fleahopper pressure were each manipulated at two levels in a randomized complete block. A high water-deficit dryland mimic (11.43 cm of irrigation) and a moderate water-deficit dryland mimic (22.86 cm of irrigation) were delivered through a low-energy precision application via center pivot irrigation system. Only trace amounts of rainfall were detected. An augmentative release of cotton fleahopper was used to directly test for yield response to cotton fleahopper as compared with a no infestation control. Square injury from

cotton fleahopper feeding was also confirmed by visual observation. The cultivar planted was Phytogen 367 WRF. The treatments were replicated three times, and plot size was 13.7 m by four rows on 101.6 cm row centers.

Plants were artificially infested during the third week of squaring at a rate of five cotton fleahopper nymphs per plant across a three meter uniform section of each plot. The source of nymphs was from the wild host plant woolly croton, *Croton capitatus* Michx. Woolly croton was collected in the fall near College Station, TX, and placed in laboratory cold storage (Lubbock, TX) until fleahoppers were needed the following year following the protocol of Hakeem and Parajulee (2015). In brief, conditions conducive to cotton fleahopper emergence were simulated in a laboratory environment in order to induce hatching of overwintered eggs embedded in the woolly croton stems, and emerged cotton fleahoppers were placed on fresh green beans. At approximately ten days post-emergence, fleahopper nymphs were provided fresh cotton squares as a training substrate prior to field release. Releases were conducted by aspirating third to fourth instar cotton fleahopper nymphs from the laboratory colony, transferring them into 1.9 cm by 3.2 cm plastic vials, then depositing them onto the terminals of plants in each treatment plot.

Measurements and analyses

At the South Texas location, insect counts using a beat bucket technique (Brewer et al., 2012) were made on a weekly basis after cotton fleahopper numbers exceeded 0.10 bugs per plant through the sixth week of squaring. A total of 20 plants were sampled per plot. Plant data included lint yield and percent boll retention measured near harvest**.** Weekly data showing treatment differences were reported here. At the Texas High Plains location, the data included number of harvestable bolls and lint yield.

All measurements were analyzed with ANOVA, conforming to the plot designs for the South Texas and Texas High Plains locations. Count data were transformed by the square root of the count + 0.5. Percent boll retention data from South Texas were transformed by the arcsine of the square root of the proportion. Based on our hypotheses, we gave special attention to cotton fleahopper density and yield patterns discerned from significant interactions between water stress and plant age, and water stress and cultivar. Cotton fleahopper-influenced effects were experimentally verified by a significant insecticide spray (South Texas) or cotton fleahopper augmentation (Texas High Plains) effect. Using the split plot design and limiting each split to two treatments in the South Texas location, differences in means were directed tested with the ANOVA. In the Texas High Plains location, Tukey's Honest Significant Difference test was used to compare means across four treatments.

Results and Discussion

Cotton fleahopper density and plant response measures of boll retention and yield were sensitive to changes in cotton water-deficit stress, age, and cultivars. Plant response was partly attributable to cotton fleahopper activity. Typical square injury caused by cotton fleahopper was observed (Anon, 2015). In South Texas, yield reduction caused by cotton fleahopper injury was experimentally verified in 2012 (spray effect: p = 0.005) and to a more limited extent in 2013 especially for Phytogen 367 WRF (cultivar by spray interaction: p = 0.028) (Fig. 1). Water-deficit stress and cotton fleahopper stress influences on cotton yield appeared to function independently (no water stress by spray interaction, $p > 0.10$).

In the Texas High Plains, yield reduction attributable to water-deficit stress and cotton fleahoppers were observed, especially in the high water-deficit regime. Synergies in water and cotton fleahopper stress occurred, with enhanced yield loss attributable to cotton fleahopper stress seen in cotton grown in high water-deficit condition artificially infested with cotton fleahopper ($p < 0.05$) but were not seen in cotton grown in a moderate water-deficit regime (Fig. 2).
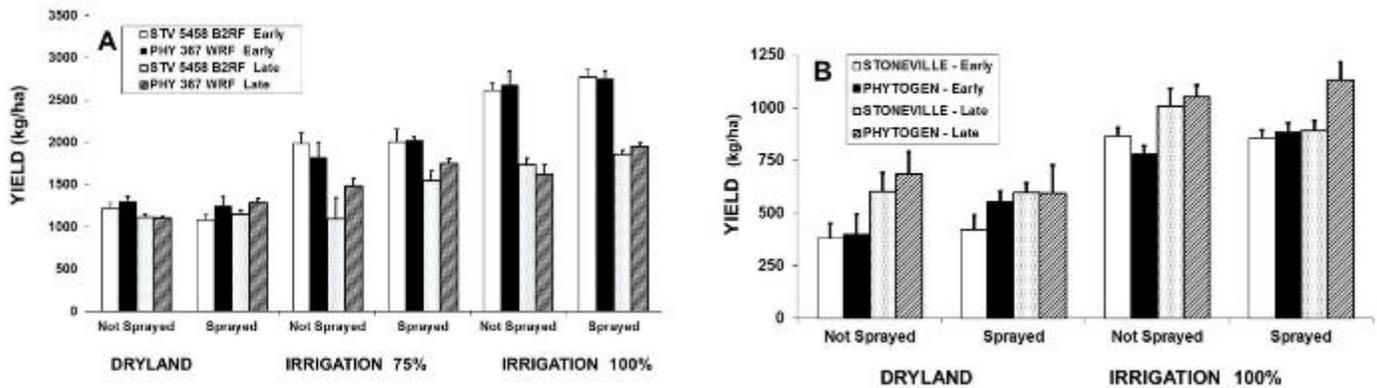
Fig. 1. Lint yield (kg/ha) across water regimes (a dryland mimic [dryland], targeting 75% crop ET replacement in 2012 only [irrigation75%], and targeting 90% crop ET replacement [irrigation 100%]), planting dates (early and late),
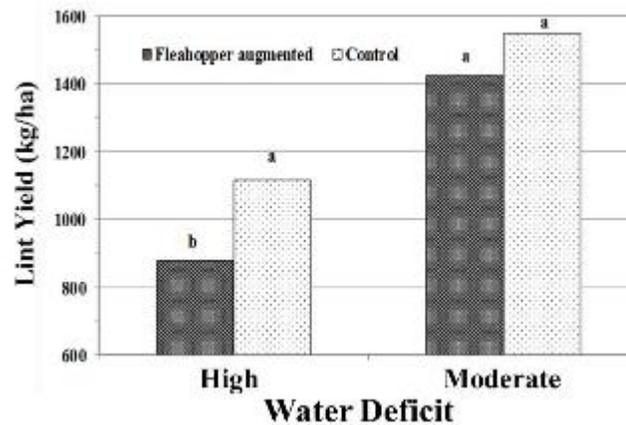
cultivars (Phytogen 367 WRF and Stoneville 5458 B2RF), and insecticide protection (sprayed and not sprayed) exposed to a natural population of cotton fleahopper in 2012 (A), and 2013 (B), Corpus Christi, TX.

Fig. 2. Lint yield (kg/ha) across high water-deficit (High) and moderate water-deficit (Moderate) regimes exposed to augmented populations of cotton fleahopper (fleahopper augmentation and control), Lamesa, TX, 2013. Different letters above bars indicated signficant differences based on Tukey's Honest Significant Difference test (p = 0.05).

South Texas location

Cotton fleahopper densities in the early planting exceeded an economic threshold of 0.30 cotton fleahoppers per plant using beat bucket sampling, which is about equal to 0.15 cotton fleahopper per terminal visually inspected (Brewer et al., 2012). Cotton fleahopper was most abundant during the fourth through sixth week of squaring (the early planting) in 2012 (planting date effect on June 1 and June 14: $p < 0.0006$) (Fig. 3), with more cotton fleahoppers occurring in the unsprayed plots (spray effect: $p < 0.0001$).

Cotton fleahopper densities were higher in the Stoneville cultivar ($p < 0.04$). They were also higher in the earlier planted cotton when grown in poorer soil moisture conditions during the fourth week of squaring (June 1 water regime by planting date interaction: $p < 0.011$) (Fig. 3).
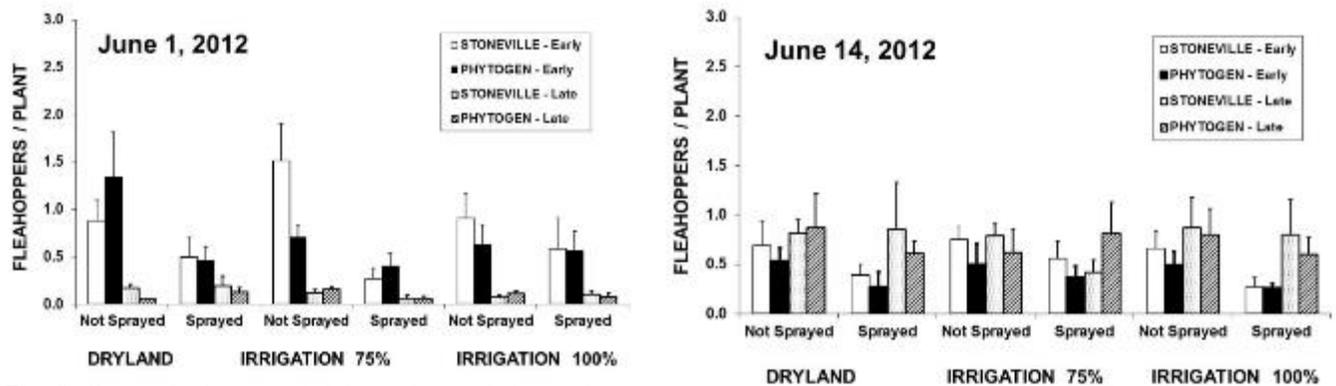
Fig. 3. Cotton fleahoppers (adults and nymphs) per plant
during two sampling dates (June 1 and June 14) taken during the first six weeks of squaring. Data taken were across water regimes (a dryland mimic [dryland], targeting 75% crop ET replacement [irrigation 75%], and targeting 90% crop ET replacement [irrigation 100%]), planting dates (early and late), cultivars (Phytogen 367 WRF and Stoneville 5458 B2RF), and insecticide protection (sprayed and not sprayed) exposed to a natural population of cotton fleahopper, Corpus Christi, TX, 2012.

For earlier planted cotton, cotton fleahopper densities were highest under irrigation targeting 90% crop ET replacement in 2013 (July 3 and July 11 water regime by planting date interaction: $p < 0.05$) (Fig. 4). Insecticide treatment significantly reduced the populations where they were found in high density under good soil moisture, on the Stoneville cultivar, and on early planted cotton (various interactions with the spray treatment were significant, $p < 0.05$) (Fig. 4).
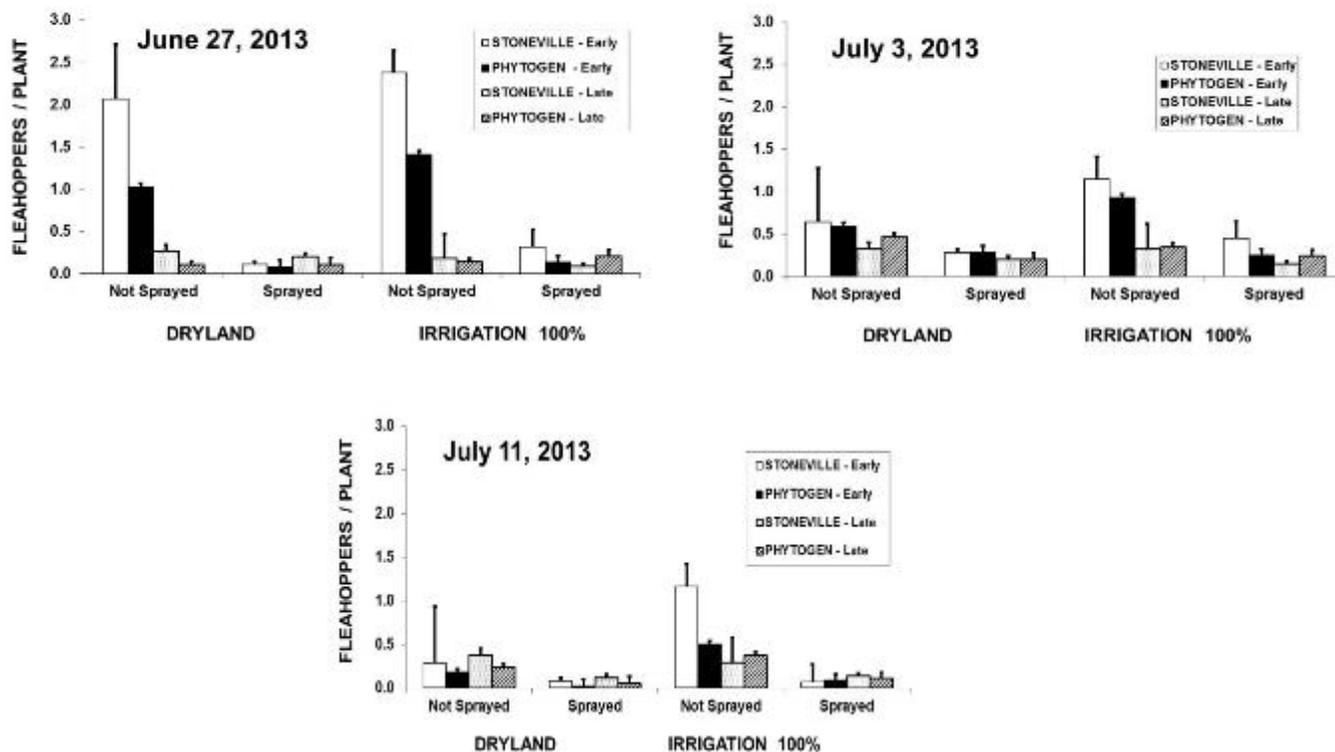
Fig. 4. Cotton fleahoppers (adults & nymphs) per plant during three sampling dates (June 27, July 3, and July 11) taken during the first six weeks of squaring. Data were taken were across water regimes (a dryland mimic [dryland] and targeting 90% crop ET replacement [irrigation 100%]), planting dates (early and late), cultivars (Phytogen 367 WRF and Stoneville 5458 B2RF), and insecticide protection (sprayed and not sprayed) exposed to a natural population of cotton fleahopper, Corpus Christi, TX, 2013.

Cotton cultivars had a strong influence on cotton fleahopper abundance. In 2012, higher densities were found on Stoneville 5458 B2RF than on Phytogen 367 WRF on June 14 (f = 4.91, d.f. = 1,24; p = 0.036) (Fig. 3). In 2013, the Stoneville cultivar planted early tended to build the highest cotton fleahopper populations (July 3 planting date by cultivar interaction: p = 0.025) (Fig. 4). Water stress had no to modest effects on cotton fleahopper densities. No water regime effects on cotton fleahopper densities nor two-way water regime interactions with other factors were observed in 2012 (p > 0.05). In 2013, cotton fleahopper densities continued to build on older cotton (the early planted cotton) grown under no water stress (July 3 and July 11 planting date by water regime interaction: p = 0.05) (Fig. 4).

In contrast, water stress had considerable influence on plant response, while cultivar influences on plant response were much reduced compared to its influence on cotton fleahopper density. Boll retention tended to be marginally higher in the early planted cotton growing under no water stress for both cultivars in 2012 (planting date by water regime interaction: $p = 0.06$) (Fig. 5). In 2013, boll retention was greater in non-water stress conditions (water regime effect: $p = 0.0037$). Boll retention did not significantly vary across cultivars ($p > 0.05$) (Fig. 5). We note that boll retention data were not taken in sprayed plots; therefore yield data was used to directly test for cotton fleahopper-induced plant response.
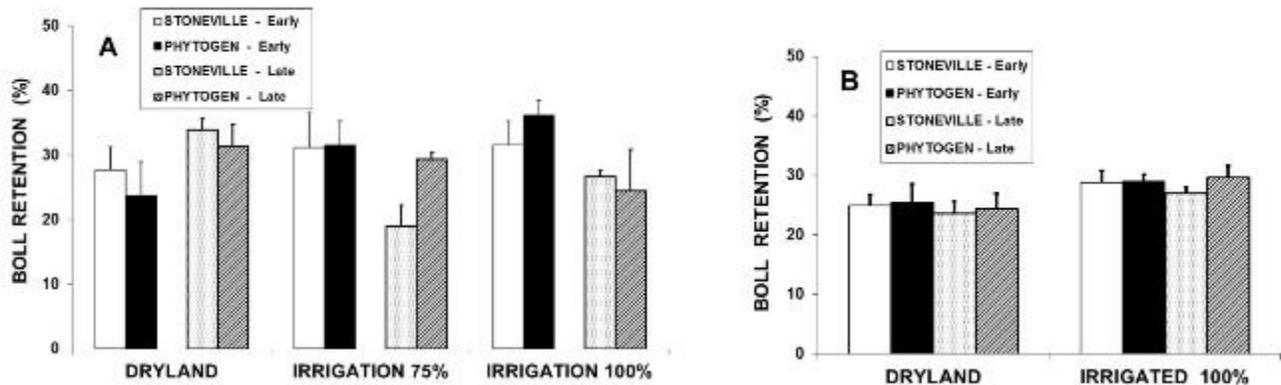
Fig 5. Percent boll retention averaged from all plant bolls taken across water regimes (a dryland mimic [dryland], targeting 75% crop ET replacement in 2012 only [irrigation 75%], and targeting 90% crop ET replacement [irrigation 100%]), planting dates (early and late), and cultivars (Phytogen 367 WRF and Stoneville 5458 B2RF) exposed to a natural population of cotton fleahopper in 2012 (A), and 2013 (B), Corpus Christi, TX.

Yield reduction was less severe in cotton grown under improved soil moisture (where boll retention was also higher), and there was comparably modest yield loss attributable to cotton fleahopper activity. The highest yields were in plots with improved soil moisture (2012 water regime effect: $p < 0.0001$, and 2013 water regime effect, $fp = 0.0008$) (Fig. 1). In 2012, the maximum yield occurred in early planted cotton grown under no water stress (water regime by planting date interaction: $p- < 0.0001$) (Fig. 1), even though cotton fleahoppers were more abundant on the early planted cotton (Fig. 3). The strong influence of soil moisture on yield was consistent across cultivars (no interaction with cultivar), even though cotton fleahopper populations varied significantly across cultivars (Figs. 3 and 4). Controlling fleahoppers modestly benefitted yield in 2012 as indicated by the significant spray factor noted above ($p = 0.005$), and modest yield benefits from controlling cotton fleahopper was also seen in 2013 for the Phytogen cultivar as noted above ($p = 0.028$). Although yield loss attributed to cotton fleahopper was greater on the Phytogen cultivar than on the Stoneville cultivar (Fig. 1), it commonly had fewer cotton fleahoppers (Fig. 3). As noted by Knutson et al. (2013), cotton resistance to cotton fleahopper includes tolerance in which cotton fleahopper presence does not induce yield loss.

High Plains location
In 2013, lint yield was lower in the fleahopper augmented treatment under the high water-deficit regime, while cotton fleahopper augmentation did not significantly lower yield under the moderate water-deficit regime ($p < 0.05$, Fig. 2). The plant may be able to compensate for fleahopper-induced fruit loss under no to modest water-deficit growing conditions. Although not significantly different ($p > 0.05$), the difference in total number of harvestable bolls attributable to the cotton fleahopper augmentation under the high water-deficit water regime (1.4 bolls per plant) was numerically greater than that for moderate water-deficit regime (0.4 bolls per plant) (Fig. 6).
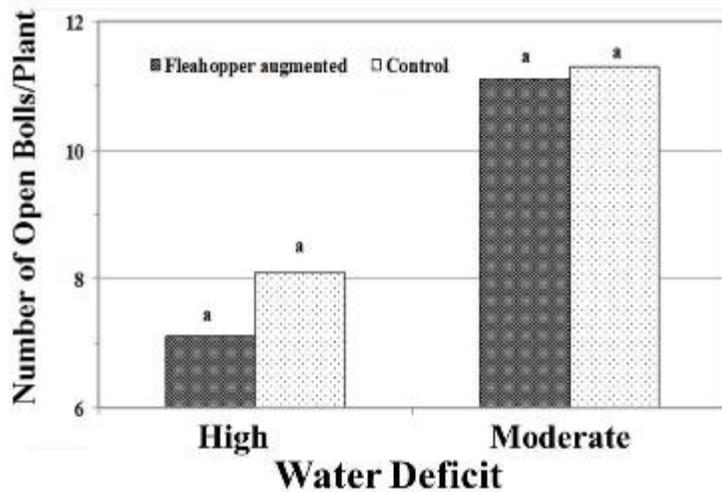
Fig. 6. Total number of harvestable bolls across high water-deficit (High) and moderate water-deficit (Moderate) regimes exposed to augmented populations of cotton fleahopper (fleahopper augmentation and control), Lamesa, TX, 2013. Different letters above bars indicated signficant differences based on Tukey's Honest Significant Difference test (p = 0.05).

Final Remarks

In our study, plant age and cultivar selection were main moderators of cotton fleahopper populations (Figs. 3 and 4), although strong cultivar effects on cotton fleahopper dynamics did not correspond to yield reduction (Fig. 1). We saw few planting date by cultivar interactions, suggesting the influence of these strategies on cotton fleahopper pest management can be considered independently. Water-deficit stress had much more modest influence on cotton fleahopper abundance (Figs. 3 and 4).

In regard to plant response, cotton fleahopper-associated yield loss was lower than water stress-associated yield loss, and the combined effects of water and cotton fleahopper stress on yield were variable. In South Texas, water stress directly affected yield with modest influence from cotton fleahopper (Figs. 1, 2, and 3). In the Texas High Plains, high water stress resulted in reduced yield and a trend toward reduced boll loads, and the effect was enhanced when cotton fleahopper was present (Figs. 2 and 6). The augmented release rate of five nymphs per plant at week three of squaring may have represented higher acute cotton fleahopper pressure than the natural populations experienced in South Texas. These results reflect the field variability seen in plant response to cotton fleahopper feeding, and the paradox of observations of different frequencies of insecticide sprays used to control cotton fleahoppers under apparently equal cotton fleahopper pressure.

