TEXAS A&M AGRILIFE RESEARCH & EXTENSION

COTTON ENTOMOLOGY RESEARCH REPORT

2013

TECHNICAL REPORT 14-4

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

COTTON ENTOMOLOGY PROGRAM

RESEARCH ACTIVITY ANNUAL REPORT

2013

SUBMITTED TO:

PLAINS COTTON IMPROVEMENT COMMITTEE
PLAINS COTTON GROWERS, INC.

JAROY MOORE, RESIDENT DIRECTOR
TEXAS A&M AGRILIFE RESEARCH-LUBBOCK

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Introduction

Plains Cotton Growers, Inc. (PCG) has been a strong supporter of cotton insect research and extension activities in west Texas for many years. Most notably, PCG was instrumental in securing state funds for the Boll Weevil Research Facility at the Lubbock Center, and provided both financial and political support to conduct boll weevil biology and ecology research even before the boll weevil became a significant economic pest of the High Plains region. After the initial entry of the boll weevil into the eastern edge of the High Plains, PCG promoted and along with USDA-APHIS administered the boll weevil diapause suppression program involving a team effort that continued to include Texas A&M University. PCG also supported Texas Cooperative Extension (now Texas A&M AgriLife Extension Service) efforts to annually evaluate the diapause suppression program, conduct applied research trials to develop boll weevil management practices that would enhance the diapause suppression program's efforts and in the 1990s supported an annual survey of High Plains overwintering sites and grid trapping of cotton across the High Plains area. Under the strong and cooperative leadership of PCG, the boll weevil eradication program for the High Plains area progressed much more rapidly than anticipated. Now, the successful boll weevil eradication program has eliminated the boll weevil from this region for over 10 years. The team effort of PCG, Texas A&M AgriLife Research and AgriLife Extension Service over many decades has resulted in a comprehensive understanding of boll weevil ecology and behavior.

With a successful boll weevil eradication program and increased adoption of the Bollgard technology (now >70%), the cotton insect research and extension program focus has changed considerably during the last 10 years. Our current research/extension focus is on developing ecologically intensive management strategies for cotton pest management. 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 our considerable time and manpower resources in investigating behavior and ecology of major cotton pests of the High Plains with the goal of developing management thresholds based on cotton production technology. Some basic research is also underway to develop some molecular techniques to accurately identify some insect species, particularly *Lygus* bugs in a mixed population or to understand their movement behavior. That will allow us to recommend appropriate insecticide and dose for that specific insect. Our Program has successfully leveraged research funds based on the funding provided by PCIC to support our Technician position. We are excited about and greatly value our Cotton Entomology research and extension partnerships with newly hired Extension Specialist, Dr. Apurba Barman, together with seasoned IPM Agents we have 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 ARTHROPOD POPULATION DYNAMICS AS AFFECTED BY NITROGEN FERTILITY; HALFWAY, TEXAS

A long-term, ongoing study investigating the effects of differential nitrogen fertility on arthropod population dynamics in a typical drip-irrigation Texas High Plains cotton production system has been conducted for the last 12 years. Differential nitrogen fertility (0, 50, 100, 150, and 200 lbs N/acre) has been shown to significantly affect cotton plant physiological parameters, thereby influencing arthropod population dynamics.

Side-dressing variable rates of nitrogen fertilizer and resulting phenotypic variation in cotton

INVESTIGATION OF GENETICALLY MODIFIED COTTON CONFERRING *LYGUS*-TOLERANCE; LUBBOCK, TEXAS

(IN COOPERATION WITH MONSANTO COMPANY)

As part of an ongoing Monsanto program to develop commerically available *Lygus*-tolerant cotton germplasm, numerous cotton lines, genetically modified to confer *Lygus* tolerance via protein expression (similar to *Bt* technology), are being evaluated for effectiveness under whole-plant cage field conditions. Initial findings have been encouraging, and some exciting entomological properties have been observed in gene-of-interest positive plants.

Field cage evaluation of transgenic cotton events

UNDERSTANDING COTTON FLEAHOPPER OVERWINTERING EMERGENCE BIOLOGY AND POPULATION DYNAMICS

Cotton fleahoppers are minor but significant pests of cotton in the Texas High Plains. They generally overwinter in wooly croton. Dead croton twigs, containing overwintered cotton fleahopper eggs, were collected from the Brazos Valley during the winter months and stored in a walk-in cooler. Current study is evaluating the influence of amount, frequency, and method of soaking of these croton twigs on fleahopper diapause breakdown, nymphal emergence, and survivorship. We plan to develop a climatic data-based model to predict the fleahopper emergence and likely pest risk on cotton based on rainfall patterns.

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. Specific objectives are to: determine the maximum potential for *Lygus* to inflict damage to cotton bolls at various boll maturity levels, characterize the cotton boll feeding biology and behavior of *Lygus*, and establish the *Lygus* economic threshold for Texas cotton.

Single-plant cages in *Lygus* economic threshold study

THRIPS MANAGEMENT IN TEXAS HIGH PLAINS COTTON: THRESHOLD DEVELOPMENT AND PRODUCT EVALUATION

Two research projects, funded by USDA NIFA Organic Research and Extension Initiative and Cotton Incorporated, are investigating ecological attributes of and management recommendations for thrips in Texas High Plains cotton. Primary goals of these projects are to characterize the cotton crop response to various levels of thrips injury and to develop/validate new economic thresholds. Comparative evaluation of available thrips management products, both organic and conventional, should help growers in making informed and economically sound thrips management decisions.

Greenhouse investigation of cotton cultivars to thrips injury

EFFECT OF NITROGEN FERTILIZER ON COTTON HOST-PLANT QUALITY AND ITS IMPACT ON ARTHROPOD ACTIVITY

M.N. Parajulee, A. Hakeem, R. Norman, S.C. Carroll, J.P. Bordovsky

Objective: The objective was to evaluate the effect of nitrogen fertilizer application rates on the population dynamics of cotton arthropods, plant growth parameters, and lint yield.

Methodology: A high-yielding FiberMax cultivar, FM 9063B2R, was planted at a targeted rate of 56,000 seeds/acre on May 23, 2013. The experiment consisted of a randomized block design with five treatments and five replications. Pre-treatment soil samples (consisting of three soil cores; 0 to 24-inch depth), were collected from each of the 25 experiment plots on June 20, 2013. The five side-dress N fertilizer application treatments at rates of 0, 50, 100, 150, and 200 lb N/acre were applied on July 11, 2013. Crop growth and insect activity were monitored during the crop season. Weekly during most of July and August, numerous plant variables were measured to evaluate the influence of residual soil nitrogen on early plant growth patterns. Examples of collected plant data variables included: 1) plant biomass, 2) plant height, 3) total leaf area, 4) percent leaf nitrogen, 5) number of 1st position cotton squares/plant, and 6) percent fruit shed.