We live in a climate that produces highly variable weather, as seen in drought conditions in Texas from 2011 to 2013. For the case of cotton fleahopper feeding on cotton, water-deficit stress affects yield substantially and directly, while our data supported a more modest water stress influence on cotton fleahopper dynamics. Cottton fleahopper-associated yield loss was lower than water stress-associated yield loss. Elevated yield loss attributable to the combined effects of cotton fleahopper and water-deficit stress was was more variable, seen under manipulated (artificial infestations) high cotton fleahopper densities. Cotton fleahopper decision-making may be more cultivar specific than as implied when reviewing regionally-based thresholds that do not mention cultivars (Anon, 2015). Cultivar sensitivity to cotton fleahopper injury leading to yield differences has been previously demonstrated for past cotton cultivars (Ring et al., 1993). For future work, use of more agriculturally representative cultivars should be emphasized, grown under a number of cotton fleahopper exposure scenarios. Including water regime scenarios remains relevant, but enhanced combined effects of water deficit-stress and cotton fleahopper stress appear to be less common than originally hypothesized.

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EFFECT OF WATER LEVELS ON COTTON FLEAHOPPER-INDUCED FRUIT LOSSES IN COTTON
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Abstract

A field study was conducted at the AG-CARES research farm located near Lamesa, Texas, to determine the effect of two irrigation levels (low and high) on cotton fleahopper-induced fruit loss and resulting cotton yield when exposed to varying cotton fleahopper densities. Cotton cultivars FM2011 and DP1454 were planted under a center pivot modified to provide "low" (3.3 inches in-season) and "high" (6.5 inches in-season) irrigation treatments. Field collected cotton fleahopper adults were released onto cotton squares in multi-plant cages. Cotton fleahopper nymphs were released onto uncaged plants. Three cotton fleahopper density treatments included 'high' (5 fleahoppers per plant), 'low' (2 fleahoppers per plant), and an uninfested control. Released cotton fleahoppers were allowed to feed for one week to mimic a natural early-season acute infestation. After one-week feeding period, cages were removed and plants were sprayed with Orthene® 97UP. Pre- and post-plant mapping were conducted to monitor fruiting patterns. The highest lint yield was recorded in control treatment, followed by low cotton fleahopper density, and the lowest lint yield was recorded in the high cotton fleahopper density treatment. Significantly more lint yield was recorded from 'high' irrigation plots compared with 'low' irrigation plots. Cotton variety DP1454 had a significantly higher yield compared to FM2011.

Introduction

Cotton fleahopper (*Pseudomatoscelis seriatus*) is an important economic pest of cotton in Texas. Cotton fleahopper is an early season pest which causes damage to cotton squares, often resulting in fruit loss, delayed crop maturity and potential yield reductions. Cotton may compensate for fleahopper-induced fruit loss by producing new fruits and adding lateral fruiting positions. It is unclear what impact varying irrigation levels will have on cotton fleahopper infestation levels and the resulting impact on crop compensation and final lint yield. It was hypothesized that irrigated cotton would exhibit a greater compensatory capability compared to a low irrigation scheme. The objective of this study was to determine the impact of cotton fleahopper density on cotton fruiting and lint yield under low and high irrigation water regimes.

Materials and Methods

The study was conducted during summer 2015 at the Agricultural Complex for Advanced Research and Extension Systems (AG-CARES) located near Lamesa, Texas. Two cotton cultivars, FM2011 and DP1454, were planted on May 16, 2015 under a center pivot modified to provide replicated "low" (3.3 inches in-season) and "high" (6.5 inches in-season) level irrigation treatments. Laboratory-reared (Hakeem and Parajulee 2015) 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, 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. 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.

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

Results and Discussion

As expected, acute infestations of cotton fleahoppers resulted in lint yield reductions in cotton. While the numerical trend in the effect of cotton fleahopper on cotton lint yield was similar between adults and nymphs (Fig. 2), treatment differences were not detected on nymphal data due to high variability in the data. Nevertheless, significantly lower lint yield was recorded in high fleahopper density treatment (5 fleahoppers adults per plant) compared to that in control (no fleahoppers released) (Fig. 2). While no significant differences were observed in nymphal release treatments, a higher numerical yield was recorded in control followed by low fleahopper density released (2 fleahoppers per plant) and then the high fleahopper density release.
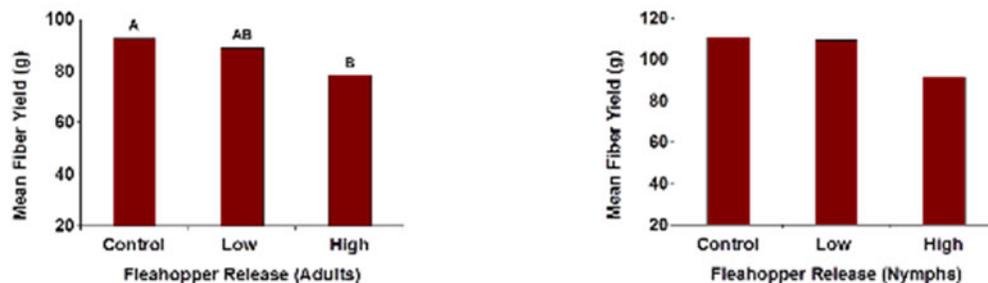
Figure 2. Cotton lint yield (g per 7-plant experimental unit) influenced by an acute infestation of cotton fleahoppers in pre-flower cotton on the AG-CARES farm, Lamesa, TX.

Regardless of the cotton fleahopper infestation, irrigation water level significantly influenced the cotton lint yield (Fig. 3). Significantly more lint yield was recorded from the higher irrigation plots compared with low irrigation plots. Cotton variety DP1454 had significantly more lint yield compared to FM2011 (Fig. 3). Although there were no significant cultivar x water level interactions in the impact of cotton fleahoppers on cotton lint yield, our data suggests that cotton grown under a higher irrigation level may compensate yield loss caused by cotton fleahoppers compared to that in a low water regime. Brewer et al. (2012) also noted this phenomenon in a similar study at both South Texas and Texas High Plains locations.
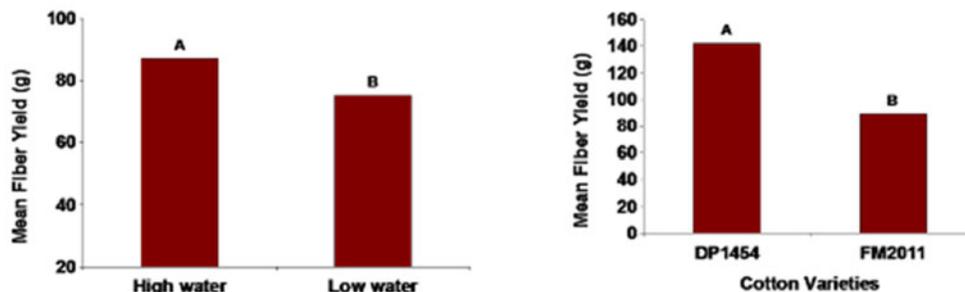

Figure 3. Cotton lint yield (g per 7-plant experimental unit) influenced by irrigation water level (left) and cotton cultivar (right) in absence of cotton fleahopper infestations on the AG-CARES farm, Lamesa, TX.

MODIFIED FIELD-CAGE STUDY TO ESTIMATE *LYGUS* TREATMENT THRESHOLDS IN TEXAS COTTON

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Abstract

A field study was conducted to generate data relevant to developing economic threshold-based management recommendations for *Lygus hesperus* in Texas High Plains cotton. Cotton plants were caged and exposed to five levels of *Lygus* (0, 1, 2, 4 and 6 adults per plant) for one week at 200, 350 and 550 HU after first flowering. One week after *Lygus* releases, cages were removed, harvested one plant from each cage for damage assessment, and sprayed the remaining crop to keep the maturing bolls free from further insect infestations. Higher *Lygus* densities caused greater boll damage as evidenced by more external lesions and internal warts compared to that for lower *Lygus* densities or control cages. Cotton compensated the early season *Lygus*-induced fruit loss, whereas mid-season infestations caused the greatest lint yield reduction. *Lygus* treatment threshold for early-season bolls would be twice the infestation level of our current threshold and up to three times for late season infestations.

Introduction

Texas is the leading cotton, *Gossypium hirsutum* L., growing state within the United States, with approximately 5 million acres (55% of U.S. cotton production) planted in recent years. *Lygus hesperus* is an emerging pest of cotton in the Texas High Plains. In 2013, an overall 2.55% reduction in U.S. cotton fiber yield was attributed to arthropod pests while 0.83% was due solely to *Lygus* species, which was ranked top among other yield-reducing pests (Williams 2014). Both adult and nymphal stages of *Lygus* can cause damage to fruiting cotton. Late-instar *Lygus* nymphs can cause greater damage to the young bolls than adults (Jubb and Carruth 1971, Parajulee et al. 2011).

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 maturity profile and characterizing *Lygus* damage risk dynamics is a necessary prerequisite for developing treatment thresholds. Current pesticide application decisions are based on field scouting, whereby spray applications are typically warranted when *Lygus* populations exceed present economic threshold levels. Generally, cotton bolls that accumulated 350 heat units are safe from piercing-sucking insects. The effect of *Lygus* feeding injuries during early, mid, and late season on lint yield is not clearly understood. The objectives of this experiment were to determine the effect of *Lygus* feeding on lint yield at three crop development stages, 200, 350 and 550 HU (>60 ºF) after first flowering.

Materials and Methods

A field study was conducted at the Texas A&M AgriLife Research and Extension Center farm located near Lubbock, Texas to develop economic threshold-based management recommendations for *Lygus hesperus*, a mid-season pest of Texas High Plains cotton (Fig. 1). Cotton cultivar ST 4946 GLB2 was planted in a field with 40-inch row spacing. The targeted seeding rate was 40,000 seeds per acre. Standard agronomic practices for the Texas High Plains were used. Multi-plant cages were used to accommodate approximately 12 plants within the cage and the cage served as a replication. This study was laid out in a split-plot randomized block design with four replications, 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]). Cage locations were marked on July 27 when 50% of the plants within the uniform stand had their first white flower. The heat unit accumulations were estimated from that point forward. Cages were installed and *Lygus* density treatments were deployed at 200, 350 and 550 HU accumulations. Cages were removed one week after actual releases and we harvested one plant from each cage to estimate *Lygus* damage on bolls. After the cages were removed,

Orthene® 97UP insecticide was sprayed to maintain the experimental row-sections of the field insect-free for the remainder of the growing season. Plant mapping was done before harvest. Bolls were hand-harvested and ginned using a table-top laboratory gin for lint and seed estimation.

Figure 1. *Lygus* adult (left); nymph (middle); and infested boll (right).

Results and Discussion

Feeding marks are indicators of *Lygus* infestation and injury. 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. 2). 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. 2). 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.
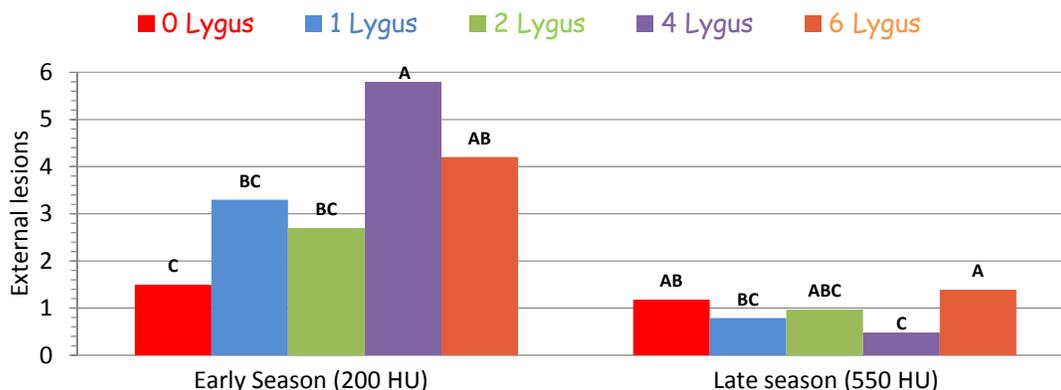
Figure 2. Cotton boll external injury (external lesions) at two phenological stages of cotton following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages, Lubbock, TX, 2015.

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. 3).
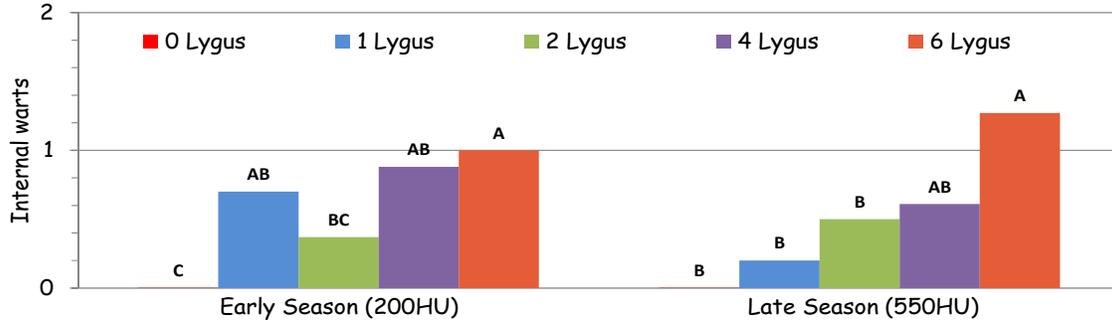
Figure 3. Cotton boll internal injury (internal warts) at two phenological stages of cotton following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages, Lubbock, TX, 2015.

Overall, lint decreased for each successive phenological stages of cotton due to an artifact of experimental logistics (Fig. 4). 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 density of *Lygus* overcompensated the yield as *Lygus* likely fed on young, non-harvestable fruits which provided an opportunity for harvestable bolls to mature (Fig. 4). The seed yield followed the same pattern as observed for lint yield (Fig. 5).
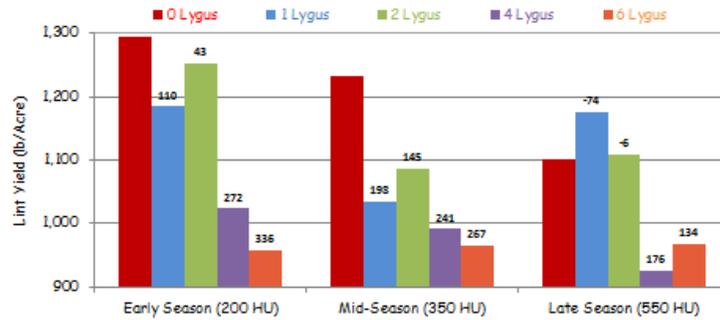
Figure. 4. Cotton lint yield following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages at three cotton phenological stages, Lubbock, TX, 2015.
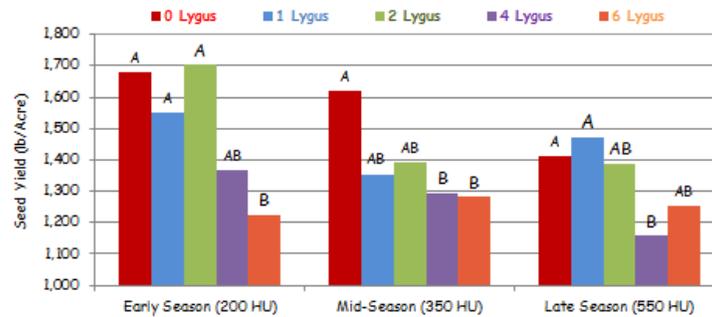
Figure. 5. Cotton seed yield following a 7-day exposure of various densities of *Lygus* adults in multi-plant cages at three cotton phenological stages, Lubbock, TX, 2015.

INFLUENCE OF NITROGEN FERTILIZER ON COTTON HOST-PLANT QUALITY AND ITS IMPACT ON COTTON APHIDS

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Abstract

The relationship between nitrogen fertilizer application in cotton and subsequent changes in lint and seed yield is well-understood. However, little research has been done to evaluate the role of nitrogen fertility in arthropod population abundance in cotton. Previous work suggests that there exists a non-linear relationship between soil nitrogen availability and cotton aphid abundance in cotton. However, interaction between plant-available soil nitrogen and moisture ultimately determines arthropod population dynamics, at least for the cotton aphid. Also, there is a lack of information on plant parameter values with respect to varying rates of available soil nitrogen in cotton production. A multi-year comprehensive field study was conducted to examine the effect of soil nitrogen (residual nitrogen plus applied nitrogen) on cotton agronomic growth parameters and arthropod abundances under a drip irrigation production system. Fixed-rate nitrogen application experimental plots, previously established and fixed for five years prior to the initiation of this study in 2008, consisted of five augmented nitrogen fertility levels (0, 50, 100, 150, and 200 lb/acre) with five replications. Each year, soil in each experimental plot was sampled for residual nitrogen analysis prior to planting. Rates of applied N exceeding 100 lb/acre resulted in higher residual nitrogen detection during the following season. However, variation in residual nitrogen did not significantly affect early plant growth (plant height, root length, or leaf area), except for 150 lb N/acre treatment. Increased N levels corresponded to increased leaf chlorophyll content, but leaf chlorophyll content was generally consistent across nitrogen levels exceeding 100 lb/acre. Aphid abundance was significantly lower in zero N plots versus other plots. Rates of N application exceeding 100 lb/acre resulted in the highest lint yield, but consistent numerical decline in yield beyond 150 lb N/acre in most years suggests that N application beyond 150 lb/acre may be unfavorable for cotton yield.

Introduction

Second to water, nitrogen fertility limits cotton production yields in the Texas High Plains. A three-year study was conducted near Lamesa, Texas, under a limited irrigation production system (Bronson et al. 2006) to characterize the effect of nitrogen application on leaf moisture and leaf nitrogen content in cotton and the resulting influence on cotton aphid population dynamics (Matis et al. 2008). Leaf nitrogen content did not vary with nitrogen application method (variable N versus blanket N application of an optimal amount), but both the blanket application and variable-rate application resulted in significantly higher leaf nitrogen contents than were noted in zero-augmented nitrogen plots. As nitrogen application rates were increased from zero to an optimum rate, a significant decrease in both aphid birth and death rates occurred, translating to a decrease in crowding and an increase in aphid survival (Matis et al. 2008). While these data help to characterize cotton aphid population dynamics between zero nitrogen fertility management and optimal nitrogen application rates, the population dynamics of cotton aphids and other cotton arthropods have not been examined under a full range of nitrogen fertility rates (Parajulee 2007; Parajulee et al. 2006, 2008). In particular, no known study has produced plant growth parameters or fruiting profile data pertaining to a spectrum of nitrogen application rates in cotton. The objective of this study was to evaluate, in cotton growing under a subsurface drip irrigation production system, cotton crop growth parameters and arthropod population abundance, as influenced by varying N fertilizer application rates.

Materials and Methods

The study was conducted at the Texas A&M AgriLife Research farm near Plainview, Texas. A 5-acre sub-surface drip irrigation system had been in place for six years prior to this study. Plot-specific nitrogen fertility treatments had been applied in a randomized block design with five replications since 2002. Five nitrogen application rates (0, 50, 100, 150, 200 lb/acre) had been deployed to the same experimental units consistently for five consecutive years to induce maximum discrimination among treatment plots through variation in soil residual nitrogen (Fig. 1)

Figure 1. Helms Farm nitrogen study experimental plot layout following a five-treatment x five-replication randomized block design. Annually, 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.

The study reported herein was conducted for six years (2008-2013). Soil residual nitrogen was monitored annually by taking two 24-inch core samples from each plot Fig. 2). The 0-12 inch portions of each core were combined to form a single, composite soil sample, and likewise, the 12-24 inch portions were combined, resulting in two samples per experimental plot. Samples were sent to Ward Laboratories, Kearny, Nebraska for analysis. Regionally well-adapted cultivars were used in this study over the duration of the study: FM960B2R was planted on May 13, 2008, May 20 2009, and May 27, 2010, DP104B2RF on June 14, 2011, and FM9063B2RF on May 17, 2012 and May 23, 2013. The experiment consisted of a randomized block design with five treatments and five replications. The five treatments included side-dress applications of nitrogen fertilizer at rates of 0, 50, 100, 150, and 200 lb N/acre. Cotton was planted (56,000 seeds/acre) in 30-inch rows and was irrigated with a subsurface drip irrigation system.

Figure 2. A) Annual pre-season soil sampling of 25 sub-surface drip irrigated cotton plots; B) Annually near the time of first bloom, each plot received the same side-dressed nitrogen application treatment rate; C) Differential cotton plant growth responses are often visually apparent between plots receiving high and low N application rates, Hale County, TX.