Results: Soil residual N levels were significantly higher in plots that received the two highest rates of N versus plots receiving lower-rate or no N augmentation. 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. The highest N augmentation plots (200 lb/acre) had significantly highest average residual N (Fig. 1). The two second highest N plots (100 and 150 lb/acre) resulted in significantly higher amount of residual N compared to that in zero and 50 lb/acre plots. Plants ceased setting additional squares in zero and 50-lb N plots 2 wk into flowering while higher N plots were actively producing squares.

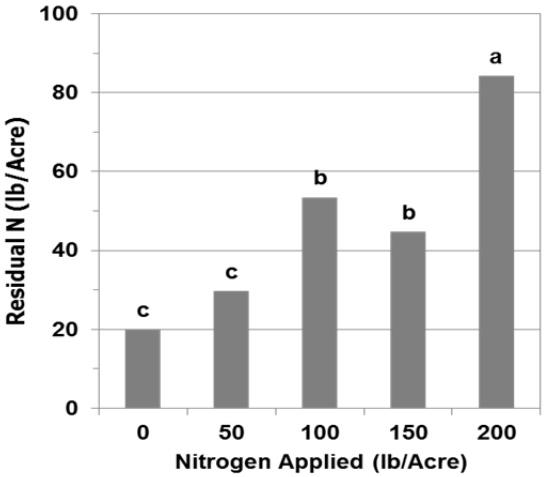

Fig. 1. Averaged over six years, effect of prior year's N application on residual N accumulation for the current crop year, 2008-2013.

Zero-N applied plots produced the lowest yield and yield increased curvilinearly, with highest average yield occurring in the 150 and 200 lb/acre (Fig. 2). Numerical decline in yield beyond 150 lb/acre in most years suggests that N application beyond 150 lb/acre may be unfavorable for cotton yield. 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 (<3.4).

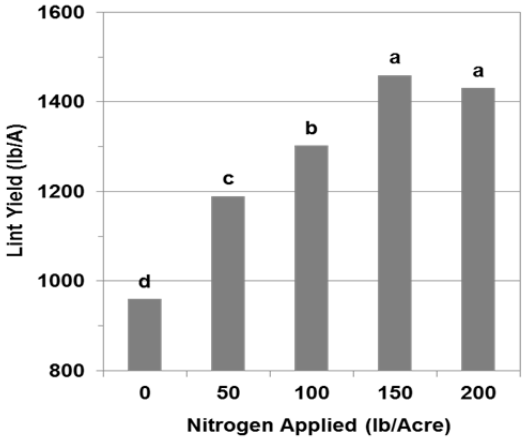

Fig. 2. Averaged over six years, effect of N application rates on lint yield, 2008-2013.

TITLE:

Cotton yield response to cotton fleahopper acute infestations as influenced by irrigation level treatments, Lamesa, TX, 2013.

AUTHORS:

Megha Parajulee, Abdul Hakeem, Stanley Carroll, and Wayne Keeling; Professor, Research Associate, Research Scientist, and Professor, Texas A&M AgriLife Research

MATERIALS AND METHODS:

Plot Size:	4 rows by 200 feet, 3 replications
Planting Date:	June 5, 2013
Varieties:	PHY 367WRF
Fertilizer:	100-35-0
In-season Irrigation:	Low = 4.1 inches; High = 8.1 inches
Insect Treatments:	Control (zero cotton fleahopper); Cotton fleahopper infested (5 nymphs per plant)
Insect Release Date:	August 6, 2013 (last effective fleahopper susceptible stage)
Harvest Date:	November 2, 2013 (hand-harvested)

Cotton fleahopper feeding injury was evaluated in a high yielding cotton cultivar, Phytogen 367 B2RF, as affected by irrigation level. Two irrigation levels were evaluated, High (8.1") and Low (4.1"), under a center pivot irrigation system. The experiment consisted of 2 irrigation levels (high and low) and two cotton fleahopper augmentation treatments (5 cotton fleahopper nymps per plant versus no fleahopper augmentation as control). Each treatment plot consisted of 10 plants and the entire test was replicated three times, with a total of 12 experimental units.

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 that were collected from the Brazos Valley, and emerged cotton fleahoppers were subsequently reared using fresh green beans as a feeding substrate. A single release of nymphal cotton fleahoppers was timed to simulate the acute late infestation of cotton fleahoppers while cotton is still vulnerable to the fleahopper injury, which is approximately around the first observation of cotton flower in test plots. The release was done on August 6 by aspirating third-to fourth-instar cotton fleahopper nymphs from the laboratory colony, transferring them into 0.75" X 1.5" plastic vials, then cautiously and methodically depositing them onto the terminals of plants in each treatment plot at the rate of 5 nymphs per plant; the control plots received no fleahoppers. There was no natural infestation of cotton fleahopper at the experimental farm, so the control plots did not require any insecticidal intervention. Post-release data collection included a pre-harvest complete plant mapping and lint yield.

RESULTS AND DISCUSSION:

Harvestable boll density (number of harvestable bolls per plant) did not significantly vary between fleahopper augmented and control plots (Fig. 1). Nevertheless, the difference in total number of harvestable bolls under 'Low' water regime (1.4 bolls per plant) was numerically greater than that for 'High' water regime (0.4 bolls per plant), suggesting that 'High' water regime compensated for the fruit loss caused by fleahopper injury. Lint yield varied with fleahopper augmentation treatment under 'Low' water regime, but it did not vary under 'High' water regime. Lint yield values were 781 and 998 lbs per acre for

'Low' water regime and 1,271 and 1,380 lbs/acre for 'High' water regime in control and fleahopper augmented plots, respectively (Fig. 2). Lint yield was significantly lower due to cotton fleahopper infestation under 'Low' water regime, but the effect was not as pronounced and not significant under 'high' water regime, indicating plants' ability to compensate for fleahopper-induced fruit loss under high irrigation production system.

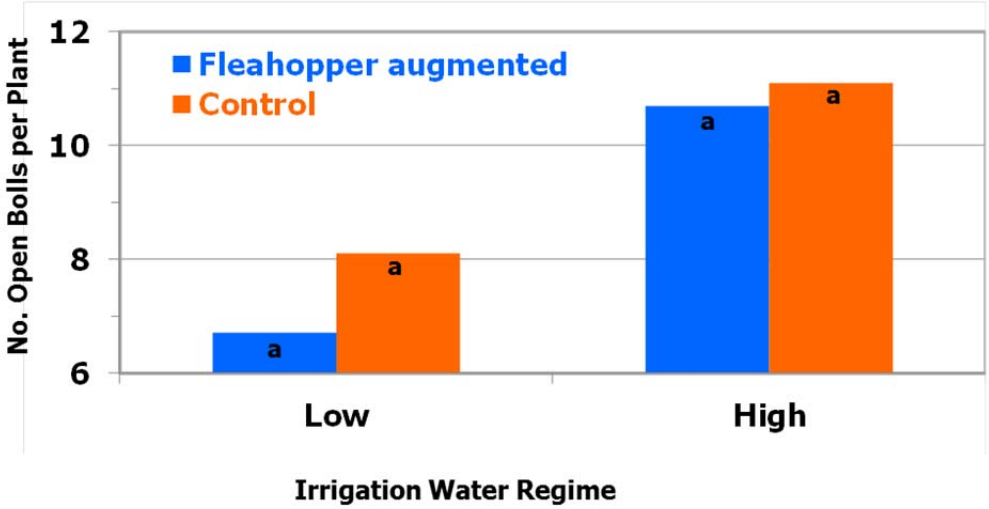

Fig. 1. Average number of total open (harvestable) bolls per plant following a simulated acute infestation of cotton fleahoppers, achieved by augmenting 5 nymphs per plant during the third week of squaring, under low and high irrigation regimes, Lamesa, Texas, 2013.

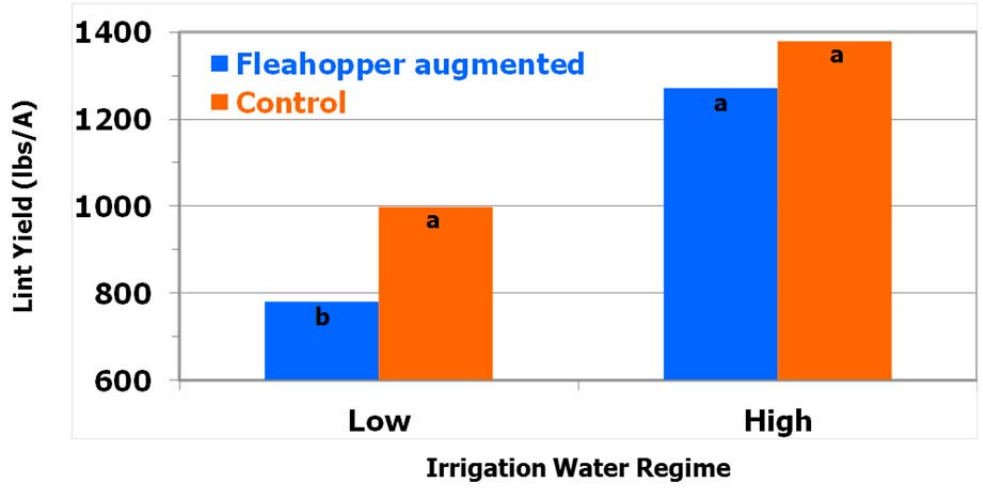

Fig. 2. Average lint yield following a simulated acute infestation of cotton fleahoppers, achieved by augmenting 5 nymphs per plant during the third week of squaring, under low and high irrigation regimes, Lamesa, Texas, 2013.

INFLUENCE OF WOOLLY CROTON SOAKING ON COTTON FLEAHOPPER EMERGENCE FROM OVERWINTERED EGGS

Abdul Hakeem and Megha Parajulee

Texas A&M AgriLife Research and Extension Center, Lubbock, TX

Abstract

The cotton fleahopper, *Pseudatomoscelis seriatus*, is an important early-season pest of cotton. Cotton fleahoppers feed on early-stage cotton squares with its piercing-sucking mouthparts which causes aborting of affected squares. Cotton fleahopper eggs predominantly overwinter on woolly croton, *Croton capitatus*, in late fall and terminate diapuse in early spring responding to minimum required temperature and moisture. The objective of this study was to evaluate the effects of different moisture levels (soaking durations of woolly croton) on the emergence of cotton fleahopper nymphs from diapausing eggs. Five moisture treatments evaluated included 1) 24-hour initial soaking and no further moistening of the substrate for the remainder of the emergence duration (T_1); 2) 2-hour initial soaking followed by mist-spraying of the substrate daily (T_2); 3) 2-hour initial soaking, followed by 30-minute soaking for the next seven days, and the mist-spraying daily (T_3); 4) 2-hour initial soaking, followed by 30-minute soaking for the next seven days, and then dipping the substrate in water daily (T_4); and 5) soaking every-other-day for 15 minutes (T_5). During this experiment, a total of 6,344 cotton fleahopper nymphs emerged from 2,200 g croton twigs in about a month period. The highest numbers (n=425) of nymphs emerged from T_4 (2-h, 30 min, dipping) followed by T_3 (2-h, 30 min, spraying) (n=404), whereas the least numbers were emerged from (T_1) (24-h) (n=173). Significantly higher (P=0.05) number of nymphs emerged from T_4 (n=425) and T_3 (n=404) compared to T_1 (n=173), T_2 (n=290), and T_5 (n=293). To maximize the fleahopper emergence from overwintered eggs, it is recommended that croton should be soaked for at least 7 days and may also need to keep it moist throughout the emergence period via mist-spraying.

Introduction

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), is an important pest of cotton in Texas and Oklahoma, and occasional pest in New Mexico, Arkansas, Louisiana, and other mid-South states (Walker et al. 1970, Esquivel and Esquivel 2009). Cotton fleahopper is a small insect with piercing-sucking mouthparts which feed on early-stage cotton squares and cause shedding of affected squares resulting in potential yield loss (Reinhard 1926, Almand 1974). In 2011, *P. seriatus* induced cotton lint yield loss was estimated at 0.15% in the United States cottonbelt and 0.24% in Texas (Fig. 1) (Williams 2013). There are at least 160 plant species representing 35 families that serve as potential host for the cotton fleahopper, including pinkladies, *Oenothera speciosa,* upright prairie coneflower, *Ratibida columnifera,* silver-leaf nightshade, *Solanum elaeagnifolium*, and woolly croton, *Croton capitatus*, among other host species (Snodgrass et al. 1984, Esquivel and Esquivel 2009). In late fall, cotton fleahoppers lay eggs on host plants and eggs overwinter until early spring. Increased temperature and spring rain events activate diapused eggs and fleahopper nymphs emerge depending on temperature and moisture conditions. In the laboratory, diapause could be terminated by providing controlled temperature, daylight and moisture (Saunders 1983). Despite its intriguing overwintering biology and host associated differentiation (Barman et al. 2012), information on cotton fleahopper overwintering ecology as it relates to semi-arid environment such as in the Texas High Plains is limited. A study was initiated to evaluate the effects of different moisture levels (soaking durations of woolly croton) on the emergence of cotton fleahopper nymphs from diapausing eggs.

Materials and Methods

This study was conducted at Texas A&M AgriLife Research and Extension Center, Lubbock, TX. Dormant twigs of croton, *Croton capitatus,* were collected from the Brazos Valley (College Station area), Texas in January 2012. Croton twigs were stored in a walk-in cooler at 4 ^{0}C. Croton twigs (110 g) were cut into ≈27 cm length and placed in cylindrical aluminum containers (ca. 27.5 cm height, 16.51 cm diameter). Temperature of growth chamber was maintained between 25 and 34 ^{0}C under a 12:12 L:D photoperiod (Fig. 2). Both ends of the rearing container were covered with coarse-mesh screens to allow for the cotton fleahopper nymphs to exit from the rearing substrate.