Leaf area, plant height, and root length were measured on July 3 (2008), July 20 (2009), July 27 (2010), July 15 (2011), July 6 (2012), and July 22 (2013) to evaluate the influence of residual nitrogen on early plant growth patterns. Except for 2008, leaf chlorophyll content was also measured from 5th mainstem node leaves (n=10 leaves per plot) weekly from July 30 to October 1 (10 weeks) in 2009, August 9 to September 9 in 2010 (5 weeks), July 21 to August 25 (6 weeks) in 2011, July 6 to August 2 (5 weeks) in 2012, and July 22 to September 27 (9 weeks). Soil samples were taken from the experimental plots on July 14 (2008), July 6 (2009), March 25 (2010), April 27 (2011), June 1 (2012, and June 20 (2013) for residual nitrogen analysis. Crop growth and insect activity were monitored throughout the season. Fertility treatments were applied on July 18 (2008), July 10 (2009), July 8 (2010), August 3 (2011), July 6 (2012), and July 11 (2013) with a soil applicator ground rig. COTMAN SQUAREMAN monitoring was used to monitor early plant growth, and was followed by measurement of Nodes Above White Flower (NAWF) for most study years. Pre-harvest plant mapping was used as an indicator of fruit load. Foliage-dwelling mobile arthropods were monitored weekly using a Keep It Simple Sampler (KISS; Beerwinkle et al. 1997) to collect insects from upper-

canopy foliage, beginning from square initiation and ending at crop cutout, for years when arthropod activity occurred (Fig. 3a-f)

Cotton aphid populations did not develop in four (2008, 2011, 2012, and 2013) of the six years of the study, despite repeated applications of cyhalothrin intended to stimulate aphid population growth. Cotton aphid abundance was monitored weekly for five weeks from August 20 to September 17 in 2009 and from August 9 to September 9 in 2010. Hand-harvested yield samples were obtained from each plot. Fiber samples were analyzed for lint quality parameters at the Cotton Incorporated Fiber Testing Laboratory (North Carolina).

Figure 3. A) Blower sampling for arthropods, B) Processing of arthropod samples in the laboratory, C) Measuring leaf chlorophyll, D) Whole-plant sample collection for parameter estimation, E) Measuring leaf area, plant root and shoot biomass, F) cotton harvesting.

Results and Discussion

In all study years, soil residual N levels were significantly higher in plots that received the two highest application rates of N fertilizer versus plots receiving lower-rate N applications or no N augmentation, excepting plots that received 100 lb/acre N in 2012 (Fig. 4). Averaged over the six-year study period, soil residual N levels were lowest in zero and 50 lb/acre plots, although the 50 lb/acre plots had numerically higher residual N than in zero N plots. The highest N augmentation plots (200 lb/acre) had significantly highest average residual N; the year-to-year residual N was always the highest amount in this treatment, at least numerically. The two second highest N augmentation plots (100 and 150 lb/acre) resulted in significantly higher amount of soil residual N compared to that in zero and 50 lb/acre plots. Even though some year-to-year variation in leaf area, plant height, and root length was noted early in the crop season, differential amounts of soil residual N generally did not influence early plant growth, except for 150 lb/acre (Figs. 5-7). The 150 lb/acre treatment was significantly favorable for plant growth during early season contributing to the highest leaf area, plant height, and root length compared to that in other N treatments. Measured leaf chlorophyll content varied with nitrogen application level, and leaf chlorophyll contents from cotton in those plots which received 0 lb N/acre or 50 lb N/acre were significantly lower than all others (Fig. 8). Cotton in plots which received the three highest nitrogen application rates (100, 150, and 200 lb N/acre) exhibited relatively consistent leaf chlorophyll readings (Fig. 8). 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. Cotton aphid activity began in late August in 2009, and densities peaked in early- to mid-September. Cotton aphid densities were significantly lower in 0 lb N/acre treatment plots compared with that in N augmented plots located only feet apart (Fig. 8). There were no significant differences in aphid densities across N augmented plots in 2009. Cotton aphid colonization occurred two weeks earlier

in 2010 compared to that in 2009. While cotton aphid densities remained below economic threshold (50 aphids/leaf for two consecutive weeks) in 2009, aphid populations surpassed economic threshold in all N-augmented plots in 2010, whereas aphids remained below 50/per leaf, except for 1 week, in zero-N plots.

Nitrogen fertility level influenced boll maturity. Bolls in zero applied N plots tended to mature significantly earlier than in N augmented plots. Laboratory measurement of boll exocarp penetrability showed that 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. Variation in soil residual N levels, coupled with variable N application, resulted in phenotypic expression of nitrogen deficiency in cotton across treatment plots, especially between zero N plots and N augmented plots (Fig. 2). The zero N plots consistently produced the lowest lint yield for every year of the six-year study, except in 2010 when 50 lb/acre plots and zero N augmented plots had similar lint yields (Fig. 9). Overall, 150 and 200 lb/acre plots produced the highest lint yield (1,460 lb and 1,430 lb lint for 150 and 200 lb N treatments, respectively), followed by 100 (1,302 lb), 50 (1,190 lb), and zero N (960 lb) plots. Yield increased curvilinearly with each additional 50 lb N added, with the numerically highest average yield (1,460 lb/acre) occurring in augmented 150 lb N/acre treatment, but the yield numerically decreased beyond 150 lb N/acre with additional N. Consistent numerical decline in yield beyond 150 lb N/acre in most years suggests that N application beyond 150 lb/acre may be unfavorable for cotton yield.
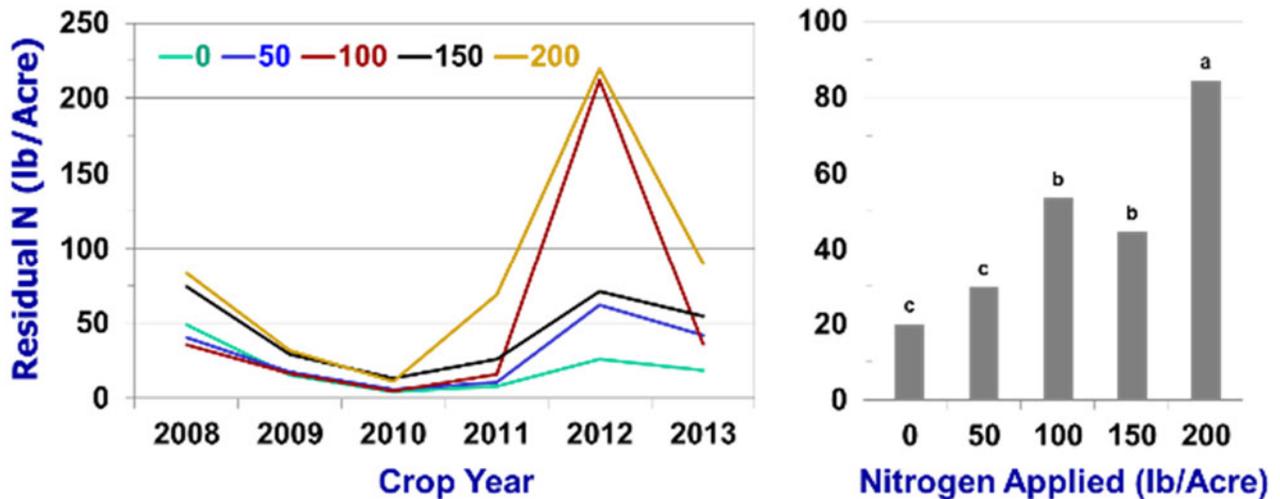
Figure 4. Effect of prior year's N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulation for the current crop year (left) and average residual N over a six-year period (right).
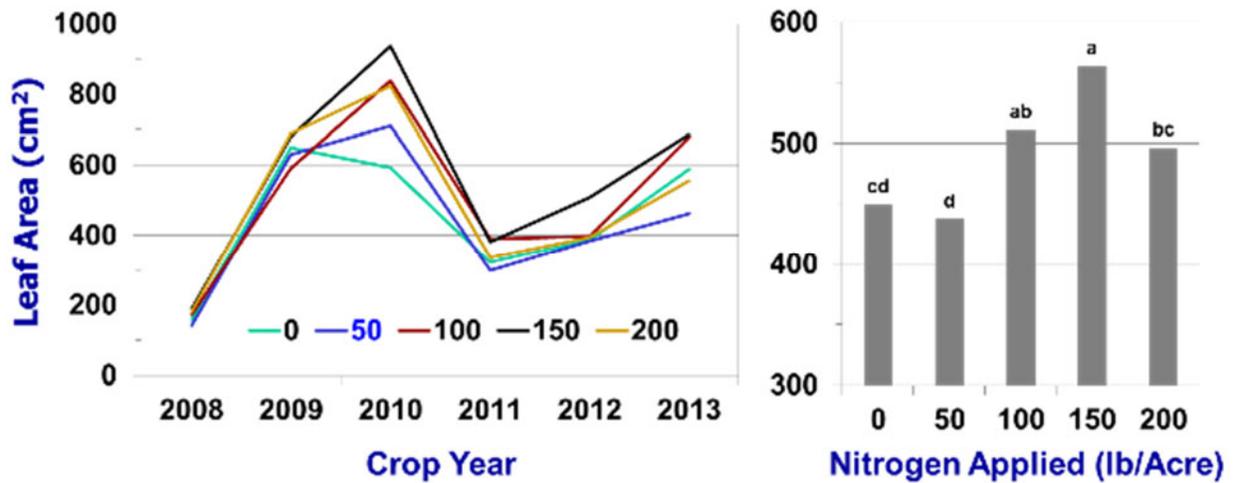
Figure 5. Effect of prior year's N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulation for the current crop year (left) and average residual N over a six-year period (right).
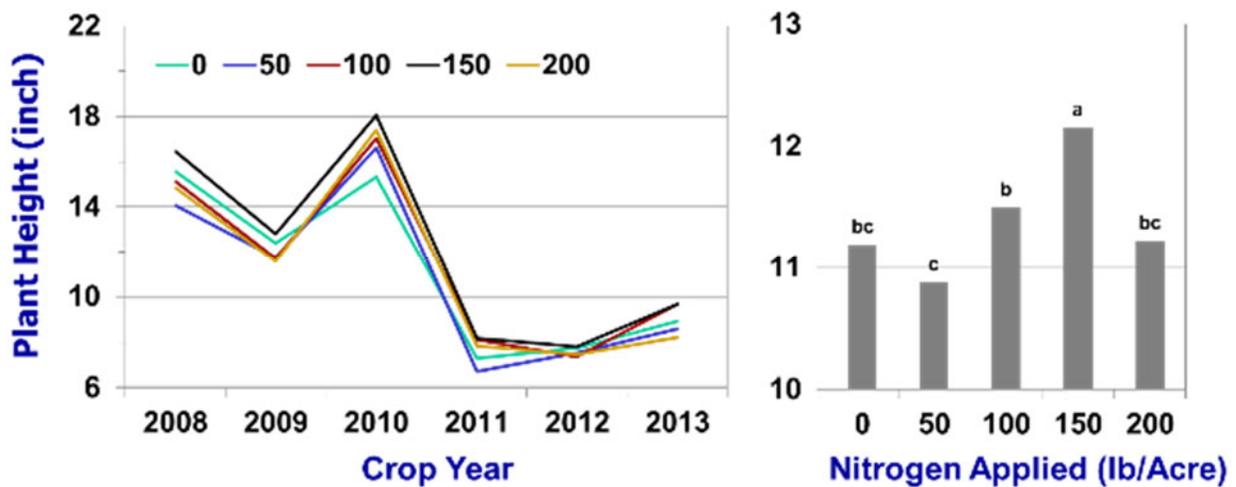
Figure 6. Effect of residual N from the previous crop year on plant height during the early crop growth period of each of the six study years (left) and average plant height over a six-year period (right).
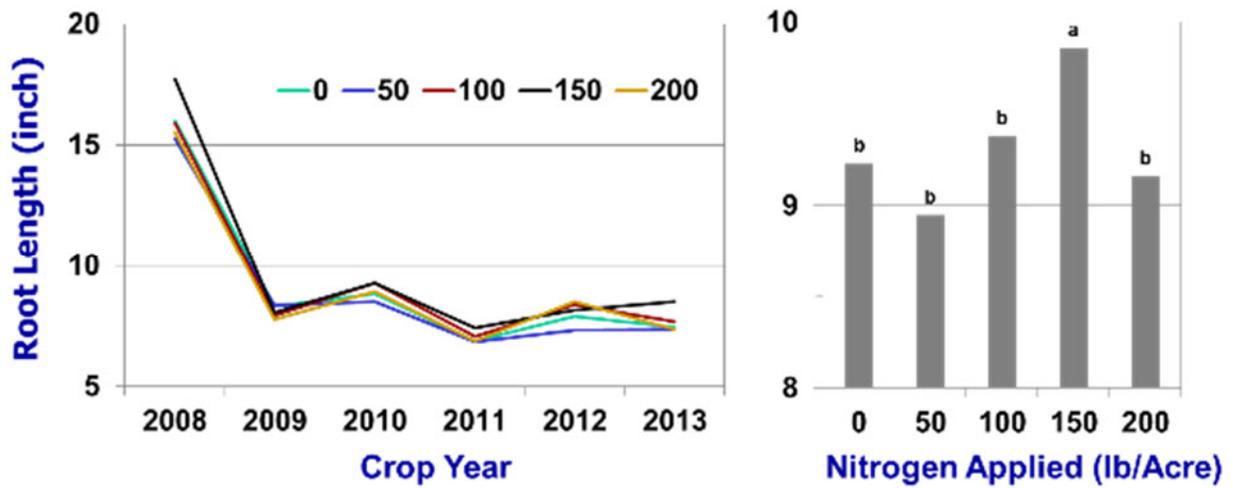
Figure 7. Effect of residual N from the previous crop year on root length during the early crop growth period of each of the six study years (left) and average root length over a six-year period (right).
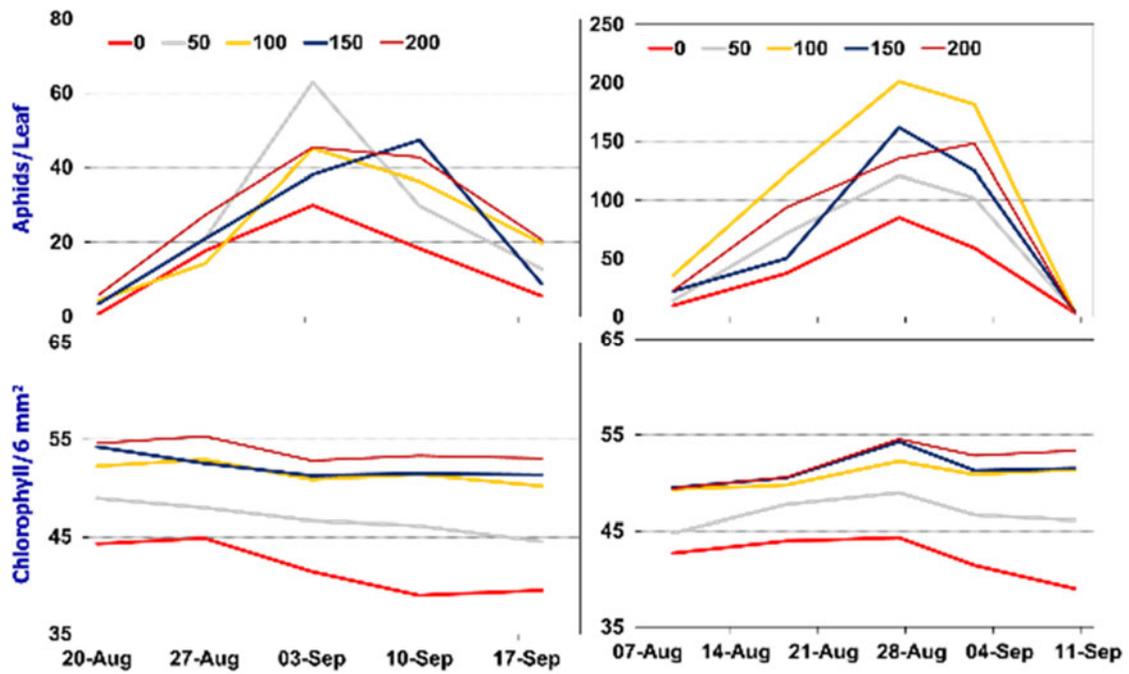
Figure 8. Temporal dynamics of cotton aphid abundance in relation to cotton leaf (5th main stem) chlorophyll content as affected by variable rates of nitrogen application (left chart – 2009, right chart – 2010).
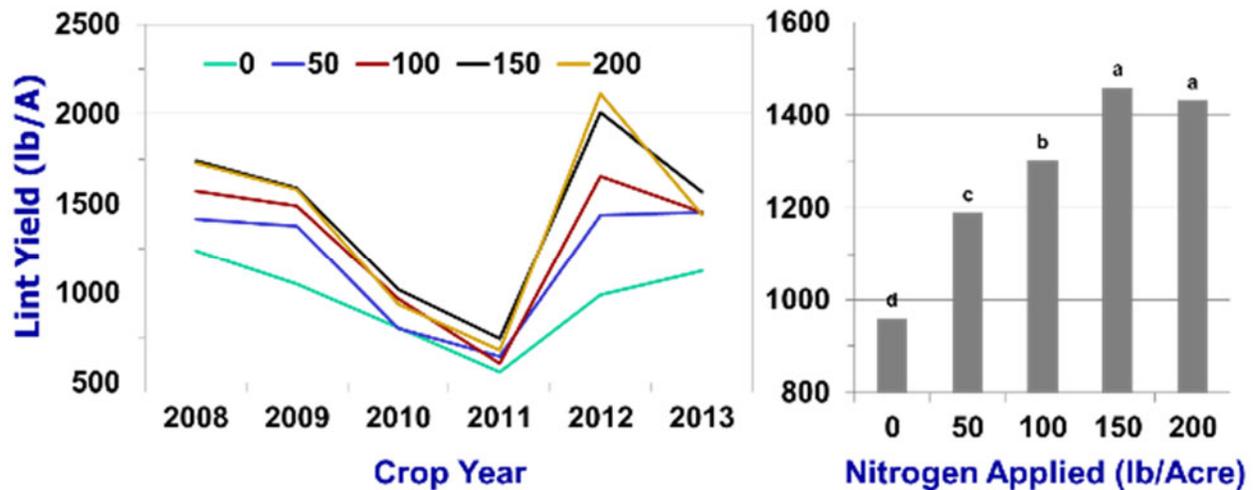
Figure 9. Year-to-year variation in the effect of nitrogen application rates on cotton lint yield (left) and average lint yield over a 6-year period (right), Helms Farm, Hale County, TX.

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ORIGINAL ARTICLE

Intercrop movement of convergent lady beetle, *Hippodamia convergens* (Coleoptera: Coccinellidae), between adjacent cotton and alfalfa

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Abstract A 2-year study was conducted to characterize the intercrop movement of convergent lady beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae) between adjacent cotton and alfalfa. A dual protein-marking method was used to assess the intercrop movement of the lady beetles in each crop. In turns field collected lady beetles in each crop were assayed by protein specific ELISA to quantify the movement of beetles between the crops. Results indicated that a high percentage of convergent lady beetles caught in cotton (46% in 2008; 56% in 2009) and alfalfa (46% in 2008; 71% in 2009) contained a protein mark, thus indicating that convergent lady beetle movement was largely bidirectional between the adjacent crops. Although at a much lower proportion, lady beetles also showed unidirectional movement from cotton to alfalfa (5% in 2008 and 6% in 2009) and from alfalfa to cotton (9% in 2008 and 14% in 2009). The season-long bidirectional movement exhibited by the beetles was significantly higher in alfalfa than cotton during both years of the study. The total influx of lady beetles (bidirectional and unidirectional combined) was significantly higher in alfalfa compared with that in cotton for both years. While convergent lady beetles moved between adjacent cotton and alfalfa, they were more attracted to alfalfa when cotton was not flowering and/or when alfalfa offered more opportunities for prey. This study offers much needed information on intercrop movement of the convergent lady beetle that should facilitate integrated pest management decisions in cotton utilizing conservation biological control.

Key words enzyme-linked immunosorbent assay; *Hippodamia convergens*; mark-capture; protein marker

Introduction

Monocultural practices have been reported to decrease the abundance and effectiveness of insect natural enemies (Altieri & Letourneau, 1982), which may result in increased pest severity in such systems (Andow, 1991;

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Landis *et al.*, 2000). One way to alleviate this problem is through the use of vegetational diversity (Gurr *et al.*, 2004). Conservation of natural enemies through enhancement of vegetational diversity has been a topic of intense study for many years (Root, 1973; Andow, 1991; Parajulee *et al.*, 1997). Increased vegetational diversity conserves natural enemies by improving the availability of food and overwintering or hibernating sites (Landis *et al.*, 2000). Numerous studies demonstrate that diversification of agricultural systems leads to higher densities and diversities of generalist predators (Sheehan, 1986; Bugg & Waddington, 1994; Parajulee *et al.*, 1997; Landis *et al.*, 2000; Langellotto & Denno, 2004). However, it is often

unclear whether higher densities or diversities of natural enemies lead to better pest control and crop protection (Bugg & Waddington, 1994; Snyder *et al.*, 2005). Understanding the underlying population processes of natural enemies such as movement, reproduction, and survival is crucial in predicting how agriculture diversification practices facilitate biological control (Corbett & Plant, 1993; Prasifka *et al.*, 1999; Schellhorn *et al.*, 2000).

Studies have shown that alfalfa, *Medicago sativa* L., provides a year-round reservoir for beneficial insects (Walker *et al.*, 1996; Mensah, 1999) and has been suggested as a best refuge habitat as part of an integrated pest management program (Mensah, 1999). Natural enemy populations often develop in alfalfa fields and expand into other crops where they contribute to suppressing pests (Flint & Roberts, 1988). It has been shown that alfalfa, whether cultivated as forage crop or growing voluntarily along with other weed hosts, serves as a significant reservoir of convergent lady beetles, in most Texas High Plains counties throughout the cotton growing season (Parajulee *et al.*, 2007). Parajulee *et al.* (2007) ranked plant species based on number of adult lady beetles captured. Alfalfa was ranked 2nd (February–March), 4th (June–July), 3rd (August–September), and 1st (October–November) among cotton and >20 weed host species. This clearly suggests that alfalfa harbors relatively large numbers of adult convergent lady beetles throughout the season. Convergent lady beetles may move from alfalfa to adjacent cotton and provide natural pest suppression. Though several studies support this, a basic understanding of predator movement in a cotton–alfalfa system is generally lacking.