Another layer of muslin cloth was placed on top of the first screen and secured by a rubber band to enclose newly emerged fleahopper nymphs until shaking to dislodge them from the substrate. Experiment included five moisture treatments and four replications (aluminum cans): 1) 24-hour initial soaking and no further moistening of the substrate for the remainder of the emergence duration (T_1); 2) 2-hour initial soaking followed by mist-spraying of the substrate daily (T_2); 3) 2-hour initial soaking, followed by 30-minute soaking for the next seven days, and the mist-spraying daily (T_3); 4) 2-hour initial soaking, followed by 30-minute soaking for the next seven days, and then dipping the substrate in water daily (T_4); and 5) soaking every-other-day for 15 minutes (T_5). The last treatment (T_5) was adapted from Breene et al. (1989) that served as a 'control'. An experimental control would have been a treatment with 'no moisture' but such treatment is unrealistic because the cotton fleahopper emergence does not occur without the moisture-activation of the diapausing eggs. Incubation was initiated on 19 July. Shaking of the rearing cans to dislodge emerging nymphs from the substrate began on Day 7 of the experiment and continued for the next 29 days. Cans were shaken twelve times to dislodge nymphs on a white poster board. Dislodged nymphs were counted and transferred into small plastic containers and fed with green beans for rearing.

Results and Discussion

A total of 6,344 cotton fleahopper nymphs emerged from 2,200 g croton substrate in about a month period. Significantly higher number of nymphs emerged from T_4 (n = 425) and T_3 (n = 404) compared with T_1 (n = 173), T_2 (n = 290) and T_5 (n = 293) (Fig. 3)**.** The highest number (n = 425) of fleahopper nymphs emerged from T_4 (2-h, 30 min, dipping) while the least numbers emerged from (T_1) (24-h) (n = 173). Cotton fleahopper emergence began 6 days after initial soaking at 24-36°C. The highest one-day emergence (153 nymphs per 110 g croton) occurred 12 days after incubation in T_4 (2 h, 30 min, dipping). The last cotton fleahopper nymph emerged from croton 32 days after the initiation of incubation. Based on this study, cotton fleahopper emergence from overwintered eggs could be maximized by soaking the overwintering substrate for at least 7 days and may also need to keep it moist throughout the emergence period.

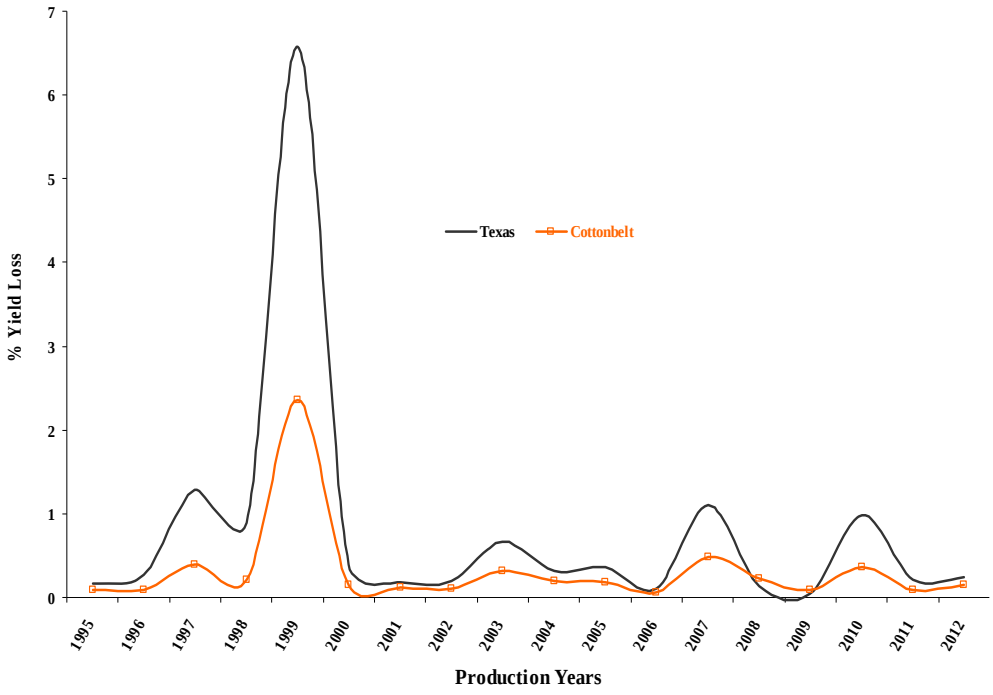

Figure 1. Lint yield losses caused by cotton fleahoppers to the U.S. cotton, 1999-2012.

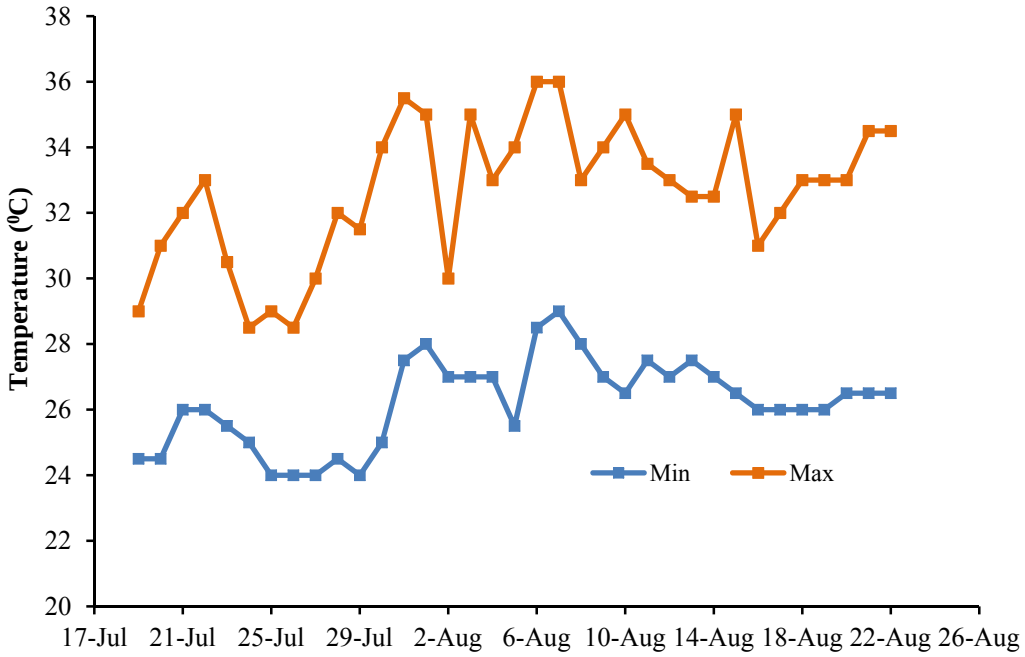

Figure 2. Daily temperatures recorded in the rearing laboratory during the study period, Lubbock, TX.

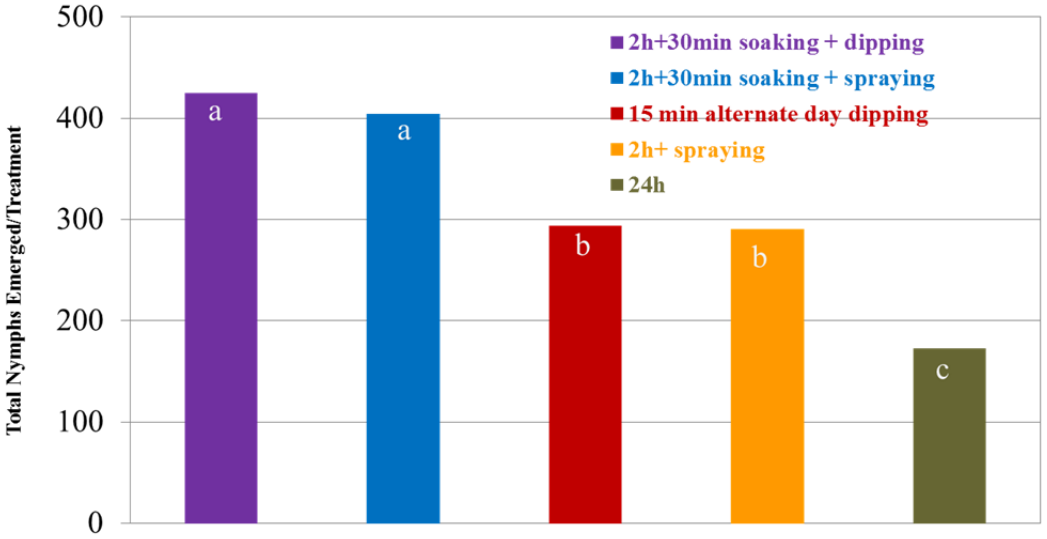

Figure 3. Effect of duration of croton soaking on the emergence of cotton fleahopper nymphs, Lubbock, TX.

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VARIATION IN COTTON VARIETAL RESPONSE TO WESTERN FLOWER THRIPS INJURY

Abdul Hakeem and Megha Parajulee
Texas A&M AgriLife Research and Extension Center, Lubbock, TX

Abstract

The western flower thrips, *Frankliniella occidentalis* Pergande, is a seedling cotton pest which can cause severe damage to seedlings resulting in significant yield loss. In 2012, thrips ranked third among arthropods-caused losses to cotton yield. To evaluate the responses of different cotton cultivars to thrips injury, a study was conducted at the Texas A&M AgriLife Research farm located near Lubbock, Texas. The study was deployed in a randomized block design with four replications and six cultivar treatments. Six cotton cultivars were planted and assigned to a 'control' or 'sprayed' treatment. Plant stand counts were performed by counting all plants in 3 row-ft per row while thrips densities were monitored by five-plant thrips washing technique. Orthene® 95S (3 oz./acre) was sprayed in all 'sprayed treatment' plots after each thrips sampling event. Flowering profiles were monitored from a 10-ft section within each of the two center rows within each plot from flowering initiation until crop cut-out. A total of 20 row-ft per plot was harvested, ginned and estimated the lint and seed yields. Plant response to thrips injury was monitored by measuring shoot length, root length, shoot biomass, root biomass, total leaf area, and total dry biomass of cotton seedlings from each plot. Plant stand counts were significantly higher in cultivars T12 07-7-1407 CT 1205, T12 07-7-1001 CT 1206, DP353 and PHY376 compared to FM 1740 B2F and SSG-HQ-212-CT; however, stand counts between the insecticide treatments were non-significant. Cultivar DP353 and PHY367 had significantly more thrips in control plots than sprayed plots but no significant differences were found between sprayed and unsprayed plots among other cultivars. Nevertheless, sprayed plots had overall lower thrips abundance than in control plots. DP353 had the longest flowering period and peak flowering occurred later in the season. In both treated and control plots, the highest number of white flowers were observed in PHY367 on July 30. Significant differences were observed in plant biomass between cultivar treatments ($P<0.1$). Significantly higher lint yields ($P<0.1$) were observed in sprayed plots compared to that in control plots in DP353 and PHY367; however, no significant differences ($P>0.1$) were observed between sprayed and control plots in other cultivars tested. Also, significant differences in seed yield ($P<0.1$) was observed between sprayed and control plots in DP353 only.

Introduction

Cotton, *Gossypium hirsutum* (L.), was probably first domesticated 5000 years ago (Brubaker and Wendel 1994) and has been grown in many parts of the world as a cash crop. China, India, and the United States of America are the most leading cotton producing countries in the world in that order. In the U.S., Texas produces 55% of the nation's cotton (Fig. 1A); of which, approximately 66% of the Texas cotton is produced in the High Plains region (Fig. 1B).

Western flower thrips, *Frankliniella occidentalis* Pergande (Fig. 2A), is a serious pest on seedling cotton in Texas and other regions of the U.S. cottonbelt. Thrips are an early season pest which can cause severe damage to seedling cotton (Figs. 2B and C). In 2012, arthropods caused 2.04% cotton yield loss in the U.S. Of which, 0.37% yield loss was caused by thrips which ranked third among arthropod-caused losses and accounted for ca. 9,000 bales loss in Texas (Fig. 3). In 2012, thrips infested an area of 8,890,673 acres in the U.S. while in Texas infestation was on an area of 3,792,718 acres (Williams 2012). Thrips cause damage to seedling cotton and excessive feeding leads to browning of leaves on the edges, develop a silvery color, or curl upward from the edges and cause the loss of leaf chlorophyll and leaf area. Several insecticides, including Orthene®, are commonly used to reduce thrips infestations during the early cotton growth stages. Improved cotton varieties may reduce production losses due to thrips injury. The objective of this study was to evaluate the responses of different cotton varieties to thrips injury.

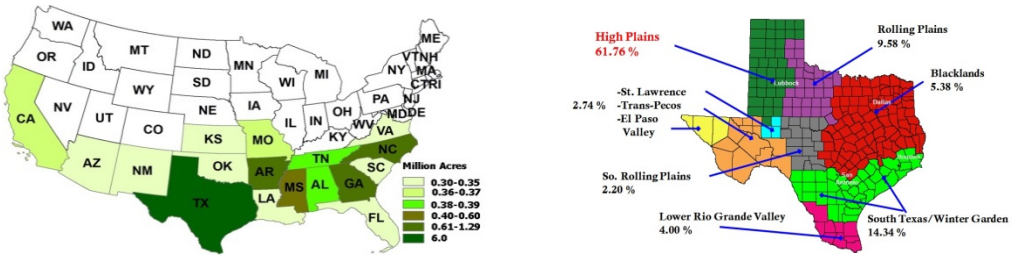

Figure 1). Cotton production acreage distribution in the United States (left) and cotton production in Texas (right).