Understanding the movement of predatory insects between crops is of great importance in using habitat manipulation to enhance biological control services (Jervis *et al.*, 2004; Lavandero *et al.*, 2004; Snyder *et al.*, 2005). Information on natural enemy movement, spatial distribution, and density can be generated through the application of marking techniques (Lavandero *et al.*, 2004). Various marking techniques are used to study the movement of insects (Hagler & Jackson, 2001). Protein marking methodology has been successfully used in various studies of insects such as convergent lady beetle (Hagler & Naranjo, 2004), pink bollworm (Hagler & Miller, 2002), pear psylla (Jones *et al.*, 2006), thrips (Jasrotia & Ben-Yakir, 2006), and honeybee (Hagler *et al.*, 2011). A field marking study using protein markers and ELISA protein detection revealed that *Lygus* move back and forth between alfalfa and cotton, and as such, alfalfa acts as both a source and a sink for *Lygus* in the cotton–alfalfa agroecosystem depending on the phenological stages of the crop (Shrestha *et al.*, 2009).

The overall objective of this project was to study the intercrop movement behavior of *H. convergens*, using a dual protein marking approach. The information gathered in this study will be useful in the development of pest management strategies to utilize naturally occurring biological control agents for insect pest suppression in cotton–alfalfa agroecosystems. Specific objectives of this study were to: (i) characterize convergent lady beetle movement between adjacent cotton and alfalfa and (ii) determine the timing and magnitude of convergent lady beetle movement from alfalfa to cotton throughout the growing season.

Materials and methods

Site selection and plot layout

A 2-year mark-capture study was conducted at the Texas A&M University AgriLife Research Farm, Lubbock, Texas to characterize season-long convergent lady beetle intercrop movement between adjacent cotton and alfalfa. A site was selected with 12 rows of cotton (180 m × 12 m) adjoined by an alfalfa field of equal area. A FiberMax® cotton variety, FM 9063 B2RF, was planted on May 19, 2008 and May 22, 2009, respectively. Alfalfa seed was sown a year in advance (April 30, 2007) to establish a crop. Both crops had similar soil type and irrigation practices. The crops were divided into three 60-m blocks. There were 4 equidistant subsampling areas (60 m × 0.508 m) per block in alfalfa in 2008 and 3 per block in 2009. Thus, in alfalfa, there were 12 subsamples in 2008 and 9 in 2009. In cotton, the 5th and 10th rows from the edge of the alfalfa plot were reserved for subsampling (60 m × 1.016 m) in each block. Thus, in cotton, there were 6 subsamples per year. Cutting the alfalfa to a lower plant height (mowing), the standard farming practice to harvest alfalfa hay, was applied to alfalfa once in 2008 on August 13 and twice in 2009 on August 3 and August 28 during the sampling periods.

Field marking

Two crude food proteins were selected for application in alfalfa and cotton. An egg white (10%) and water solution, hereinafter referred to as EW (10% EW, Papetti Farm® AllWhites® 100% Liquid Egg Whites, Michael Foods, Minnetonka, MN, USA), was sprayed in alfalfa, while a nonfat dry milk (10%) and water solution, hereinafter referred to as NFDM (10% NFDM, Great Value™ Nonfat Dry Milk, Wal-Mart Stores, Inc.), was sprayed in cotton, each at a rate of 285 L/ha. Spray applications were made, approximately at weekly intervals, from the 7–8 true leaf stage of cotton through boll development during

the cotton growing seasons of 2008 (9 applications) and 2009 (7 applications). Extra care was taken to avoid protein marker solution cross-contamination. Separate tanks were used to load and spray the markers. Marker solution applications were performed with a tractor-driven, 4-row boom sprayer, which was thoroughly cleaned after each use. Insect marker acquisition occurred either during spray application or after by residual contact by the insects with the marked plant tissue (Hagler *et al.*, 2014).

Insect sampling

Insect samples were collected from each subsampling area via a "keep it simple" (KIS) sampler after 24 h of protein marker application. A KIS sampler is a handheld pneumatic sampling device, in this case, made locally by modifying an Echo® PB-265 backpack leaf blower (nominal air flow rating: 458 cfm) with an insect collecting mesh net (Beerwinkle *et al.*, 1997). Clean insect collecting mesh net was used at each sampling time for each individual sample to avoid contaminations. Insect sampling was performed in cotton and alfalfa on 9 sampling dates in 2008 and 7 sampling dates in 2009. Insect samples were stored in 3.78 L Zip-Loc® bags and insects in samples were killed by freezing at −20 °C. Soon after freezing, the convergent lady beetles were placed individually into 1.5-mL microcentrifuge tubes and stored at −20 °C for further processing via ELISA assay.

Insect sampling was similar to survey sampling where samples were taken from various plots in 2 crop fields on several dates. The experimental design consisted of sampled areas as main plots with sampling dates as subplots. Each subsample from cotton was taken from a 60 m × 1.016 m sampling section (0.00619 hectare) and each subsample in alfalfa was taken from a 60 m × 0.508 m sampling section (0.003095 hectare). In order to report convergent lady beetle counts on a "per hectare" basis, the reciprocal of each hectare sampling area value was taken. The resulting multiplication factors were 322.80 and 161.40 for alfalfa and cotton, respectively. Subsequent analysis was done considering insects per hectare.

Indirect enzyme-linked immunosorbent assay (ELISA)

Indirect ELISA was performed for each individual beetle to examine protein marker adherence using the protocols described by Jones *et al.* (2006). Since the 2 markers are not cross-reactive, testing of a single lady beetle specimen for both markers was possible, allowing for tracking of intercrop movement. The antigen solution from each convergent lady beetle specimen was obtained

by washing the beetle in 300 μL of 1× TBS buffer. Eighty microliter of antigen solution thus obtained by washing individual beetle was loaded into a single well in the 96-well microtiter plate. When testing for NFDM, a bovine casein standard was used as a positive control, whereas when testing for EW, a chicken egg albumen standard was used. Negative controls were included for each test which consisted of 80 μL solution obtained by washing individual unmarked beetle with 300 μL of 1× TBS buffer. The spectroscopy was carried out on the microtiter plate, with absorbance readings taken at a light wavelength of 650 nm using a Stat Fax 3200 plate reader (Awareness Technology, Inc., FL, USA). Absorbance values or optical density (OD) for each sample was then compared with the absorbance values of 8 known negative samples from unmarked beetles. The cutoff threshold OD value of the sample was equal or greater than the mean absorbance value of the known negatives, plus 3 times the standard deviation from the mean. A positive reading occurred when the absorbance value of the individual insect sample was equal or greater than the cutoff value and samples were interpreted as negative when the absorbance value was less than the cutoff value (Hagler, 1997).

Categorization of the marked convergent lady beetles

Based on the marker detection on convergent lady beetle samples via indirect ELISA, the total captured convergent lady beetles were categorized into 5 categories. These categories provided the basis for characterization of convergent lady beetle movements. These categories were: (i) Double-marked immigrant: captured convergent lady beetles testing positive for both crop-specific protein markers applied to cotton and alfalfa were classified as "double-marked immigrants." These convergent lady beetles showed bidirectional movement (back and forth) between the crops. (ii) Single-marked immigrants: captured convergent lady beetles testing positive only for a protein marker not applied to the capture source host were classified as "single-marked immigrants." These insects showed unidirectional movement into the capture source host. "Capture source host" refers to the host crop from which the convergent lady beetles were collected. (iii) Total immigrants: captured convergent lady beetles testing positive for both protein markers applied to cotton and alfalfa plus captured convergent lady beetles testing positive only for a protein marker not applied to the capture source host were classified as "total immigrants." Thus, this group, "total immigrants," is the combination of bidirectional and unidirectional movement shown by convergent lady beetles which represent the total convergent

lady beetle influx in a crop from the other crop within the 24-h postapplication time frame. (iv) Resident: captured insects testing positive only for the protein marker applied to the capture source host were classified as "resident." These insects showed no movement from 1 host crop to another and chose to remain in the same crop within the 24-h postapplication time frame. (v) Unmarked: captured insects testing negative for both protein markers, regardless of the capture source host, were classified as "unmarked." It might be possible that these insects were in the experimental plot and did not get marked or that our detection technique failed to detect the marker adhering to their bodies. However, the most likely scenario is that these insects moved into the experimental field from the surroundings.

Data analysis

Proc TTEST (SAS Institute, 2008) was used to separate the means for years and host. The effect of cotton phenology on the convergent lady beetle intercrop movement behavior was also determined by grouping the data into 3 cotton phenological stages: cotton squaring, flowering, and boll growth. All sample data from each phenological stage were averaged and the effect of cotton phenology on the movement behavior was analyzed. Because crop phenology contained 3 levels, an LSD test with $\alpha = 0.05$ was used to separate the means.

Data were also analyzed using days after planting (DAP) of cotton as the regression variable. Following Draper and Smith (1981), the zero was moved to the mean DAP for each year: 2008, center was at 78.11 (August 5) so all DAP had the mean subtracted ($X - \bar{x}$); and for 2009, the center was at 70.15 (July 31). The weekly lady beetle captures were essentially independent of each other. This fact allowed for the accumulation of the counts from sample time to sample time. This cumulative presentation of the data was divided into 4 sets based on crop habitat and year of the study (2008 alfalfa, 2008 cotton, 2009 alfalfa, and 2009 cotton), and each set was fitted with linear or quadratic (curvilinear) regression (Proc MIXED; SAS Institute, 2008) models. The regression coefficients from the various fitted lines were tested using the *t*-test.

Results

Convergent lady beetle abundance

During the 2 years of study, a total of 267 samples were collected (96 from cotton and 171 from alfalfa).

A total of 1414 adult convergent lady beetles were retrieved from these samples. Out of the total captured, 345 convergent lady beetles were from cotton while 1069 were from alfalfa. Total numbers of adult convergent lady beetles captured in cotton in 2008 and 2009 were 184 and 161, respectively. Similarly, numbers of adult convergent lady beetles caught in alfalfa in 2008 and 2009 were 716 and 353, respectively (Table 1). In 2008, convergent lady beetle populations showed conspicuous fluctuations in abundance across sampling dates (Fig. 1A). The highest numbers were encountered in late July and early September. The lady beetle population increased in mid-July and declined from late August to early September (Fig. 1A). In 2009, the number of convergent lady beetles captured in alfalfa was higher in early July; the numbers then declined and remained relatively low (<1000/hectare) until mid-August when the convergent lady beetle numbers sharply increased from late August to early September (Fig. 1B). For 2009, the number of convergent lady beetles in cotton increased slightly in late July and then slowly declined from late August to early September.

The total abundance of convergent lady beetles was significantly higher in alfalfa ($P = 0.011$, 2008; $P = 0.016$, 2009) compared to that in cotton (Table 2). In 2008, the number of total captured convergent lady beetles from alfalfa remained constant across all cotton phenological stages (Table 3). In cotton, however, it was significantly higher during the flowering stage compared to that in boll development stage. In 2009, the lady beetle abundance in alfalfa was significantly higher during cotton boll development than squaring and flowering, while in cotton, it was significantly higher during cotton flowering than squaring. Interestingly, in 2009, a numerically higher number of convergent lady beetles was captured in cotton than alfalfa during cotton flowering. The total captured lady beetles in alfalfa were significantly higher than cotton ($P = 0.005$) during squaring stage of cotton in 2009 (Table 3).

The rate of population increase in convergent lady beetles in alfalfa was progressively higher than cotton in 2008 from the beginning of the season. The slope of the predicted mean density for alfalfa, based on the regression of days after cotton planting, was significantly greater than for cotton in 2008 ($P < 0.0001$; Table 4; Fig. 2A). In 2009, the rate of increase in number of lady beetles in alfalfa and cotton remained similar until mid-August and increased sharply thereafter in alfalfa compared to that in cotton. The slope of the predicted mean density of lady beetles from alfalfa was similar to that from cotton in 2009 (Table 4; Fig. 2A).

Table 1 Variously marked and categorized convergent lady beetles captured in adjacent alfalfa and cotton fields expressed as number per hectare and percentage, Lubbock, Texas, USA.

	Alfalfa			Cotton		
	Actual count	Count/ha	%	Actual count	Count/ha	%
2008						
Resident (a)	214	69 079	30	45	7 263	24
Double-marked immigrant (b)	330	106 524	46	84	13 558	46
Single-marked immigrant (c)	36	11 621	5	18	2 905	10
Total immigrant (b + c)	366	118 145	51	102	16 463	56
Total marked (a + b + c)	580	187 224	81	147	23 726	80
Unmarked (d)	136	43 901	19	37	5 972	20
Total captured (a + b + c + d)	716	231 125		184	29 698	24
2009						
Resident (a)	48	15 494	14	30	4 842	19
Double-marked immigrant (b)	252	81 346	71	90	14 526	56
Single-marked immigrant (c)	22	7 102	6	23	3 712	14
Total immigrant (b + c)	283	91 352	77	113	18 238	70
Total marked (a + b + c)	322	103 942	91	143	23 080	89
Unmarked (d)	31	10 007	9	18	2 905	11
Total captured (a + b + c + d)	353	113 948		161	25 985	

$N = 27$ (9 weeks and 3 plots) in 2008 and $N = 21$ (7 weeks and 3 plots) in 2009. Samples from alfalfa and cotton had hectare conversion factors of 322.80 and 161.40, respectively.

Table 2 Comparison of hosts for total captured and variously marked and categorized convergent lady beetles (number per hectare) averaged over sampling dates found in 2 adjacent fields (alfalfa and cotton), Lubbock, Texas, USA.

Variables	Alfalfa (mean ± SE)	Cotton (mean ± SE)	df	t	P
2008					
Total captured	2140 ± 343	550 ± 80	4	4.53	<0.0106
Double-marked immigrant	987 ± 227	252 ± 55	4	3.16	<0.0343
Single-marked immigrant	108 ± 40	54 ± 14	4	1.30	<0.2640
Total immigrant	1267 ± 1763	304 ± 58	4	5.19	<0.0066
Resident	640 ± 48	135 ± 34	4	8.61	<0.0016
Unmarked	407 ± 67	111 ± 45	4	3.98	<0.0165
2009					
Total captured	1809 ± 292	619 ± 50	4	4.02	<0.0158
Double-marked immigrant	1292 ± 162	346 ± 14	3	5.82	<0.0043
Single-marked immigrant	113 ± 41	89 ± 37	4	0.44	<0.6816
Total immigrant	1386 ± 182	418 ± 29	4	5.24	<0.0063
Resident	246 ± 68	115 ± 35	4	1.91	<0.1284
Unmarked	159 ± 51	70 ± 14	4	1.72	<0.1609

Means and SE are from Proc TTEST (SAS Institute, 2008). Reduced degrees of freedom are the consequence of Satterthwaite test which results from the unequal variance test.

Field marking efficiency

The cotton and alfalfa fields were marked with 10% NFDM and 10% EW protein markers. The sampling for adult convergent lady beetles was carried out 24-h postapplication. The result showed that the majority of the lady beetles from the sprayed fields could be successfully marked with this technique; cotton (80% in 2008
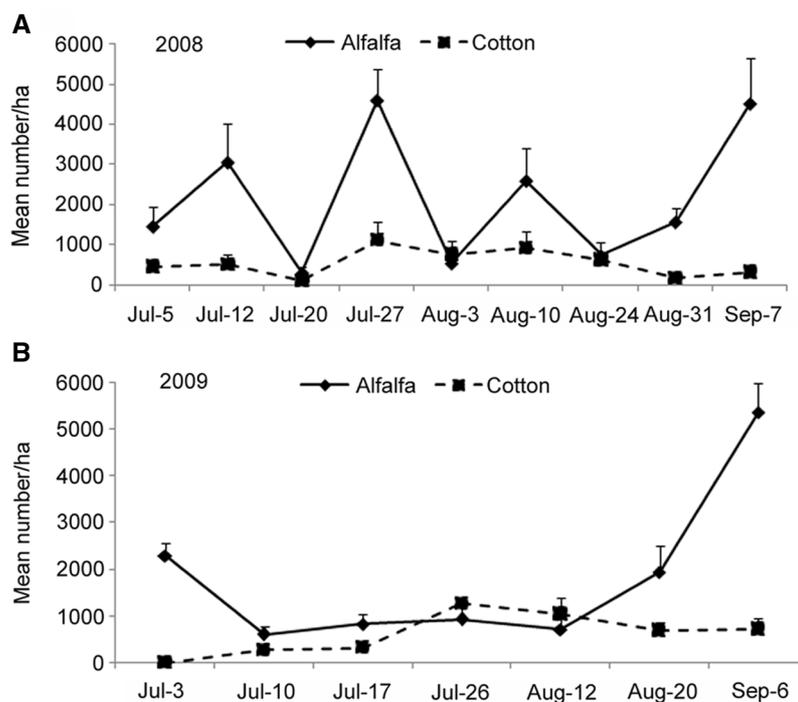
Fig. 1 Seasonal abundance of convergent lady beetles in adjacent alfalfa and cotton fields, Lubbock, Texas, USA.

and 89% in 2009) and alfalfa (81% in 2008 and 91% in 2009, Table 1).

A high percentage of the captured convergent lady beetles in adjacent cotton and alfalfa were marked with both protein markers sprayed in the crops. For 2008 and 2009, the percentages of double-marked convergent lady beetles were 46% and 56% in cotton and 46% and 71% in alfalfa, respectively (Table 1).

Convergent lady beetle movement between cotton and alfalfa

The field marking technique clearly indicated 2 movement patterns shown by convergent lady beetles: bidirectional and unidirectional. Some of the lady beetles also showed no movement from their respective host where they were already present. The season long bidirectional movement as shown by double-marked immigrants was significantly higher in alfalfa ($P = 0.03$, 2008; $P = 0.004$, 2009) compared to that in cotton (Table 2). However, the unidirectional movement as shown by single-marked immigrant lady beetles did not vary between treatment crops in both years. The total influx of convergent lady beetles (bidirectional and unidirectional combined) was significantly higher in alfalfa ($P = 0.0066$, 2008; $P = 0.0063$, 2009). Resident convergent lady beetles (those beetles

that stayed in the source host and did not show movement) were significantly higher in alfalfa ($P = 0.0016$) compared to that in cotton in 2008 (Table 2).

The rate of increase in double-marked immigrant lady beetles was higher in alfalfa as compared to cotton in 2008. The slope of the predicted mean densities for alfalfa was significantly higher than for cotton ($P < 0.0001$, Table 4, Fig. 2B). In 2009, the rate of increase in double-marked immigrant lady beetles was rapid in alfalfa compared to that in cotton toward the end of the season. However, the slopes of the predicted mean densities for alfalfa and cotton were not significantly different (Fig. 2B).

In 2008, the rate of increase of resident lady beetles in alfalfa was higher than cotton. The slope of the predicted mean based on the regression for alfalfa was significantly higher than cotton ($P < 0.0001$, Fig. 2C). In 2009, the rate of increase in resident convergent lady beetles in alfalfa and cotton was similar as indicated by the similar slope of the predicted mean for alfalfa and cotton (Fig. 2C).

The rate of increase in single-marked immigrant lady beetles in alfalfa was much more rapid compared to that in cotton in 2008 and the slope of the predicted mean based on regression of DAP for alfalfa was significantly higher ($P < 0.0001$, Table 4, Fig. 2D) than for cotton. In 2009, the rate of increase in immigrant convergent lady beetles was similar in alfalfa and cotton (Fig. 2D).

Table 3 Cotton phenological stage-specific comparison of hosts for total captured and variously marked convergent lady beetles (number per hectare) averaged over sampling dates found in adjacent alfalfa and cotton fields, Lubbock, Texas, USA.

Variable	Year	Phenology	Alfalfa (mean ± SE)	Cotton (mean ± SE)	df	t	P value
Total captured	2008	Squaring	1596 ± 511 a	421 ± 114 ab	4	2.48	0.068
		Flowering	2565 ± 748 a	924 ± 219 a	4	2.27	0.085
		Boll growth	2260 ± 763 a	305 ± 86 b	3	2.74	0.110
	2009	Squaring	1243 ± 277 b	197 ± 61 b	4	10.50	0.005
		Flowering	825 ± 175 b	1157 ± 220 a	4	−0.94	0.398
		Boll growth	3641 ± 927 a	713 ± 101 ab	3	4.13	0.052
Double-marked immigrant	2008	Squaring	72 ± 32 b	63 ± 33 b	4	0.24	0.820
		Flowering	1426 ± 412 a	502 ± 176 a	4	2.30	0.082
		Boll growth	1462 ± 593 a	188 ± 53 ab	3	3.75	0.063
Single-marked immigrant	2009	Squaring	813 ± 192 b	108 ± 31 c	4	12.97	0.002
		Flowering	574 ± 127 b	632 ± 181 a	4	−0.31	0.770
		Boll growth	2726 ± 875 a	417 ± 77 b	4	5.50	0.005
	2008	Squaring	45 ± 28 a	99 ± 49 a	4	−1.61	0.183
		Flowering	206 ± 96 a	45 ± 36 a	4	1.55	0.195
		Boll growth	72 ± 40 a	18 ± 18 a	4	1.06	0.348
	2009	Squaring	96 ± 49 a	27 ± 14 a	4	1.50	0.207
		Flowering	36 ± 23 a	148 ± 64 a	4	−1.53	0.201
		Boll growth	215 ± 108 a	121 ± 78 a	4	0.74	0.502
Total immigrant	2008	Squaring	162 ± 82 b	162 ± 55a	4	0.00	0.999
		Flowering	2386 ± 172 a	547 ± 197a	4	7.05	0.002
		Boll growth	1256 ± 517 b	207 ± 24a	3	2.03	0.179
	2009	Squaring	909 ± 94 b	135 ± 27 a	4	7.97	0.001
		Flowering	609 ± 187 b	780 ± 140 a	4	−0.73	0.507
		Boll growth	2941 ± 496 a	538 ± 13 a	2	4.85	0.039
	2008	Flowering	673 ± 180 a	224 ± 80 a	4	2.29	0.084
		Boll growth	530 ± 226 a	81 ± 27 a	3	1.98	0.183
	2009	Squaring	251 ± 95 a	54 ± 27 a	4	2.27	0.085
		Flowering	128 ± 67 a	162 ± 121a	4	−0.24	0.822
		Boll growth	359 ± 100 a	162 ± 84 a	4	1.51	0.205

Proc TTEST (SAS Institute, 2008). *P* values indicate if differences between hosts are significant at different phenological stages of cotton. Means with different letters at different phenological stages of cotton within each host are significantly different at *P* = 0.05.