Figure 2A). Adult western flower thrips, Frankliniella occidentalis, B) Severe damage caused by F. occidentalis to seedling cotton, C) Stunted cotton seedlings due to thrips injury.

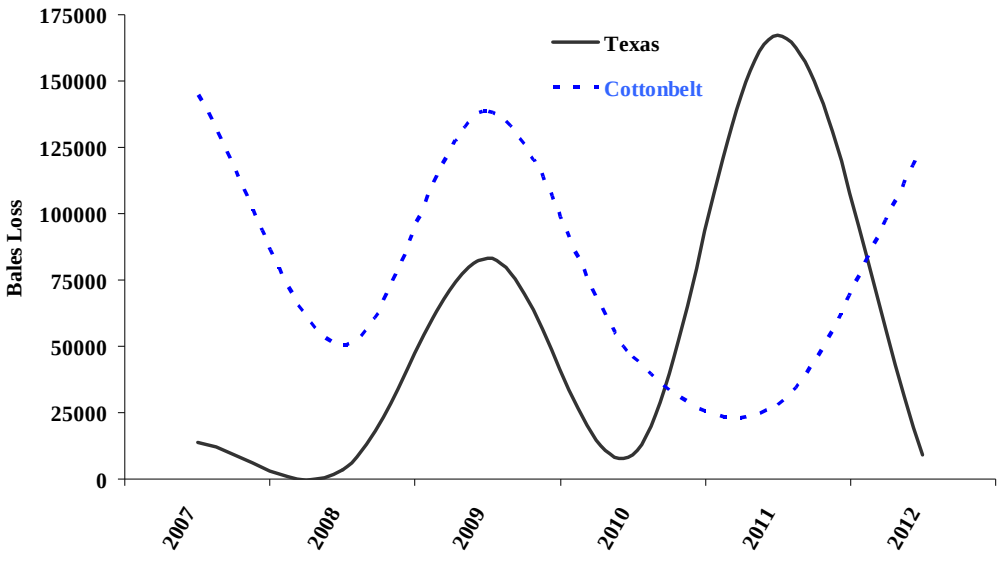

Figure 3. Annual cotton lint yield loss attributed to thrips injury in the U.S. (Williams 2007-2012).

Materials and Methods

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

assigned to a 'control' or 'sprayed' treatment. Thus, the entire study consisted of 48 experimental units (six cultivars x two treatments x four replications). Plant stand counts were performed on May 23 and June 3 by counting all plants in 3 row-ft per row in all 48 plots. Thrips visual counts in the field were also performed on May 23 and June 3 by counting thrips on 10 plants per plot. Thrips densities were also monitored in all 48 plots using a five-plant thrips washing technique. Orthene® 97S (3 oz/acre) was sprayed in all 24 'sprayed treatment' plots immediately after each thrips sampling event. A 10-ft section was marked on each of the two center rows within each plot and flowering profile was monitored 2-3 times per week. This type of phenological monitoring began prior to the initiation of flowering and continued until crop cut-out. Flowering profile was monitored 10 times during the flowering period. Two 10-ft sections from the middle two rows (20 total row-ft) were harvested to estimate the cotton lint and seed yields from each experimental plot. Plant response to thrips injury was monitored by measuring shoot length, root length, shoot biomass, root biomass, total leaf area, and total dry biomass of cotton seedlings from each plot on June 24, coinciding with cotton plants attaining approximately 5 true-leaf stage.

Results and Discussion

Visual thrips counts did not significantly vary between treatments or cultivars. Stand counts between treatments were also non-significant; however, plant counts were significantly higher in CT1205, CT1206, DP353 and PHY376 compared to FM1740 and SSGHQ. Cultivar DP353 and PHY367 had significantly more thrips in control plots than sprayed plots. No significant thrips population densities or lint yield differences were found between the insecticide-treated and untreated control portions of the other four cultivars (Fig. 4). DP353 had the longest flowering period and peak flowering occurred later in the season compared with other cultivars examined (Fig. 5). In both treated and control plots, the highest number of white flowers were observed in PHY367 on July 30 (Figs. 5 and 6) and peak flowering continued from mid-July through August. Several significant differences were observed between plant biomass and cultivar treatments ($P<0.1$) in control and sprayed plots (Tables 1 and 2); however, interactions between insecticide and cultivar treatments were non-significant. Significantly lower lint yield in untreated control plots ($P<0.1$) was observed between sprayed and control plots in DP353 and PHY367 which might be due to presence of significantly more thrips in control plots than insecticide-sprayed plots in these two cultivars (Fig. 7). Significant differences in seed yield ($P<0.1$) was observed between sprayed and control plots in DP353 only, however, no significant differences in seed yield ($P>0.1$) were observed between sprayed and control plots in other cultivars tested (Fig. 8).

During this study, we observed that field colonization of thrips was low during the study period, varied with cultivars, with DP353 attracting the most adult thrips and lowest densities observed in FM1740 and SSGHQ. However, drastic varietal difference in plant growth and yield masked the subtle difference in thrips tolerance across these tested varieties.

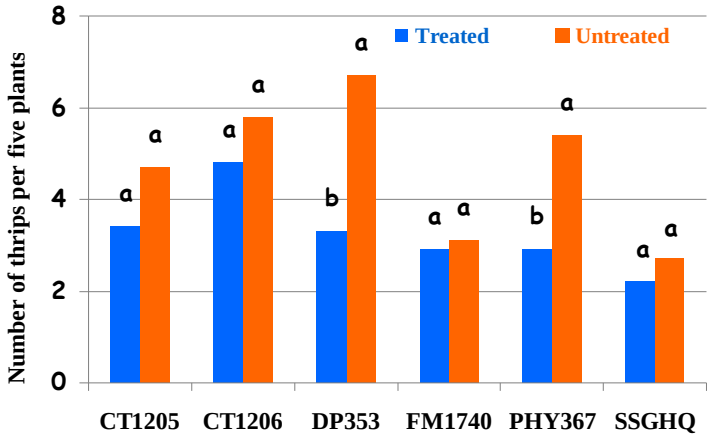

Figure 4. Thrips densities recovered using whole-plant washing procedure.