Effect of cotton phenology on convergent lady beetle movement

In 2008, the number of double-marked immigrant lady beetles captured in alfalfa was significantly higher during the cotton flowering and boll growth stages than during squaring, whereas in cotton it was significantly higher during flowering than the squaring (Table 3). In 2009, the number of double-marked immigrant lady beetles captured in alfalfa was significantly higher during cotton boll development stage than squaring and flowering. In cotton, it was significantly higher during flowering, followed by boll development and squaring. The num-ber of double-marked immigrant lady beetles captured in 2009 was significantly higher in alfalfa than cotton at squaring (*P* = 0.002) and boll development (*P* = 0.005) stages.

In both years, densities of single-marked immigrant lady beetles within and between the 2 crops did not significantly vary in all 3 cotton phenological stages. On the other hand, the total lady beetle immigration in alfalfa was significantly higher during cotton flowering stage compared to cotton squaring and boll growth stages in 2008, whereas it was significantly higher in boll growth stage than during squaring and flowering stages in 2009. There was no significant difference in total influx of

Table 4 Regression coefficients for the fitted lines in Figures 2A–D.

Year	Host	Variable	N	Intercept	Linear	Quadratic	Cubic	$P > F$
2008	Alfalfa	Total	27	9941	244	na	na	0.0001
		DMI	27	3357	122	na	na	0.001
		Residents	27	3796	73.57	−0.6278	na	0.0093
		SMI	27	475	15.01	na	na	0.001
2009	Alfalfa	Total	21	4653	66.56	1.3463	0.0748	0.0783
		DMI	21	2087	29.27	1.009	0.0714	0.0092
		Residents	21	958	18.62	na	na	0.001
		SMI	21	420	9.25	na	na	0.001
2008	Cotton	Total	27	3270	77.13	−0.6544	na	0.0935
		DMI	27	1202	39.33	na	na	0.001
		Residents	27	717	18.64	na	na	0.001
		SMI	27	374	4.52	na	na	0.001
2009	Cotton	Total	21	1937	72.06	na	na	0.001
		DMI	21	1080	39.24	na	na	0.001
		Residents	21	350	12.65	na	na	0.001
		SMI	21	269	11.02	na	na	0.001

DMI, double-marked immigrants; SMI, single-marked immigrants. Regression coefficients are from Proc MIXED (SAS Institute, 2008) determined from each set of data. $N = 27$ (3 plots $\times$ 9 weeks) in 2008 and $N = 21$ (3 plots $\times$ 7 weeks) in 2009.

lady beetles from alfalfa into cotton during any of the 3 cotton phenological stages in both years. However, the total lady beetle influx from cotton to alfalfa varied with cotton phenological stages. The number of total immigrant convergent lady beetles in alfalfa from cotton was significantly higher during cotton flowering ($P = 0.002$) in 2008. However in 2009, the number of total immigrant convergent lady beetles in alfalfa from cotton was significantly higher during squaring ($P = 0.001$) and boll development ($P = 0.039$) stages (Table 3). In both years, the number of resident convergent lady beetles captured within either of the 2 crops did not vary across cotton phenological stages. Also, there was no difference in resident beetle densities between crops in all 3 phenological stages for both years (Table 3).

Seasonal dynamics of intercrop movement and net movement

The data illustrated in Figure 3 show the lady beetle influx into cotton from alfalfa and vice versa on all sampling dates. The total convergent lady beetle influx into alfalfa from cotton was significantly higher in late July (2008) and early September (2008 and 2009) (Figs. 3A and B). Net lady beetle intercrop movement between cotton and alfalfa was calculated by subtracting total immigrant lady beetles in cotton from total immigrant lady beetles in alfalfa. Positive net movement values indicated a net lady beetle gain in alfalfa. Likewise, negative net movement

values indicated a net lady beetle gain in cotton. The net movement of convergent lady beetles was generally toward alfalfa on most of the sampling dates for both years; nevertheless, it was toward cotton on July 5 and 20 in 2008 and on July 26 and August 12 in 2009 (Figs. 3A and B). The net movement was consistently and greatly toward alfalfa in the later part of the growing season in both years.

Discussion

Data yielded from this 2-year study showed that the total convergent lady beetle captured during the entire sampling period was significantly higher in alfalfa than cotton. This corroborates with previous reports from this region (Parajulee *et al.*, 2007). In 2008, the fluctuation in convergent lady beetle numbers on alternate sampling dates during most of the season have been due to the high mobility of lady beetles among alfalfa and other surrounding habitats affected by habitat quality and availability of prey. In both years, lady beetles were more abundant in alfalfa during the late cotton season when cotton was near senescence, which might have left only alfalfa in the landscape as a preferable crop to colonize by lady beetles. However, the total captures were not significantly different between the 2 crops across cotton phenological stages, except for cotton squaring period of 2009, which could be attributed to the differences in the crop quality at that time. Within the treatment crop, alfalfa had uniform lady beetle
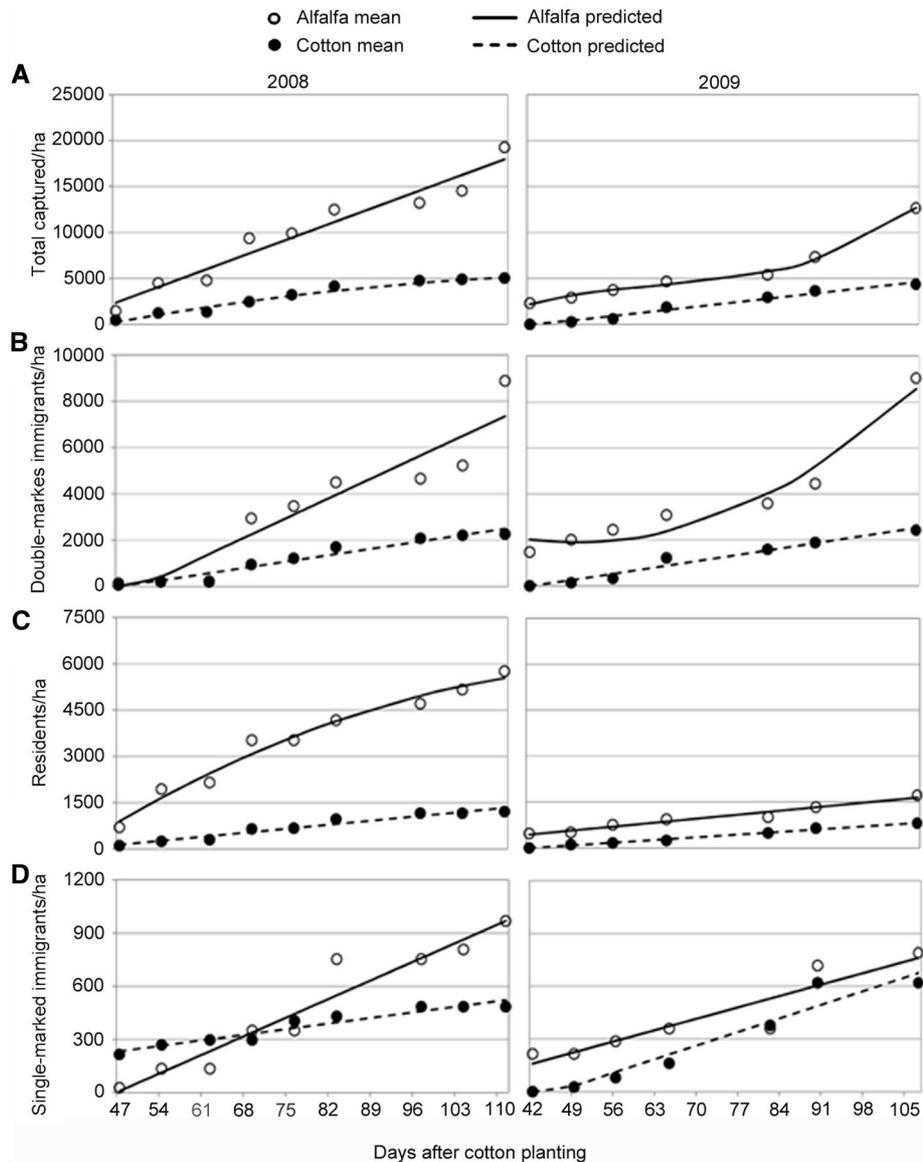
Fig. 2 Comparison of alfalfa and cotton on cumulative total captured, double-marked immigrant, resident, and single-marked immigrant convergent lady beetles with predicted lines (see Table 4 for parameter values).

abundance across cotton phenological stages except for the boll development stage in 2009. This observed difference in 2009 might be due to the maturity of surrounding crops including cotton whereas alfalfa was blooming at the same time. In cotton, total lady beetles captured was significantly higher in flowering stage compared to that in boll development (2008) and squaring (2009) stages, clearly suggesting that the abundance of lady beetles in cotton is affected by its phenological stages, coupled with crop quality. The cotton flowering stage was more attractive to retain these beetles as flowering cotton provides

better food resources in the form of nectar, pollen, and presumably higher prey activity.

The slope of the cumulative abundance curve of the total lady beetles was significantly greater in alfalfa compared to that in cotton throughout the growing season in 2008, but the difference was significant only toward the end of the season in 2009. This disparity between years might be attributed to the planting of a small cowpea field near the alfalfa in 2009, unrelated to this study, which could have drawn these beetles away during the growing season, and the numbers sharply increased in alfalfa compared
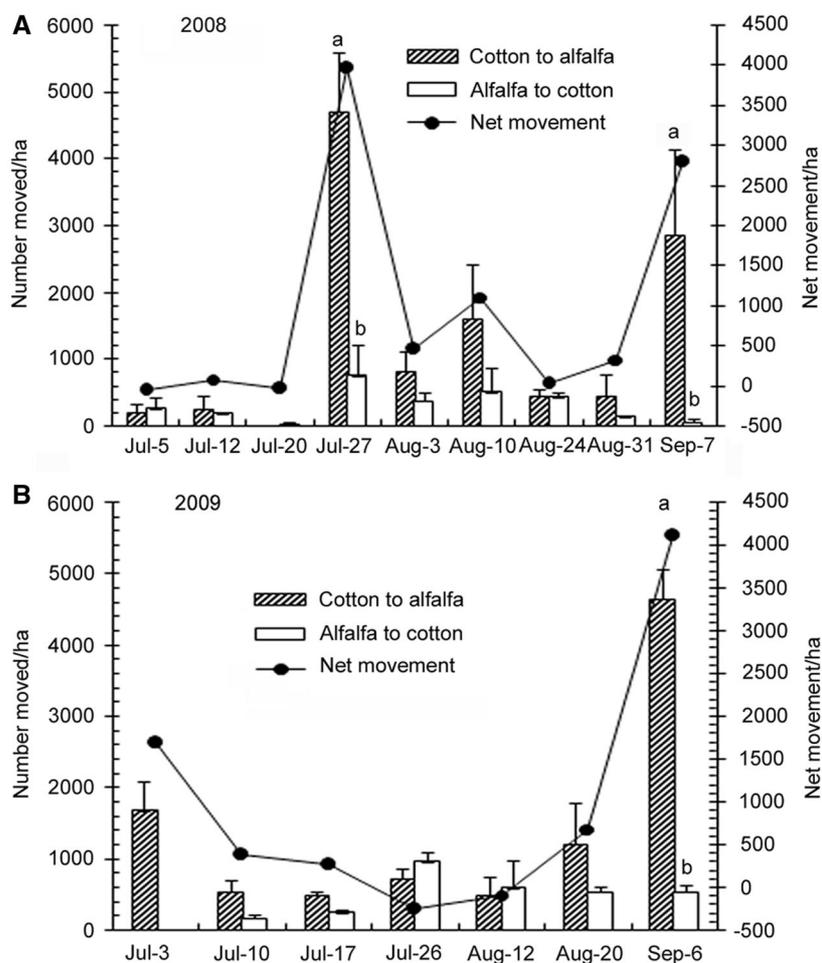
Fig. 3 Average weekly profiles of convergent lady beetle movement and net intercrop movement between cotton and alfalfa in 2008 (A) and 2009 (B).The left *y*-axis represents the number of insects moving from one treatment crop to other and the right *y*-axis represents the net movement between the treatment crops. Different lowercase letters within sampling date indicate significant difference in movement between the crops ($P = 0.05$).

to that in cotton when cowpeas senesced (Fig. 3B). These data suggest that alfalfa serves as a sink for lady beetles when it is adjacent to cotton, but other flowering crops (and associated lady beetle prey) in the vicinity such as cowpeas may be more attractive to lady beetles than alfalfa. Although not recorded, we noticed good cowpea aphid populations in cowpeas which must have attracted the lady beetles that were dispersing from cotton.

Protein-marking data revealed that a major proportion of convergent lady beetles retrieved from both cotton and alfalfa were double-marked immigrants, indicating that they were moving between alfalfa and cotton. In other words, the movement behavior of convergent lady beetles was bidirectional in this cotton–alfalfa agroecosystem. Prasifka *et al.* (1999) also showed that movement of

convergent lady beetle is bidirectional between sorghum and cotton. In 2009, the double-marked immigrants in alfalfa from cotton was significantly higher in squaring and boll development stages of cotton and no difference was observed in the flowering stage with numerically more double-marked immigrants recorded from cotton. These data suggest that although lady beetles moved between alfalfa and cotton, alfalfa was a more preferred habitat for convergent lady beetles to colonize during cotton squaring and boll growth stages due to a higher concentration of prey in alfalfa, none or reduced level of flowering in cotton, more lush and flower-rich alfalfa, or a combination of all these factors.

Tracking similar to the total lady beetle abundance curve, the slope of the cumulative abundance curve of the

double-marked convergent lady beetles was significantly greater in alfalfa compared to that in cotton in 2008, but the difference was significant only toward the end of the season in 2009. This difference between the years might be due to the fewer number of ladybeetles recovered in alfalfa in 2009. It is likely that the movement is affected by the densities of the lady beetle.

The total immigrant or total influx of convergent lady beetles for the entire season was toward alfalfa from cotton. This observation supports the notion that alfalfa is a more preferred host over cotton for lady beetle colonization. In cotton, higher lady beetle influx was recorded in late July and August in both years, and in 2009, numerically higher total influx was towards cotton rather than alfalfa during the period when cotton was flowering. This suggests that the total influx can be affected by the quality of crops and flowering cotton appears to be more attractive to lady beetles than an alfalfa crop of the same period, which might be due to the availability of more food resources.

The data on net movement or net balance of inflow and outflow of convergent lady beetles also favored alfalfa over cotton on most of the seasonal sampling dates. There was no particular pattern observed based on the phenology for the two study years. It is highly likely that net movement from alfalfa to cotton or vice versa is also affected by crop quality and availability of prey during the sampling periods. Prasifka *et al.* (1999) reported that predators may move in response to increasing temperature and decreasing food availability. It is possible that alfalfa, in our study, provided a cooler and dense environment, possibly leading to more food resources for lady beetles.

This study showed that convergent lady beetles moved between adjacent cotton and alfalfa, but the lady beetles would be more attracted to alfalfa when cotton is not flowering and/or when alfalfa offers more opportunities for prey. This study offers much needed information on intercrop movement behavior of the convergent lady beetle, a dominant arthropod predator in cotton–alfalfa agroecosystems, and facilitates integrated pest management decisions in cotton utilizing conservation biological control. Future research studies to investigate the possible biological, physical, or ecological factors that are responsible for specific movement behavior of lady beetles between alfalfa and cotton are recommended.

Acknowledgments

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Feeding behavioral response of cotton aphid, *Aphis gossypii*, to elevated CO_2: EPG test with leaf microstructure and leaf chemistry

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Abstract

Effect of elevated CO_2 on feeding behavior of the cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae), was investigated using electrical penetration graphs (EPG) on cotton, *Gossypium hirsutum* L. (Malvaceae). Leaf microstructures and foliar soluble constituents were also measured simultaneously to quantify the impact of foliar changes on leaf nutritional quantity and quality, owing to elevated CO_2, on stylet penetration and food-quality plasticity of *A. gossypii*. Significant increases in fresh body weight, fecundity, and population abundances of *A. gossypii* were found in elevated CO_2 in contrast to ambient CO_2. Elevated CO_2 influenced the feeding behavior, as evidenced by altered EPG recordings, including the increased non-penetration period (walking and finding the feeding site), $E2_{<8\ min}$ (probes with sustained ingestion of <8 min), and first $E2_{>8\ min}$ (first occurrence of probes with sustained ingestion of >8 min), and decreased $E2_{>8\ min}$ recordings. Moreover, leaf microstructures were significantly affected by CO_2 levels, with thinner upside epidermis (UPE) and thicker underside epidermis (UDE), sponge tissues (ST), and fence tissues under elevated CO_2 compared to that in ambient CO_2. Therefore, it is expected that *A. gossypii* spend more time penetrating the thicker leaf UDE and ST when the host plant is exposed to elevated CO_2. Furthermore, elevated CO_2 significantly enhanced foliar soluble matter, including soluble sugars (SS), free amino acids and fatty acids (FFA), and total soluble matter (TSM), which was congruent with significant increase or decrease in leaf turgor or osmotic potential. Increased leaf turgor and leaf soluble constituents favored ingestion in *A. gossypii*, resulting in increases in fresh body weight, fecundity, and population abundances under elevated CO_2. These feeding behaviors and resulting population growth parameters are consistent with the significant positive correlations between aphid fresh body weight and foliar FFA/TSM, between *A. gossypii* fecundity and foliar SS of cotton plants, and between the time of $E2_{<8\ min}$ recordings and leaf turgor.

Introduction

Global climate change, especially the rising of atmospheric CO_2 levels, has been an item of great concern for the scientific community for about half a century. The rising atmospheric CO_2 concentration may have a variety of direct and indirect effects on the trophic relationships among plants, their herbivores, and the herbivores' natural enemies (Stiling et al., 2002; Chen et al., 2005a, 2007a; Sudderth & Sudderth, 2014). The direct physiological effects of enriched CO_2 on individual plant species are well documented. In general, increased atmospheric CO_2 concentrations can increase photosynthesis, growth, yield, and C:N ratios of most plant species, particularly C_3 plants (e.g., Pritchard et al., 1999; Ge & Chen, 2006). However,

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the responses of herbivorous insects to elevated CO_2-grown foliage are frequently idiosyncratic (e.g., Bezemer & Jones, 1998; Newman et al., 2003), thereby warranting species-specific studies of their behavior.

Phloem-feeding insects, such as the cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae), have shown a consistently more positive response in population growth to rising atmospheric CO_2 concentrations (Sun et al., 2015), than leaf-chewers and leaf-miners (Bezemer & Jones, 1998). Most leaf-chewing insects, e.g., grasshoppers (Thunberg) (Johnson & Lincoln, 1991), *Orgyia leucostigma* (Smith) (Lindroth et al., 2002), *Chilo suppressalis* (Walker) (Chen et al., 2011), *Spodoptera exigua* (Hübner) (Coviella et al., 2002), and cotton bollworm, *Helicoverpa armigera* (Hübner) (Chen et al., 2005b,c, 2007b, 2011; Wu et al., 2007a; Coll & Hughes, 2008), exhibit compensatory increases in food consumption and/or reduced growth, survival rates, and reduction in density, presumably because of the negative effects of elevated CO_2 on plant nutritional quality. However, most phloem-feeders – e.g., peach potato aphid, *Myzus persicae* (Sulzer) (Hughes & Bazzaz, 2001; Stacey & Fellowers, 2002), wheat aphids *Rhopalosiphum padi* (L.) (Zhang et al., 2003) and *Sitobion avenae* (Fabricius) (Chen et al., 2004a), cotton aphid, *A. gossypii* (Chen et al., 2004b, 2005a,d), sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Li et al., 2011), and brown planthopper, *Nilaparvata lugens* (Stål) (Chang et al., 2011) – became much more serious threats under elevated CO_2, due to shorter life span, higher body weight and fecundity, and increased population abundances in these species. Here, the question is what mechanisms elicit the positive responses of phloem-feeders to elevated CO_2.

Aphids are sap-feeding pest insects that derive nutritional matter from their host plants through passive sucking of phloem content (Xue et al., 2008). A well-established experimental method to quantify sap-feeding behavior of aphids is electrical penetration graphing (EPG), which monitors the stylet penetration behavior via variation in electrical recording signals (McLean & Kinsey, 1964; Tjallingii, 1988, 1990; Jiang et al., 2015). The EPG technique has been successfully employed to compare aphids' feeding behavior on host and non-host plants (McLean & Kinsey, 1968) and to investigate the acceptance process of host plants by aphids (Tjallingii & Mayoral, 1992). EPG research has also been used to study feeding behavioral responses of aphids to elevated CO_2-grown host plants (Zhang et al., 2009; Liu et al., 2013). Previous research also noted that the elevated CO_2 significantly affected leaf morphology and microstructure of plants (Yang et al., 1997), with significant increases in leaf thickness of C_3 plants (Thomas & Harvey, 1983), owing to marked increases in foliage cell division under elevated CO_2 (Masle, 2000). Nevertheless, plant species might vary in their response to elevated CO_2, e.g., soybean and loblolly pine exhibited significant increases in palisade thickness from two cell layers to three (Thomas & Harvey, 1983), whereas the common bean, *Phaseolus vulgaris* L., showed significant increases in spongy thickness owing to elevated CO_2 (Radoglou & Jarvis, 1992). It is presumed that the variation in general patterns of leaf structure under elevated CO_2 levels might affect the stylet probing and ingestion by aphids on host plants. Moreover, leaf stomatal resistance increased about 33–55% and leaf stomatal conductance decreased about 20–40% when plants were grown under increased atmospheric CO_2 concentrations (Cure & Acock, 1986; Xie et al., 2006). Elevated CO_2 has also been shown to stimulate plant growth with higher total nonstructural carbohydrates, soluble sugars, proteins, free amino acids, and fatty acids for cotton and wheat plants (Chen et al., 2004a, 2005b,c; Wu et al., 2007b,c), most likely resulting in increased mesophyll osmotic pressure and turgor (Masle, 2000). Increased turgor would be more favorable for the ingestion of plant sap by phloem-feeders.

In this study, an EPG experiment was carried out with *Gossypium hirsutum* L. (Malvaceae) cv. Deltapine 5415 and its sap-feeding pest, *A. gossypii*, to examine the effects of elevated CO_2 on stylet ingestion by phloem-feeding insects on host plants. Utilizing assays for foliage microstructures and leaf nutritional parameters, the specific objectives were to elucidate the mechanisms of feeding behavioral response of phloem-feeders to the rising atmospheric CO_2 concentrations.

Materials and methods

Closed-dynamic CO_2 chamber (CDCC)

This study was conducted in six identical growth chambers that were retrofitted to provide a known volume of CO_2 in a closed system (RXZ-380; Jiangnan Life Apparatus, Ningbo, China). A periodic regime was maintained in these chambers: 28 °C and 60% r.h. during the day, 24 °C and 70% r.h. at night, and L14:D10 photoperiod, with light at 9 000 lux, supplied by 12 60-W fluorescent lamps. Two levels of constant atmospheric CO_2 concentrations were applied, including ambient level (375 µl l^{-1}) and the level predicted to occur in about 100 years (750 µl l^{-1}) (Houghton et al., 2001). Three chambers were used for each CO_2 treatment, but the number of plants across the three chambers within each CO_2 treatment determined the number of replications for each test. Concentrations in separate chambers were monitored continuously, and adjusted with an automatic control system for CO_2 levels. Details of the automatic control system and growth chambers are provided in Chen & Ge (2004c).

Cotton cultivar and planting setup

Cotton cv. DP 5415 was planted in white plastic pots (18 cm diameter, 21 cm high) filled with 4:1 loam soil: cow dung (vol:vol). Upon seedling emergence, cotton plants were thinned to two plants per pot and exposed to the CO_2 treatments. Pots were watered to saturation with tap water twice a week; no chemical fertilizers or insecticides were used. Twenty-four pots were randomly placed in each CDCC and rerandomized once a week to minimize position effects within the chamber.

Aphis gossypii laboratory culture and inoculation setup

Apterous *A. gossypii* were collected from field-planted cotton as soon as they appeared in the Nanjing Agricultural University farm (Nanjing, China), and then were reared in the laboratory for at least 10 generations on 30- to 60-day-old cotton seedlings (cv. DP 5415) to obtain uniform colonies. On the 30th day after planting (DAP), eight pots were randomly selected from the 24 pots in each CDCC and infested with 10 adult aphids per plant randomly selected from the colony. Population densities (i.e., number of aphids per plant) of *A. gossypii* were monitored every 5 days, beginning 45 DAP until 60 DAP. Another five pots were randomly selected from the remaining 16 pots in each CDCC on 40 DAP, and then two newborn aphid nymphs from the above colony were caged singly on the undersurface of the third leaf above the cotyledon of each cotton plant (2 aphid nymphs per plant × 2 plants per pot × 5 pots per chamber × 3 chambers = 60 aphids per CO_2 treatment) following the method of Parajulee (2007). Individual aphid nymphs (20 individually caged newborn aphids per chamber, F_1 generation) were allowed to develop into reproductive adults. The first five newly emerged adults from each chamber were individually measured for adult fresh body weight using a Cahn 20 automatic electrobalance (Cahn, St. Louis, MO, USA). These adults were returned to their original individual cages and monitored for their daily fecundity until each of the adult aphids had died, to determine the lifetime fecundity. Once the remaining 15 F_1 adults began reproducing, the mother aphid and all but one newborn aphids were removed from their cages. As for F_1, five of these 15 F_2 aphids were weighed and returned to their cages for lifetime fecundity assessment. The mother aphid and all but one offspring were removed from the remaining 10 cages. This process was repeated for the F_3 and F_4 generations.

Electrical penetration graphs to monitor aphid feeding

On 60 DAP, five pots were randomly selected from the remaining 11 pots in each CDCC, and *A. gossypii* adults from the laboratory colony were used to study the feeding activities using a Giga-8 DC-EPG amplifier system with 1 GΩ input impedance, 50× amplification, and <1 pA input bias current (Wageningen University, Wageningen, The Netherlands). Test insects were carefully connected to a gold wire (18.5 mm diameter, 3 cm long) with conductive silver glue on their dorsum. After 1 h starvation, the wired aphid adults were placed on the abaxial surface of the leaf (n = 15 aphids for 'ambient CO_2' and 17 aphids for 'elevated CO_2' grown plants randomly obtained from the six chambers), and the other side of the gold wire was connected to the amplifier. The experiment was conducted in a greenhouse at 26.5 ± 1 °C, 70 ± 10% r.h., and L14: D10 photoperiod. Probing behavior was recorded for 6 h continuously, and 4 h of records from the beginning of feeding were analyzed using EPG Stylet software (EPG Systems, Wageningen, The Netherlands). All recorded signals were analyzed, including non-penetration period (NP waveform; aphid walking and stylet not probing the host substrate), pathway phase (C waveform; aphid stylet probed the host substrate to locate the feeding site), phloem phase (E waveform, including two events: E1 waveform, salivation into phloem sieve elements; E2 waveform, ingestion of the phloem content), and xylem phase (G waveform; ingestion of the xylem sap). In this study, we only report the recordings of NP, $E2_{>8\ min}$, $E2_{<8\ min}$, first $E2_{>8\ min}$, and P_{rest}. Waveform parameters $E2_{>8\ min}$ and $E2_{<8\ min}$ indicate sustained phloem ingestion for more and less than 8 min, respectively (Kimmins & Tjallingii, 1985; Tjallingii, 1990; Davis & Radcliffe, 2008), first $E2_{>8\ min}$ indicates the duration of the first occurrence of $E2_{>8\ min}$, and P_{rest} represents all other waveforms of feeding activities.

Leaf microstructure and turgor

Six remaining plants from each CDCC chamber were used to measure leaf microstructure and foliar soluble constituents. Third fully expanded mainstem node leaves from the plant canopy were selected to measure the leaf microstructure parameters. Light microscopy was used to measure the thickness of upside epidermis (UPE), underside epidermis (UDE), fence tissues (FT), sponge tissues (ST), and total thickness of leaf (TTL). Selected leaves were cut into pieces of about 2 mm squares (n = 100 for each CO_2 treatment combined over three chambers) and fixed for 24 h in fresh FAA (50 ml 95% ethanol, 5 ml glacial acetic acid, 10 ml 37% formaldehyde, 35 ml H_2O). Then, all samples were rinsed in water and dehydrated in a graded ethanol series to 100% ethanol, embedded in paraffin, sectioned and mounted on glass slides, and treated with a safranin and fast-green stain procedure (Clark, 1981; Wise et al., 2000; Takahashi et al., 2010). Subsequently, the transverse sections (8–10 μm thick) were embedded using an acrylic resin (LR White; London

Resin, Reading, UK) (Slaton et al., 2001). Leaf turgor (LT) was calculated based on the difference between leaf water potential (WP) and osmotic potential (OP): LT = WP − OP (Chartzoulakis et al., 2002; Navarro et al., 2007). WP and OP were determined with a portable PSYPRO Water Potential System (Wescor, Logan, UT, USA). As the sensor, a C-52 sample chamber (Wescor) was connected to the PSYPRO. The measurements were recorded following the procedures of Ebner et al. (2011) and Miranda et al. (2013).

Foliar soluble components

To assess the biochemical changes of the treated cotton leaves, leaf soluble components, such as soluble sugars (SS), soluble proteins (SP), free amino acids (FAA), free fatty acids (FFA), and total soluble matter (TSM) were quantified. For SS and FAA determinations, 1 g of leaf samples were cut into 5 ml of 80% ethanol and the mixture was boiled for 10 min and centrifuged at 376 g for 10 min. The supernatant was collected and the pellet was re-extracted in 5 ml of hot 80% ethanol, then the supernatant was collected again. The supernatants were pooled, and SS were estimated by an enzymatic analysis using phenol sulfuric acid (Dubois et al., 1956; Mohotti & Lawlor, 2002); FAA was determined in the remaining supernatant according to the method of Moore & Stein (1954) using leucine as standard (Satyanarayana et al., 2011). SP was determined following the method of Bradford (1976), in which 5 ml of the protein reagent was added into 0.1 ml of the extraction and the contents mixed on a vortex mixer. The absorbance was measured at 595 nm after 1 h. The SP concentration was calculated from a constructed standard curve for bovine serum albumin. The estimation of FFA was carried out following the extraction procedure of Garcia-Lopez et al. (1994), transformed to methyl esters using the method of Metcalfe et al. (1966), and then quantified FFA using a gas chromatograph (GC-2014; Shimadzu, Kyoto, Japan) equipped with a FID detector (Choudhary & Grover, 2013; Shoghi-Kalkhoran et al., 2013). TSM was estimated as the sum of SS, SP, FAA, and FFA.

Data analysis

All data sets were analyzed using IBM-SPSS v.20.0 (IBM, Armonk, NY, USA). Parameters including EPG recordings (NP, E2$_{>8 \text{ min}}$, E2$_{<8 \text{ min}}$, first E2$_{>8 \text{ min}}$, and P$_{rest}$), leaf microstructures (UPE, UDE, FT, ST, and TTL), osmotic adjustments (WP, OP, and LT), and foliar soluble components (SS, SP, FAA, and FFA) were analyzed separately using a one-way ANOVA with CO$_2$ treatment (ambient

vs. elevated) as source of variability. Aphid fresh body weight, lifetime fecundity, and population abundances of four successive generations were analyzed separately using a two-way ANOVA with CO$_2$ level (ambient vs. elevated CO$_2$) and aphid generation (four generations) as sources of variability. Tukey's least significant difference (LSD) test was used to separate the means between treatments (α = 0.05). In addition, Pearson's correlation analysis was conducted to investigate the relationships between the EPG recordings of aphid ingestion and leaf microstructure (excluding UPE and FT because aphid feeding takes place on the abaxial surface of the host leaf), leaf turgor, and foliar soluble components of cotton plants grown in ambient vs. elevated CO$_2$. For this analysis, individual plant data were averaged across chambers within each CO$_2$ level that produced five data points per CO$_2$ treatment (five plants per chamber), but the missing values on some chambers reduced the data points to four for final analysis.

Results

Aphid body weight, fecundity, and population dynamics

CO$_2$ level and aphid generation both significantly affected aphid fresh body weight (CO$_2$: $F_{1,112}$ = 5.23; generation: $F_{1,112}$ = 10.28, both P<0.05), fecundity (CO$_2$: $F_{1,104}$ = 12.26; generation: $F_{1,104}$ = 7.04, both P<0.001), and population dynamics (CO$_2$: $F_{1,184}$ = 26.04; generation: $F_{1,184}$ = 68.81, both P<0.0001), with a significant interaction between CO$_2$ level and aphid generation on aphid fresh body weight ($F_{1,112}$ = 9.05, P = 0.006). Compared with ambient CO$_2$, elevated CO$_2$ increased aphid fresh body weight, number of nymphs laid per aphid, and population abundances, and the values increased with each successive generation of *A. gossypii* for all four generations evaluated in this study (Figures 1 and 2). Elevated CO$_2$ significantly increased aphid fresh body weight of the fourth generation (Figure 1A), number of nymphs laid per aphid of generations 2–4 (Figure 1B), and the population abundances from 15 to 30 days after inoculation (Figure 2).

EPG waveforms of *Aphis gossypii* feeding

The EPG technique was used to detect possible differences in feeding behavior of *A. gossypii* under elevated vs. ambient CO$_2$. The EPG recordings including NP, E2$_{<8 \text{ min}}$, E2$_{>8 \text{ min}}$, and the first E2$_{>8 \text{ min}}$ were all significantly affected by CO$_2$ level (Figure 3, Table 1), with increases in total duration of the EPG waveforms of NP, E2$_{<8 \text{ min}}$, and the first E2$_{>8 \text{ min}}$, and a decrease in total duration of the E2$_{>8 \text{ min}}$ recording for elevated CO$_2$ compared with ambient CO$_2$ (Figure 3).
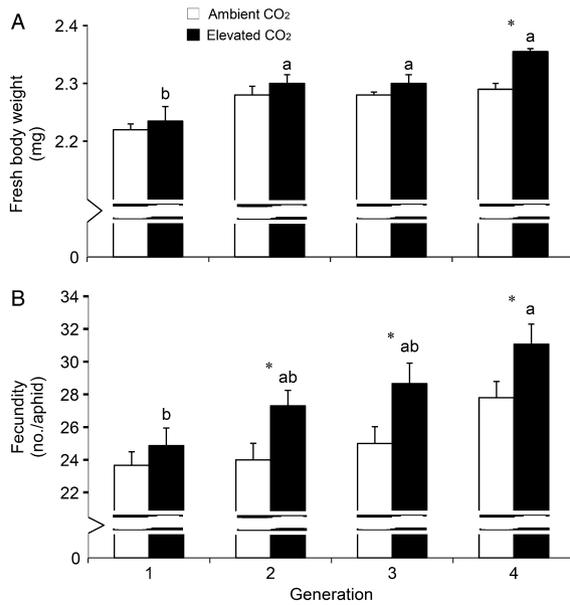
Figure 1 Mean (+ SE) (A) fresh body weight (mg) and (B) lifetime fecundity of four generations of *Aphis gossypii* fed on cotton plants under ambient and elevated CO$_2$. Asterisks indicate significant differences between ambient and elevated CO$_2$, and different letters capping the bars indicate significant differences among aphid generations within CO$_2$ level (LSD tests: P<0.05).
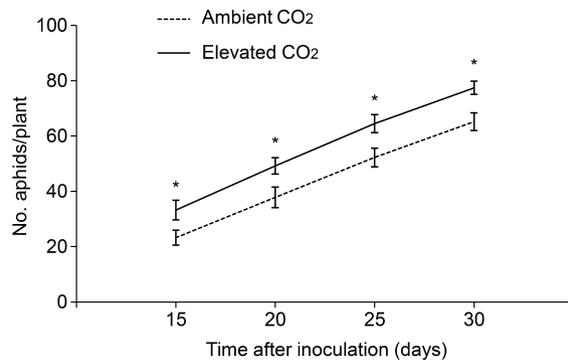

Figure 2 Population density of *Aphis gossypii* (mean number per plant ± SE) fed on cotton plants grown in ambient and elevated CO$_2$ at four generation-equivalent durations (adult aphids were inoculated on treatment plants and population densities measured every 5-day intervals for 30 days). Asterisks indicate significant differences between ambient CO$_2$ and elevated CO$_2$ (LSD test: P<0.05).

Leaf microstructure and turgor

CO$_2$ level significantly altered the foliar microstructure of cotton plants (Figure 4, Table 1). Increases in the thickness of the UDE, FT, ST, and TTL, and a decrease in the thickness of UPE were observed when cotton plants were grown in elevated vs. ambient CO$_2$ (Figure 4). Moreover,
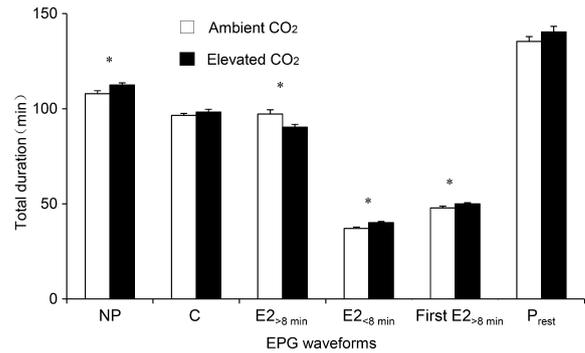

Figure 3 Mean (+ SE) duration (min) of behavioral parameters of *Aphis gossypii* fed on cotton leaves in ambient and elevated CO$_2$ (NP, non-penetration; C, pathway phase; E2$_{>8\ min}$ and E2$_{<8\ min}$, sustained phloem ingestion for more and less than 8 min, respectively; first E2$_{>8\ min}$, duration of the first E2$_{>8\ min}$ from all E2$_{>8\ min}$ occurrences during the 4-h EPG recording; P$_{rest}$, all other waveforms of feeding probes). Asterisks indicate significant differences between ambient CO$_2$ and elevated CO$_2$ (LSD tests: P<0.05).

elevated CO$_2$ significantly increased the leaf turgor of cotton plants, due to marginal increase in water potential (LSD test: P = 0.11) and significant decrease in osmotic potential for cotton plants grown in elevated compared to ambient CO$_2$ (Figure 5).

Foliar soluble components

All the measured foliar nutritional components (SS, SP, FAA, FFA, and TSM) significantly increased for cotton plants grown in elevated compared to ambient CO$_2$ (Figure 6, Table 1).

EPG waveforms and leaf microstructure parameters

The Pearson's correlation analysis indicated significantly positive correlations between the duration of E2$_{<8\ min}$ recording and the thickness of UDE, ST, UDE + ST, and leaf turgor (Table 2). Also, the time of the first E2$_{>8\ min}$ recording and leaf turgor were positively correlated (Table 2). The duration of E2$_{>8\ min}$ recording showed a negative (but not significant) correlation with leaf microstructure parameters.

Relationships among EPG waveforms of aphid feeding, aphid growth parameters, and foliar soluble components of cotton plants

The duration of E2$_{>8\ min}$ recording was negatively correlated with the fecundity of *A. gossypii*, and it was not significantly correlated with the population abundance of *A. gossypii* (Table 3). The duration of NP recording was positively correlated with fresh body weight of *A. gossypii*. Fresh body weight of *A. gossypii* was positively correlated with all foliar soluble components evaluated (Table 3).

Table 1 One-way ANOVA for the effects of CO_2 levels on *Aphis gossypii* growth, EPG recordings, cotton leaf microstructure, osmotic adjustment, and foliar soluble components

Parameter	d.f.	F	P
Aphid growth indices			
Fresh body weight (mg)	1,112	5.23	0.032
Fecundity (no. offspring/adult)	1,104	12.26	0.0007
Population abundance (no. aphids/plant)	1,184	26.04	<0.0001
EPG recordings (min)			
NP	1,30	5.36	0.028
$E2_{>8 \ min}$	1,30	7.29	0.044
$E2_{<8 \ min}$	1,30	9.67	0.0039
First $E2_{>8 \ min}$	1,30	4.19	0.049
P_{rest}	1,28	1.55	0.22
Leaf microstructure (μm)			
Upside epidermis	1,198	40.98	<0.0001
Underside epidermis	1,198	45.34	<0.0001
Fence tissues	1,198	137.70	<0.0001
Sponge tissues	1,198	202.78	<0.0001
Total thickness of leaf	1,198	293.23	<0.0001
Osmotic adjustment (MPa)			
Water potential	1,22	2.86	0.11
Osmotic potential	1,22	16.72	0.0005
Leaf turgor	1,22	19.17	0.0002
Foliar soluble matter (mg g^{-1} fresh weight)			
Soluble sugar	1,10	9.80	0.011
Soluble proteins	1,10	8.31	0.016
Free amino acids	1,10	5.71	0.038
Free fatty acids	1,10	6.09	0.033
Total soluble matter	1,10	22	0.0009

NP, non-penetration; $E2_{>8 \ min}$ and $E2_{<8 \ min}$, successful probes of sustained ingestion for more and less than 8 min, respectively; first $E2_{>8 \ min}$, duration of the first occurrence of $E2_{>8 \ min}$ from all $E2_{>8 \ min}$ occurrences in the 4-h EPG recording; P_{rest}, all other waveforms of feeding probes.