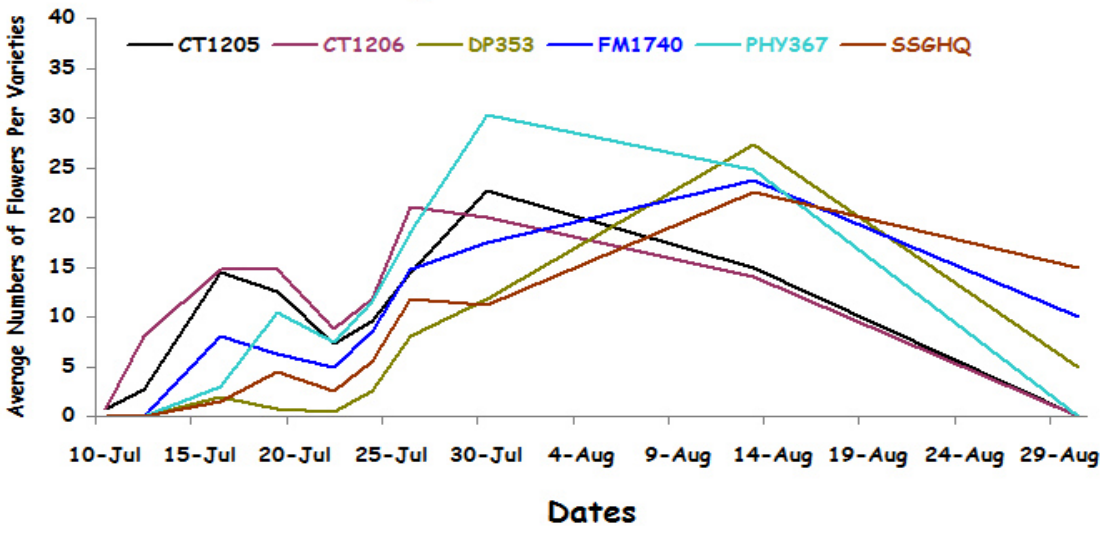

Figure 5. Flowering profile of cotton cultivars in untreated control plots.

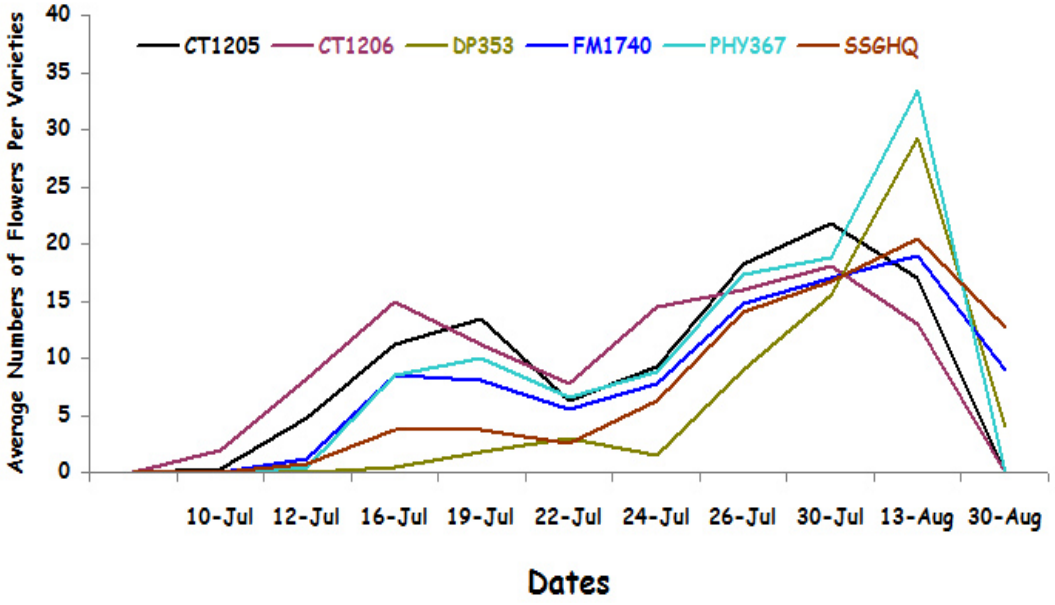

Figure 6. Flowering profile of cotton cultivars in insecticide sprayed plots.

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

Plant Parameters	Varieties/Lines					
	T12 07 7-1407 CT1205	T12 07 7-1001 CT1206	DP353	PHY367 WRF	FM1740 BRF 212 CT	SSG HQ
Shoot length (cm)	9.10a	8.97a	8.32a	8.37a	7.90a	6.52a
Root length (cm)	17.35a	16.47a	14.32a	16.37a	16.25a	14.07a
Shoot biomass (g)	2.06a	2.36a	1.42ab	1.31ab	1.67ab	0.94b
Root biomass (g)	1.76ab	2.05a	1.06bc	1.20bc	1.49abc	0.93c
Leaf biomass (g)	4.69ab	5.50a	3.73ab	3.04b	3.94ab	2.56b
Leaf area (cm^2)	135.6ab	163.41a	134.19ab	103.22ab	114.86ab	85.15b
Leaf chlorophyll	54.39a	53.60a	49.75a	55.12a	55.24a	51.14a

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

Plant Parameters	Varieties/Lines					
	T12 07 7-1407 CT 1205	T12 07 7-1001 CT 1206	DP353	PHY367 WRF	FM1740 BRF	SSG HQ 212 CT
Shoot length (cm)	8.32ab	8.97ab	8.72ab	9.47a	8.25ab	6.22b
Root length (cm)	19.57a	19.19ab	15.35b	17.50ab	15.90ab	16.10ab
Shoot biomass (g)	2.88a	2.47a	1.90ab	2.23ab	1.58ab	0.88b
Root biomass (g)	2.44a	2.15a	1.40ab	2.02a	1.56ab	0.91b
Leaf biomass (g)	6.61a	6.29a	4.77ab	4.59ab	3.85ab	2.70b
Leaf area (cm^2)	163.83a	170.01a	162.86a	128.96a	111.14a	73.19a
Leaf chlorophyll	53.91a	54.38a	51.47a	54.64a	53.30a	51.10a

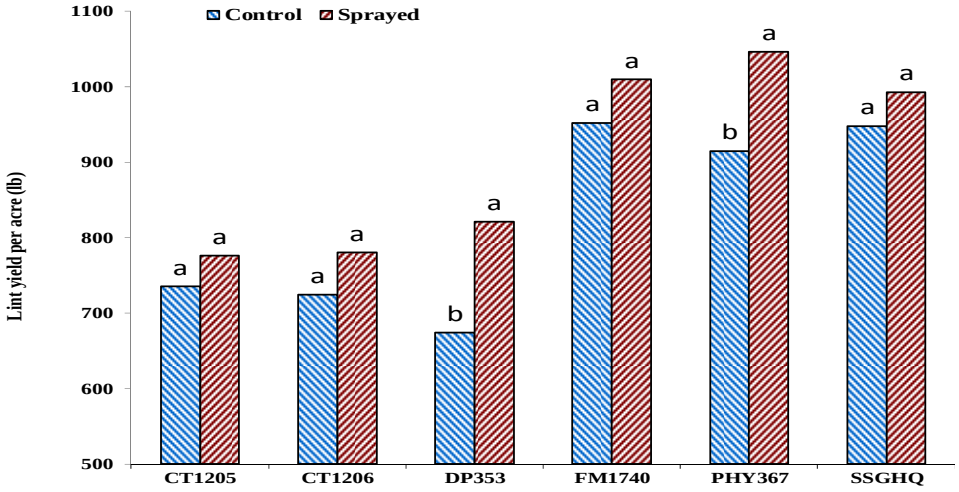

Figure 7. Lint yield (lb per acre) across tested cultivars and breeding lines.