Discussion

Phloem-feeders (e.g., aphids) are the insect group shown to have a positive response to rising atmospheric CO_2 concentrations, in contrast with leaf-chewers and leaf-miners (Bezemer & Jones, 1998). This study showed a positive response of the cotton aphid exposed to the elevated CO_2 rearing conditions, with higher fresh body weight, fecundity, and population abundances. The leaf microstructure, leaf turgor, and foliar soluble components of cotton plants were also significantly affected by CO_2 treatments, with significant increases in the thickness of UDE, FT, ST, and TTL, significant decrease in the thickness of UPE, and significant increases in leaf turgor, SS, SP, FAA, FFA, and TSM of cotton plants grown in elevated CO_2 in contrast to
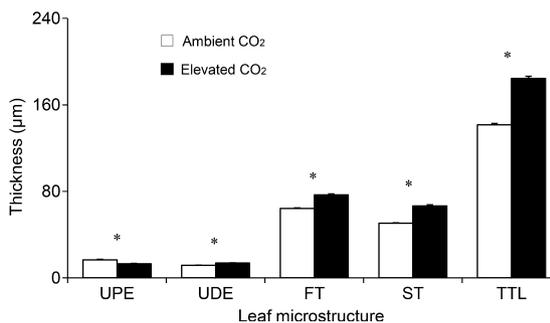

Figure 4 Mean thickness (+ SE) of leaf microstructure parameters of cotton grown in ambient and elevated CO_2 (UPE, upside epidermis; UDE, underside epidermis; FT, fence tissues; ST, sponge tissues; TTL, total thickness of leaf). Asterisks indicate significant differences between ambient CO_2 and elevated CO_2 (LSD tests: P<0.05).
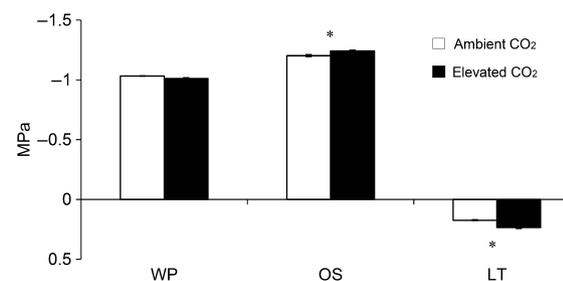

Figure 5 Mean (+ SE) water potential (WP), osmotic potential (OP), and leaf turgor (LT) of cotton plants grown in ambient and elevated CO_2. Asterisks indicate significant differences between ambient CO_2 and elevated CO_2 (LSD tests: P<0.05).
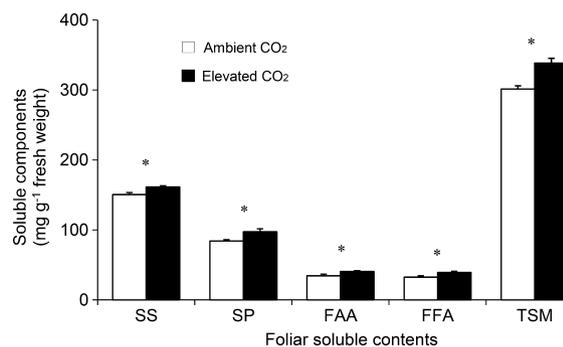

Figure 6 Mean (+ SE) foliar soluble contents, including soluble sugar (SS), soluble proteins (SP), free amino acids (FAA), free fatty acids (FFA), and total soluble matter (TSM) of cotton plants grown in ambient and elevated CO_2. Asterisks indicate significant differences between ambient CO_2 and elevated CO_2 (LSD tests: P<0.05).

Table 2 Coefficients of Pearson's correlation between EPG-waveform recordings of *Aphis gossypii* and leaf microstructure and turgor of cotton plants grown in ambient and elevated CO$_2$ (n = 8 data points)

EPG waveforms (min)	Leaf microstructure (μm)			Leaf turgor (MPa)
	UDE	ST	UDE + ST	
E2$_{>8\ min}$	−0.539	−0.640	−0.628	−0.781*
E2$_{<8\ min}$	0.916**	0.936**	0.935**	0.941**
First E2$_{>8\ min}$	0.579	0.656	0.648	0.850**
P$_{rest}$	0.237	0.248	0.247	0.072

UDE, underside epidermis; ST, spongy tissues; NP, non-penetration; E2$_{>8\ min}$ and E2$_{<8\ min}$, successful probes of sustained ingestion for more and less than 8 min, respectively; first E2$_{>8\ min}$, duration of the first occurrence of E2$_{>8\ min}$ from all E2$_{>8\ min}$ occurrences in the 4-h EPG recording; P$_{rest}$, all other waveforms of feeding probes.
Asterisks indicate level of significance: *0.01<P<0.05, **P<0.01.

Table 3 Coefficients of Pearson's correlation between *Aphis gossypii* growth and reproduction indices and EPG waveforms and foliar soluble components of cotton plants grown in ambient and elevated CO$_2$ (n = 8 data points)

	Fresh body weight (mg)	Fecundity (no. offspring/ aphid)	Population abundance (no. aphids/ plant)
EPG waveforms (min)			
NP	0.865**	0.343	0.145
E2$_{>8\ min}$	−0.636	−0.294	0.081
E2$_{<8\ min}$	0.939**	0.536	0.273
First E2$_{>8\ min}$	0.617	0.405	0.085
P$_{rest}$	0.432	−0.332	−0.471
Foliar soluble components (mg g^{-1} fresh weight)			
Soluble sugar	0.772*	0.439	0.196
Soluble proteins	0.824*	0.309	0.117
Free amino acids	0.839**	0.460	0.340
Free fatty acids	0.888**	0.473	0.116
Total soluble matter	0.893**	0.436	0.640

UDE, underside epidermis; ST, spongy tissues; NP, non-penetration; E2$_{>8\ min}$ and E2$_{<8\ min}$, successful probes of sustained ingestion for more and less than 8 min, respectively; first E2$_{>8\ min}$, duration of the first occurrence of E2$_{>8\ min}$ from all E2$_{>8\ min}$ occurrences in the 4-h EPG recording; P$_{rest}$, all other waveforms of feeding probes.
Asterisks indicate level of significance: *0.01<P<0.05, **P<0.01.

ambient CO$_2$. Significant change in leaf microstructure and turgor is expected to alter the feeding behavior of aphids (Schoonhoven et al., 2005).

The analysis of EPG recordings revealed significant changes in aphid feeding and growth behavior under increased CO$_2$ rearing conditions. Significant increases in total time of NP, E2$_{<8\ min}$, and the first E2$_{>8\ min}$ recordings, and a significant decrease in total time of the E2$_{>8\ min}$ recording were observed for *A. gossypii* under elevated compared with ambient CO$_2$. There were significant positive correlations between the time of E2$_{<8\ min}$ recording and the thickness of UDE, ST, and UDE + ST, and leaf turgor, and between the time of first E2$_{>8\ min}$ recording and leaf turgor. The changes in the thickness of leaf microstructure and leaf turgor caused by elevated CO$_2$ could significantly influence the feeding behavior of *A. gossypii*. More specifically, *A. gossypii* would spend more time on the recordings of NP, E2$_{<8\ min}$, and first E2$_{>8\ min}$, but less time on the E2$_{>8\ min}$ recording (sustained feeding phase), indicating that rising CO$_2$ level shortens feeding time. Consequently, the probability of successful probing events and removal of sap content from the feeding host would be lower (Kimmins & Tjallingii, 1985; Tjallingii, 1990; Davis & Radcliffe, 2008). Moreover, significant positive correlations between E2$_{<8\ min}$ recording and aphid population abundance, and between NP recording and aphid fresh body weight suggest that the elevated CO$_2$ favors cotton aphid population growth while shortening the feeding duration compared to that in ambient CO$_2$. Furthermore, significant negative correlation between E2$_{>8\ min}$ recording and fecundity of *A. gossypii* indicated that the successful probes of sustained ingestion

for more than 8 min are unfavorable for its reproductive efficiency.

In general, elevated CO$_2$ stimulates photosynthesis, plant growth, and yield, particularly in C$_3$ plants (Cure & Acock, 1986; Vara Prasad et al., 2005). An increased rate of net photosynthesis was conducive to the accumulation of biomass when grown under elevated CO$_2$ (Thomas & Strain, 1991; Zhao et al., 2004; Ainsworth & Long, 2005; Baig et al., 2012), which would likely result in the increase of leaf thickness. Radoglou & Jarvis (1992) had shown that the leaf thickness of *P. vulgaris* responded positively to elevated CO$_2$, particularly, the mesophyll area via cell enlargement. As a typical C$_3$ plant, cotton leaf microstructures significantly responded to elevated CO$_2$, with increased thickness of UDE, ST, and whole leaf, and thinner UPE. Thus, we hypothesize that elevated CO$_2$ can stimulate higher photosynthesis level due to thinner leaf UPE and easier absorption of solar light and atmospheric CO$_2$. The thinner leaf UPE and higher leaf turgor are more favorable for the ingestion of sap content by the phloem-feeders as they consume more sap while spending less time probing the host surface (i.e., E2$_{>8\ min}$ vs. E2$_{<8\ min}$).

Previous studies suggested that the photosynthetic carbon uptake of C_3 plants was enhanced by elevated CO_2 despite acclimation of photosynthetic capacity (Leakey et al., 2009). It was also found that elevated CO_2 concentration had a positive effect on carbohydrate accumulation, namely an increase in sucrose, glucose, and starch content of spruce needles (Urban & Marek, 2000; Cabálková et al., 2007; Teslova et al., 2010). These phenomena are likely caused by higher assimilatory function because of the increasing photosynthetic efficiency under elevated compared to ambient CO_2 (Baig et al., 2012). Moreover, significant positive correlations between *A. gossypii* fresh body weight and foliar FFA/TSM, and between *A. gossypii* fecundity and foliar SS suggest that the changes in phloem sap composition of cotton plants due to elevated CO_2 presumably have direct effects on the feeding behavior of *A. gossypii*. Furthermore, leaf turgor can be enhanced by reduced osmotic potential when water potential is increased (Chartzoulakis et al., 2002; Navarro et al., 2007). In this study, leaf soluble components including SS, SP, FAA, FFA, and TSM were all significantly enhanced by elevated CO_2 in contrast to ambient CO_2, which significantly reduced OP as WP increased, causing higher leaf turgor in elevated relative to ambient CO_2. It is expected that the increased leaf turgor in elevated CO_2 enables aphids to ingest the same amount of phloem content while spending significantly less time probing the host substrate.

Acknowledgements

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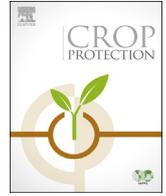
Cotton water-deficit stress, age, and cultivars as moderating factors of cotton fleahopper abundance and yield loss

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ABSTRACT

Field experiments were conducted in 2012 and 2013 during drought conditions in South Texas and the Texas High Plains to test whether cotton water-deficit stress, age, and cultivars are moderating and interacting factors that affect cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), abundance and yield loss. Irrigation and sequential plantings of several cultivars were used to simulate a range of water stress, plant ages, and cultivar variability. Cotton grown under these experimental conditions were exposed to cotton fleahopper using natural and artificial infestation. Cotton cultivars had a strong influence on cotton fleahopper abundance, with higher densities on Stoneville cultivar 5458 B2RF, which is relatively pubescent, than on the Phytogen cultivar 367 WRF, which is relatively glabrous, in South Texas ($p < 0.04$). But the strong cultivar effects on cotton fleahopper abundance did not correspond to yield reduction. No water stress effects on cotton fleahopper densities were observed in 2012 ($p > 0.05$), whereas cotton fleahopper densities increased on older cotton grown under no water stress in 2013 in South Texas ($p < 0.05$). In contrast, yield response was primarily sensitive to soil moisture conditions (up to 50% yield reduction when grown in dryland mimic conditions below 75% crop ET replacement, $p < 0.0009$). Water and cotton fleahopper stress synergies were detected but variable, with greatest lint yield loss attributable to cotton fleahopper seen in cotton grown in high water stress conditions in the High Plains ($p < 0.05$). Yield trends were consistent across cultivars (no interaction with cultivar), even though cotton fleahopper populations varied significantly across cultivars and exceeded regional economic thresholds beginning the second week of squaring ($p < 0.05$).

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1. Introduction

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), feeding on squares (i.e., pre-floral buds) of cotton, *Gossypium hirsutum* L. (Malvaceae), has reduced yield by up to 6% and also has delayed harvest in the southwest and mid-south (USA) cotton growing regions (Williams, 2000). But variability in the relationship of cotton fleahopper-induced square loss to subsequent yield loss under similar cotton fleahopper feeding pressure occurs and presents a challenge to cotton fleahopper management using traditional sampling and economic threshold methods (Ring et al., 1993; Brewer et al., 2012). In practice, field history of cotton fleahopper damage, weather conditions, and IPM practitioner

sensitivity to square loss have been used to adjust decision-making locally. In South Texas, one to four foliar sprays for cotton fleahopper control are common across cotton fields that have apparently similar pest risk based on similar cotton fleahopper density estimates generated from pest monitoring (Brewer, pers. obs.).

In review of the literature, cotton yield loss variability to cotton fleahopper feeding has been partly associated with cultivar differences (Holtzer and Sterling, 1980), including heritable traits considered for plant resistance (Knutson et al., 2013; McLoud et al., 2016). Ring et al. (1993) calculated visual-based cotton fleahopper economic injury levels (EIL) of between 0.015 and 0.45 insects per plant. The wide range was attributed to cultivar influences, based on comparison of yield—cotton fleahopper density relationships. Parajulee et al. (2006) partly attributed severity of cotton square loss to susceptibility differences across stages of cotton development and age of the reproductive tissues when cotton fleahopper migrated into fields from overwintering sites. Cotton may also compensate for early square loss (Anon, 2015). Cotton water deficit-

induced stress (water stress) also has been associated with square retention rates (Stewart and Sterling, 1989), which may influence plant sensitivity to cotton fleahopper feeding. These factors may be the underpinning of why thresholds in outreach materials vary across cotton growing regions of the southwest (i.e., 0.10 to 0.30 insects per terminal visually inspected during the first three weeks of squaring) (Anon, 2015), and why this insect is a minor pest in other locations (Williams, 2000). But if management strategies (i.e., planting time and cultivar selection) and weather conditions (i.e., poor rainfall in dryland production areas) influence cotton sensitivity to cotton fleahopper feeding, direct density estimation of cotton fleahopper for decision-making may give false indication of damage potential and improperly trigger insecticide applications using economic thresholds based on insect population estimates.

Therefore, square and subsequent yield loss variability has direct implications to in-season cotton fleahopper management that would benefit from further study. Here, we hypothesize that cotton water stress, age, and cultivars affect cotton fleahopper abundance and yield loss. As noted above, individual effects have been shown in past studies, but joint assessment of these factors may shed light on their comparative individual influences and their potential synergistic effects. The practical goal of understanding these relationships is to improve our assessment of cotton risk from cotton fleahopper and begin generation of a data base to make objective economic threshold adjustments under variable weather and management practices.

2. Methods

Drought conditions in Texas, 2012 and 2013, provided opportunity to assess cotton fleahopper activity and cotton response in a high contrast of water stress conditions manipulated by using irrigation in a field setting. Cotton fleahopper abundance and cotton response including yield were evaluated in several water regimes in two widely separated cotton growing regions: the coastal region of South Texas and the Texas High Plains. Standard agronomic practices were used (Morgan, 2015). Insect pest effects were largely restricted to cotton fleahoppers by using cotton cultivars with Bt-transgenes to control boll-feeding lepidopterans and by selecting study sites in areas where boll weevil has been eliminated and cotton fleahopper is a pest problem (Parajulee et al., 2006; Brewer et al., 2012; Luttrell et al., 2015). Cultivars, planting dates, and natural and artificial infestations of cotton fleahopper were used to optimize contrast in cotton fleahoppper pressure and cotton response. Experimental manipulation varied between South Texas and the Texas High Plains per opportunities and constraints outlined below.

2.1. South Texas location

A natural cotton fleahopper population was followed across time at a Corpus Christi, TX, location. Another plant bug, verde plant bug, that can affect square retention was detected during the study, but it never exceeded an economic threshold of 0.22 bugs per plant through peak bloom (Brewer et al., 2013). A split plot design was used to expose a natural population of cotton fleahopper to a soil moisture gradient of three (2012) and two (2013) water regimes (main plot), to two different plant ages by planting twice (sub-plot), and to two cotton cultivars (sub-sub-plot). An insecticide treatment was added as a final split plot in the design to directly test for cotton fleahopper-induced yield loss. Water regimes were established by using an above-ground drip irrigation system. Square injury from cotton fleahopper feeding was also confirmed by visual observation. The specific plot site was moved yearly so that the previous year crop was either sorghum or corn.

There were five replications, and individual plot size was four 15.24 m rows on 96.5 cm centers.

In 2012, cumulative rainfall from planting to harvest was 15.5 cm for both plantings. The water regimes used were a high water stress dryland mimic using minimal irrigation (2.9 cm of irrigation, or 18.4 total water input with rainfall), a moderate water stress dryland mimic using irrigation targeting 75% crop evapotranspiration replacement (crop ET) (6.24 cm of irrigation, or 21.74 cm total water input with rainfall), and a light water stress mimic using irrigation targeting 90% crop ET (10.85 cm of irrigation, or 26.35 cm total water input with rainfall). The surface irrigation drip tubes were 17 mm (dia.) and emitted 3.4 L per h (Netafim, Fresno, CA). The two planting dates were April 12 and 30. The two cultivars were the early season maturing Phytogen 367 WRF (Dow AgroSciences, Indianapolis, IN) and the mid to full season maturing Stoneville 5458 B2RF (Bayer CropScience, Research Triangle Park, NC). The Stoneville cultivar was relatively pubescent or hairy to very hairy, a trait which has been associated with high cotton fleahopper populations (Knutson et al., 2013; Bourland et al., 2003), while the Phytogen cultivar was more glabrous or smooth to lightly hairy (Brewer, pers. obs., Bourland et al., 2003). The last split was a foliar insecticide treatment: no insecticide and acephate (Amvac Chemical, Newport Beach, CA) applied two times weekly at a rate of 560.4 g per ha beginning at second week of squaring.

In 2013, cumulative rainfall was 31.0 cm and 27.9 cm for the earlier and later planting, respectively, measured from planting to harvest. The two water regimes used were a moderate light water stress dryland mimic (15.49 cm of irrigation or 46.49 cm total water input with rainfall for an earlier planting, and 20.07 cm of irrigation or 47.97 cm total water input with rainfall for a later planting) and the non-water stress mimic using irrigation targeting 90% crop ET replacement (26.42 cm of irrigation or 57.42 cm total water input with rainfall for an earlier planting, and 35.05 cm of irrigation or 62.95 cm total water input with rainfall for a later planting). Comparing years, total water inputs doubled from the previous year due to the increased rains, but at planting soil moisture was more depleted in 2013 than in 2012. The two planting dates in 2013 were moved later this year (April 22 and May 6) to further encourage cotton fleahopper movement into the crop. The same cultivars were used as in 2012. The insecticide treatment was changed to thiamethoxam (Centric 40 WG, Syngenta Crop Protection, Greensboro, NC) applied four times weekly at a rate of 87.6 g per ha weekly beginning at second week of squaring.

2.2. High plains location

The Lamesa, TX, location experienced barely detectable cotton fleahopper populations in 2013 likely due to the extended drought; therefore we focused on boll retention and subsequent yield using an augmented population of cotton fleahopper. Water stress and cotton fleahopper pressure were each manipulated at two levels in a randomized complete block. Only trace amounts of rainfall were detected. A very high water stress dryland mimic (11.43 cm of irrigation/total water input) and a moderate water stress dryland mimic (22.86 cm of irrigation/total water input) were delivered through a low-energy precision application via center pivot irrigation system. For study site comparison, the total water inputs of the very high water stress here were nearly 50% lower than those of the high water stress level in the South Texas location in 2012, and the water inputs of the moderate water stress level here was similar to those of the moderate water stress in South Texas in 2012. An augmentative release of cotton fleahopper was used to directly test for yield response to cotton fleahopper as compared with a no infestation control. Square injury from cotton fleahopper feeding was also confirmed by visual observation. The cultivar planted was

Phytogen 367 WRF. The treatments were replicated three times, and plot size was 13.7 m by four rows on 101.6 cm row centers.

Plants were artificially infested during the third week of squaring (one week prior to beginning of flowering) at a rate of five cotton fleahopper nymphs per plant across a 3 m uniform section of each plot. Preliminary study and field observations suggested that the survivorship of field released cotton fleahoppers was about 20%. Release during the third week of squaring was consistent with field observations when cotton fleahopper naturally infests cotton in the High Plains. For study site comparison, this infestation exceeded the regional economic threshold of 0.30 cotton fleahoppers per plant in the High Plains during this infestation period, while the south Texas location natural infestation also exceeded the economic threshold of 0.15 cotton fleahopper per plant for infestations beginning earlier during squaring (Anon, 2015). The source of nymphs was from the wild host plant woolly croton, *Croton capitatus* Michx. Woolly croton was collected in the fall near College Station, TX, and placed in laboratory cold storage (Lubbock, TX) until fleahoppers were needed the following year following the protocol of Hakeem and Parajulee (2015). In brief, conditions conducive to cotton fleahopper emergence were simulated in a laboratory environment in order to induce hatching of overwintered eggs embedded in the woolly croton stems, and emerged cotton fleahoppers were placed on fresh green beans. At approximately ten days post-emergence, fleahopper nymphs were provided fresh cotton squares as a training substrate prior to field release. Releases were conducted by aspirating third to fourth instar cotton fleahopper nymphs from the laboratory colony, transferring them into 1.9 cm by 3.2 cm plastic vials, then depositing them onto the terminals of plants in each treatment plot.

2.3. Measurements and analyses

At the South Texas location, insect counts using a beat bucket technique (Brewer et al., 2012) were made on a weekly basis after cotton fleahopper numbers exceeded 0.10 bugs per plant and continued through the sixth week of squaring. A total of 20 plants were sampled per plot. Weekly data showing treatment differences were reported here. Plant data included lint yield and percent boll retention measured near harvest. Cotton from 45 foot of row from the interior plot rows was machine picked. Percent boll retention was estimated from six plants per plot. At the Texas High Plains location, the data included number of harvestable bolls and lint yield from 3-m section of the cotton row. Pre-harvest plant mapping was conducted by counting number of harvestable bolls per plant. Cotton was hand-harvested. Cotton was ginned at both locations using a 10-saw Continental Eagle laboratory gin.

All measurements were analyzed with ANOVA, conforming to the plot designs for the South Texas and Texas High Plains locations (Littell et al., 1991). Count data were transformed by the square root of the count +0.5. Percent boll retention data from South Texas were transformed by the arcsine of the square root of the proportion (Neter et al., 1985). These designs allowed focus on cotton water stress, age, and cultivars using natural cotton fleahopper infestations (South Texas) and on water stress using augmented infestations (Texas High Plains). Based on our hypotheses, we gave special attention to cotton fleahopper density and yield patterns discerned from significant interactions between water stress and plant age, and water stress and cultivar. Cotton fleahopper-influenced effects were experimentally verified by a significant insecticide spray (South Texas) or cotton fleahopper augmentation (Texas High Plains) effect. Differences in means were directed tested with the ANOVA for the South Texas location. In the Texas High Plains location, Tukey's Honest Significant Difference test was used to compare means across four treatments (Littell et al., 1991).

3. Results and discussion

Generally, cotton fleahopper density and plant response measures of boll retention and yield were sensitive to changes in cotton water stress, cotton age, cultivars, and insect stress. Yield decline was partly attributable to cotton fleahopper activity, and was very sensitive to water stress. Typical square injury caused by cotton fleahopper was observed (Anon, 2015). These general results were consistent at the two locations, but the experimental set up and environmental conditions at each location provided different opportunities to detect and compare individual and combined experimental effects.

3.1. South Texas location

By the second week of squaring, cotton fleahopper injury was detected on the cultivars. Cotton fleahopper densities in the early planting exceeded 0.20 cotton fleahoppers per plant using beat bucket sampling at this time for at least some treatments both years. At 50% efficiency of the sampling technique, the cotton fleahopper activity detected was approaching the designated threshold of 0.15 cotton fleahopper per terminal for the South Texas region (Brewer et al., 2012) and persisted through the following four weeks. This insect activity along with use of insecticide-treated plots allowed for comparison of cotton fleahopper activity across cultivars and yield across cultivars attributed to cotton fleahopper activity.

Cotton fleahopper was most abundant during the fourth through sixth week of squaring (the early planting) in 2012 (planting date effect on June 1 and June 14: $f > 13.5$, d.f. $= 1, 48$; $p < 0.0006$) (Fig. 1), with more cotton fleahoppers occurring in the unsprayed plots (spray effect: $f > 19.0$, d.f. $= 1, 48$; $p < 0.0001$). Cotton fleahopper densities were higher in the Stoneville cultivar ($f > 4.9$, d.f. $= 1, 24$; $p < 0.04$). They were also higher in the earlier planted cotton when grown in poorer soil moisture conditions during the fourth week of squaring (June 1 water regime by planting date interaction: $f \geq 5.47$, d.f. $= 2, 24$; $p < 0.011$) (Fig. 1).

For earlier planted cotton, cotton fleahopper densities were highest under irrigation targeting 90% crop ET replacement in 2013 (July 3 and July 11 water regime by planting date interaction: $f > 4.8$, d.f. $= 1, 8$; $p < 0.05$) (Fig. 2). Insecticide treatment significantly reduced the populations where they were found in high density under good soil moisture, on the Stoneville cultivar, and on early planted cotton (various interactions with the spray treatment were significant, $p < 0.05$) (Fig. 2).

Cotton cultivars had a strong influence on cotton fleahopper abundance. In 2012, higher densities were found on Stoneville 5458 B2RF than on Phytogen 367 WRF on June 14 ($f = 4.91$, d.f. $= 1,24$; $p = 0.036$) (Fig. 1). In 2013, the Stoneville cultivar planted early tended to build the highest cotton fleahopper populations (July 3 planting date by cultivar interaction: $f = 6.09$, d.f. $= 1, 16$; $p = 0.025$) (Fig. 2). Water stress had no to modest effects on cotton fleahopper densities. No water stress effects on cotton fleahopper densities nor two-way water regime interactions with other factors were observed in 2012 ($p > 0.05$). In 2013, cotton fleahopper densities continued to build on older cotton (the early planted cotton) grown under no water stress (July 3 and July 11 planting date by water regime interaction: $f > 4.8$, d.f. $= 1, 8$; $p = 0.05$) (Fig. 2).

In contrast, water stress had considerable influence on plant response, while cultivar influences on plant response were much reduced compared to its influence on cotton fleahopper density. Boll retention tended to be marginally higher in the early planted cotton growing under no water stress for both cultivars in 2012 (planting date by water regime interaction: $f = 3.41$, d.f. $= 2, 12$; $p = 0.06$) (Fig. 3). In 2013, boll retention was greater in non-water
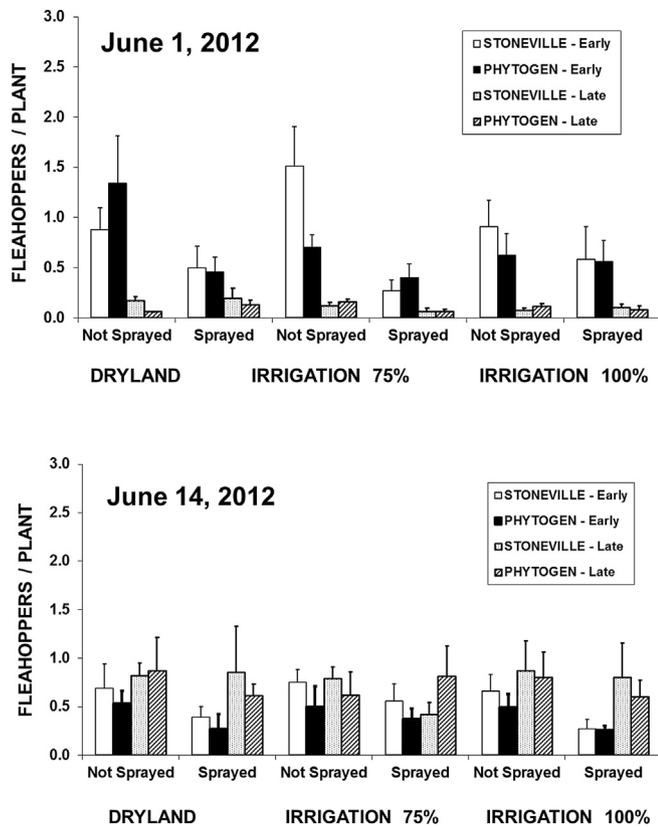
Fig. 1. Cotton fleahoppers (adults & nymphs) per plant from a field experiment with manipulation (five replications) of water regimes, planting dates, cultivars, and insecticide protection in 2012, Corpus Christi, TX. Insect data shown for two sampling dates (June 1 which is fourth and second week of squaring for the early and late planting, respectively; and two weeks later on June 14). Water regimes were a dryland mimic (dryland), 75% crop ET target (in 2012 only, irrigation 75%), and 90% crop ET target [irrigation 100%]. Planting dates were April 12 (early) and April 30 (late). Cultivars were Phytogen 367 WRF and Stoneville 5458 B2RF. Insecticide protection was sprayed for two weeks beginning at second week of squaring and not sprayed.

stress conditions (water regime effect: $f = 11.8$, d.f. $= 1, 15$; $p = 0.0037$). Boll retention did not significantly vary across cultivars ($p > 0.05$) (Fig. 3). We note that boll retention data were not taken in sprayed plots; therefore yield data was used to directly test for cotton fleahopper-induced plant response.

Yield reduction caused by cotton fleahopper injury was experimentally verified in 2012 (spray effect: $f = 8.49$, d.f. $= 1, 46$; $p = 0.005$) and to a more limited extent in 2013 especially for Phytogen 367 WRF (cultivar by spray interaction: $f = 5.37$, d.f. $= 1, 29$; $p = 0.028$) (Fig. 4). Synergies in water stress and cotton fleahopper stress on cotton yield were not detected (no water stress by spray interaction, $p > 0.10$). Yield reduction was less severe in cotton grown under improved soil moisture (where boll retention was also higher), and there was comparably modest yield loss attributable to cotton fleahopper activity. The highest yields were in plots with improved soil moisture (2012 water regime effect: $f = 97.3$, d.f. $= 2, 8$; $p < 0.0001$, and 2013 water regime effect, $f = 84.5$, d.f. $= 1, 4$; $p = 0.0008$) (Fig. 4). In 2012, the maximum yield occurred in early planted cotton grown under no water stress (water regime by planting date interaction: $f = 26.5$, d.f. $= 2, 12$; $p < 0.0001$) (Fig. 4), even though cotton fleahoppers were more abundant on the early planted cotton (Fig. 1). The strong influence of soil moisture on yield was consistent across cultivars (no interaction with cultivar) (Fig. 4), even though cotton fleahopper
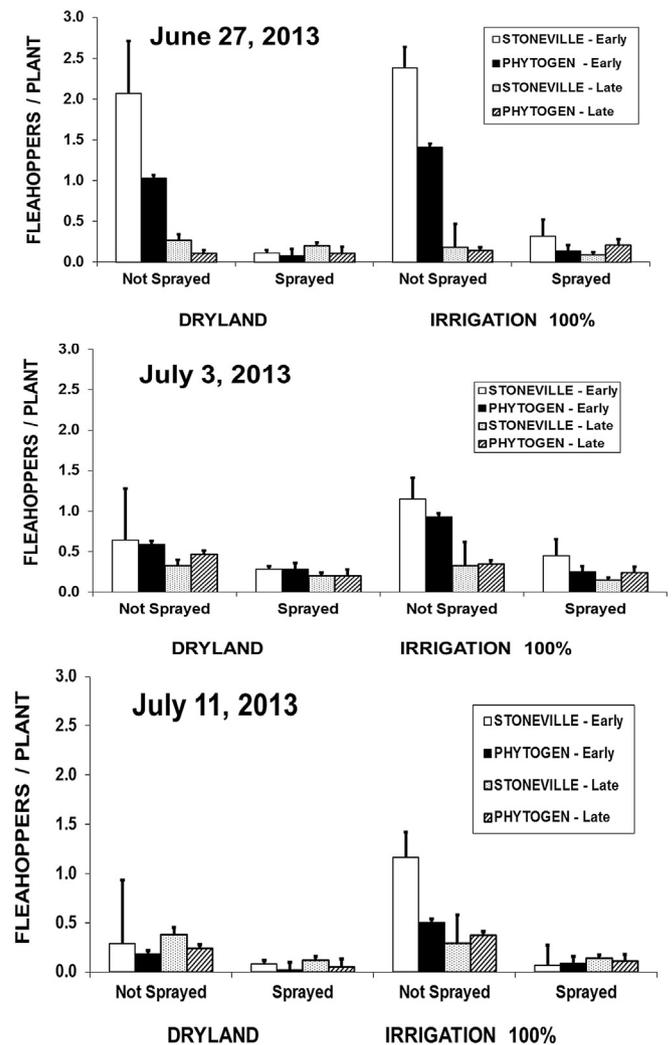

Fig. 2. Cotton fleahoppers (adults & nymphs) per plant from a field experiment with manipulation (five replications) of water regimes, planting dates, cultivars, and insecticide protection in 2013, Corpus Christi, TX. Insect data shown for three sampling dates (June 27 which is the fifth and third week of squaring for the early and late planting, respectively; and one [July 3] and two [July 11] weeks later). Water regimes were a dryland mimic (dryland) and 90% crop ET target [irrigation 100%]. Planting dates were April 22 (early) and May 6 (late). Cultivars were Phytogen 367 WRF and Stoneville 5458 B2RF. Insecticide protection was sprayed for five weeks beginning second week of squaring and not sprayed.

populations varied significantly across cultivars (Figs. 1 and 2). Controlling fleahoppers modestly benefitted yield in 2012 as indicated by the significant spray factor noted above ($p = 0.005$), and modest yield benefits from controlling cotton fleahopper was also seen in 2013 for the Phytogen cultivar as noted above ($p = 0.028$). Although yield loss attributed to cotton fleahopper was greater on the Phytogen cultivar than on the Stoneville cultivar (Fig. 4), it commonly had fewer cotton fleahoppers (Fig. 2). As noted by Knutson et al. (2013), resistance to cotton fleahopper includes tolerance in which cotton fleahopper does not induce yield loss.

3.2. High plains location

Synergies were detected in water and cotton fleahopper stress in the High Plains location. The difference in total number of harvestable bolls attributable to the cotton fleahopper augmentation under very high water stress (1.4 bolls per plant) was greater
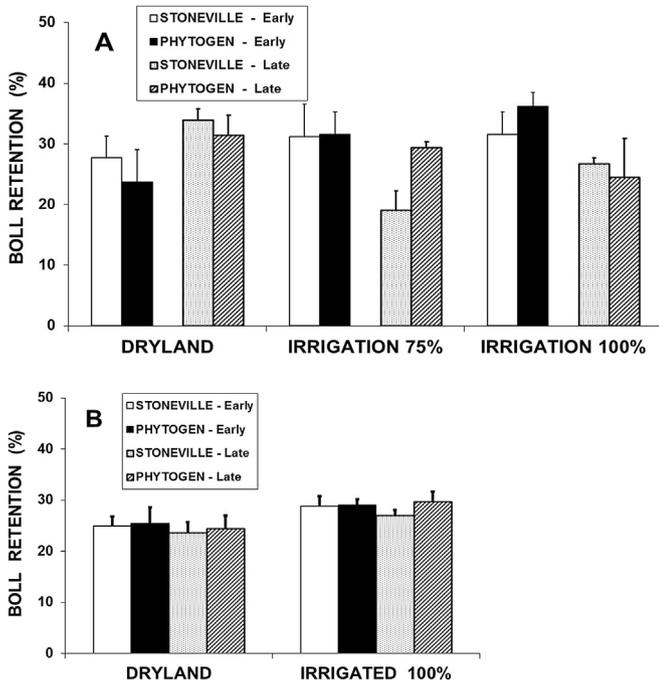
Fig. 3. Average percent boll retention from a field experiment with manipulation (five replications) of water regimes, planting dates, cultivars, and insecticide protection in 2012 (A), and 2013 (B), Corpus Christi, TX. Water regimes were a dryland mimic (dryland), 75% crop ET target (in 2012 only, irrigation 75%), and 90% crop ET target [irrigation 100%]. Planting dates were early and late (see Figs. 1 and 2 for dates). Cultivars were Phytogen 367 WRF and Stoneville 5458 B2RF.
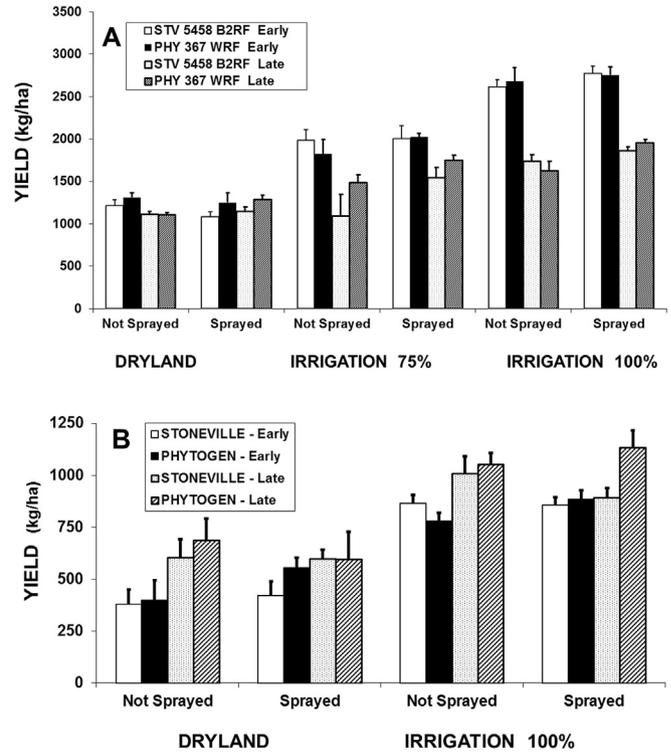
Fig. 4. Lint yield (kg/ha) from a field experiment with manipulation (five replications) of water regimes, planting dates, cultivars, and insecticide protection in 2012 (A), and 2013 (B), Corpus Christi, TX. Water regimes were a dryland mimic (dryland), 75% crop ET target (in 2012 only, irrigation75%), and 90% crop ET target [irrigation 100%]. Planting dates were early and late (see Figs. 1 and 2 for dates). Cultivars were Phytogen 367 WRF and Stoneville 5458 B2RF. Insecticide protection was sprayed for five (A) and two (B) consecutive weeks beginning at first squaring and not sprayed.

than that for moderate high water stress (0.4 bolls per plant) ($p < 0.05$, Fig. 5). Yield loss attributable to cotton fleahopper stress was observed in cotton grown in the very high water stress condition when artificially infested with cotton fleahoppers ($p < 0.05$) at a rate of five nymphs per plant (which at 20% estimated survival exceeded the High Plains threshold of 0.30 cotton fleahoppers per plants [Anon, 2015]). In contrast, yield loss was not seen in cotton grown in a more moderate water-deficit regime, even when infested with cotton fleahopper (Fig. 6).

4. Conclusion

Crop plants under water stress have been observed to incur more insect injury which can adversely affect yield (Haile, 2000), although mechanistic explanations including relationships to insect population dynamics are more limited (Huberty and Denno, 2004). In our study, plant age and cultivar selection were main moderators of cotton fleahopper populations (Figs. 1 and 2), although strong cultivar influences on cotton fleahopper dynamics did not correspond to yield reduction (Fig. 4). We saw few planting date by cultivar interactions, suggesting the influence of these strategies on cotton fleahopper pest management can be considered independently. Water stress had much more modest influence on cotton fleahopper abundance (Figs. 1 and 2).

In regard to plant response, cotton fleahopper-associated yield loss was lower than water stress-associated yield loss, and the combined effects of water and cotton fleahopper stress on yield were variable. In South Texas, water stress directly affected yield with modest influence from cotton fleahopper (Figs. 1, 2 and 4). In the Texas High Plains, very high water stress resulted in reduced yield and reduced boll loads, and the effect was enhanced when cotton fleahopper was present (Figs. 5 and 6). Water stress was
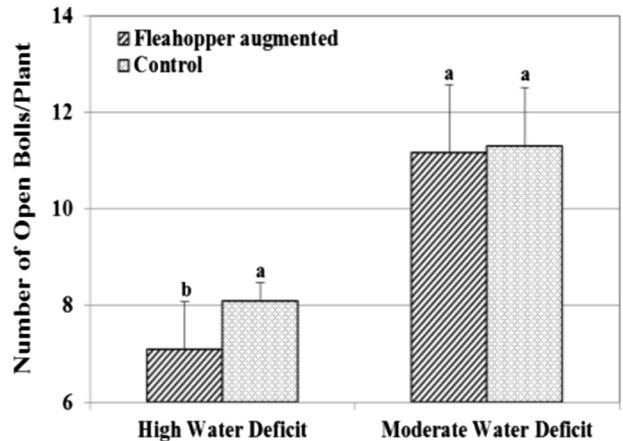

Fig. 5. Number of open bolls per plot from a field experiment with manipulation (four replications) of water stress and insect stress in 2013 (Lamesa, TX). Water regimes were very high water stress and moderate water stress exposed to augmented populations of cotton fleahopper (fleahopper augmentation and control). Different letters above bars indicated signficant differences based on Tukey's Honest Significant Difference test (p = 0.05).

more severe at this site, and the major pulse of artificial cotton fleahopper infestation may have contributed to resolution of this water and insect stress interaction. More broadly, these results likely reflect the field variability seen in plant response to cotton fleahopper feeding, and the paradox of observations of different
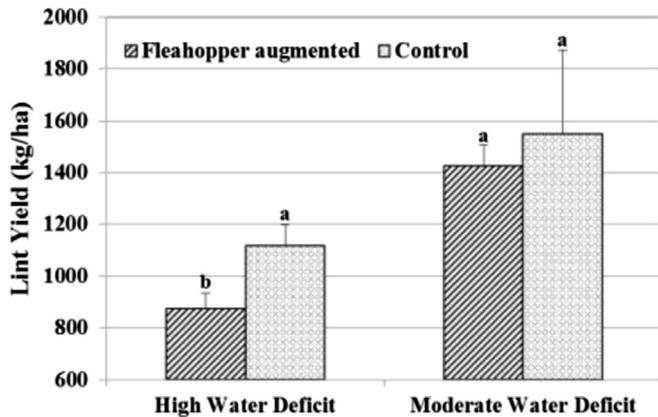
Fig. 6. Lint yield (kg/ha) from a field experiment with manipulation (four replications) of water stress and insect stress in 2013 (Lamesa, TX). Water regimes were very high water stress and moderate water stress exposed to augmented populations of cotton fleahopper (fleahopper augmentation and control). Different letters above bars indicated signficant differences based on Tukey's Honest Significant Difference test (p = 0.05).

frequencies of insecticide sprays used to control cotton fleahoppers under apparently equal cotton fleahopper pressure.

We live in a climate that produces highly variable weather, as seen in drought conditions in Texas from 2011 to 2013. Drought has been implicated in changes in pest population dynamics and plant sensitivity to insect feeding (Haile, 2000). For the case of cotton fleahopper feeding on cotton, water stress affects yield substantially and directly. The South Texas data supported a more modest water stress influence on cotton fleahopper dynamics, where cotton fleahopper-associated yield loss was lower than water stress-associated yield loss. Elevated yield loss attributable to synergistic effects of cotton fleahopper and water-deficit stress was more variable. Synergistic effects were best seen under manipulated (artificial infestations) cotton fleahopper densities in very high water stress conditions at the High Plains location. But a reduction of naturally occurring cotton fleahoppers was also seen at this location which had been experiencing extreme drought. In more moderate drought conditions at the South Texas location where cotton fleahopper persisted and naturally infested the experiment, synergies in insect and water stress were not detected.

Cotton fleahopper decision-making may be more cultivar specific than as implied when reviewing regionally-based thresholds (Anon, 2015). Cultivar sensitivity to cotton fleahopper injury leading to yield differences has been previously demonstrated for past cotton cultivars (Ring et al., 1993). For future work, use of more agriculturally representative cultivars should be emphasized, grown under a number of cotton fleahopper exposure scenarios. Including water regime scenarios remains relevant, but synergistic effects of water stress and cotton fleahopper stress appear to be less common than originally hypothesized.

Conflicts of interest

Acknowledgment

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