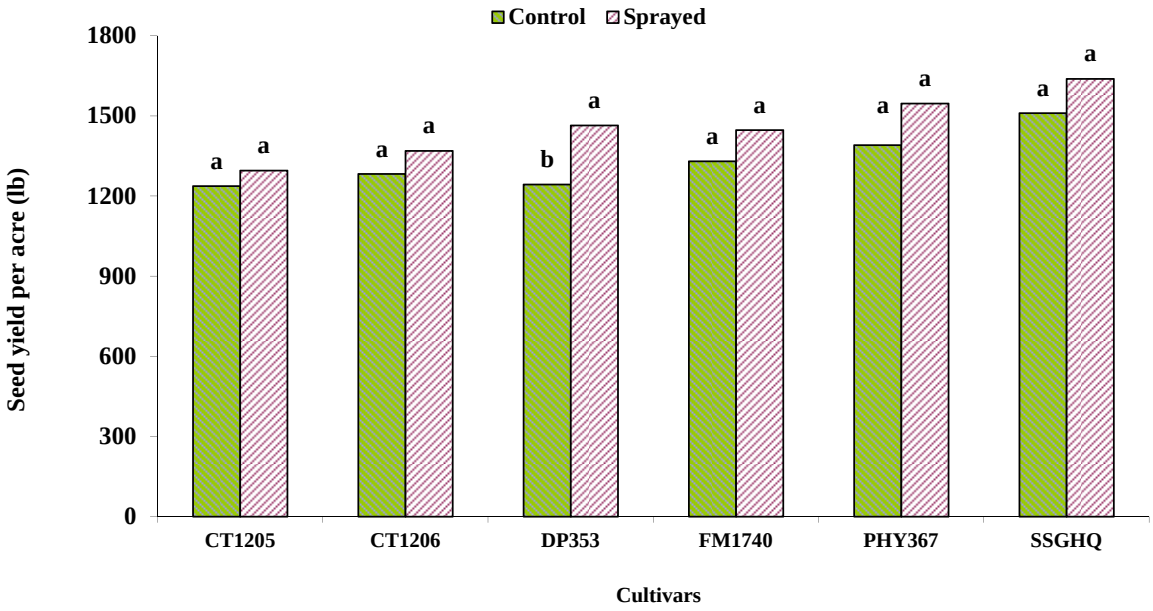

Figure 8. Seed yield (lb per acre) across tested cultivars and breeding lines.

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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, particularly in a high yield potential subsurface drip irrigation production system. 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.

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. 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.

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. 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.

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.

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.

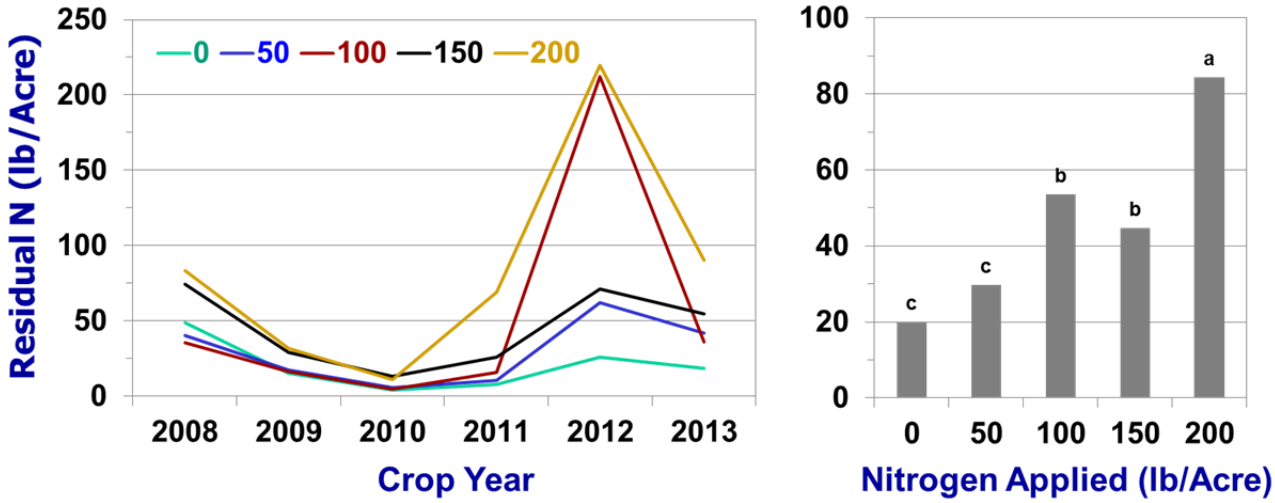

Figure 4. Effect of prior year's N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulation for the current crop year (left) and average residual N over a six-year period (right).

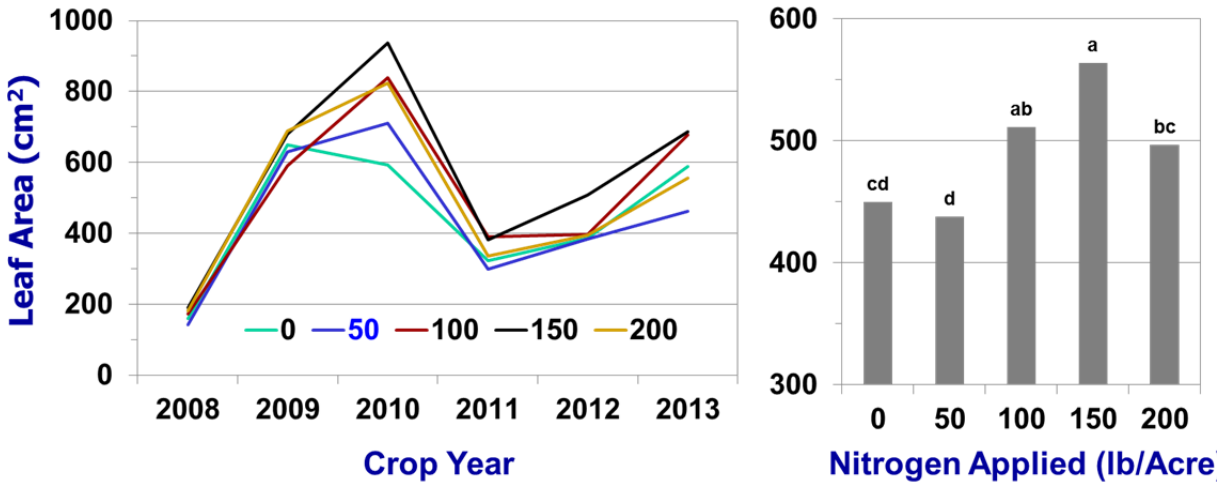

Figure 5. Effect of prior year's N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulation for the current crop year (left) and average residual N over a six-year period (right).

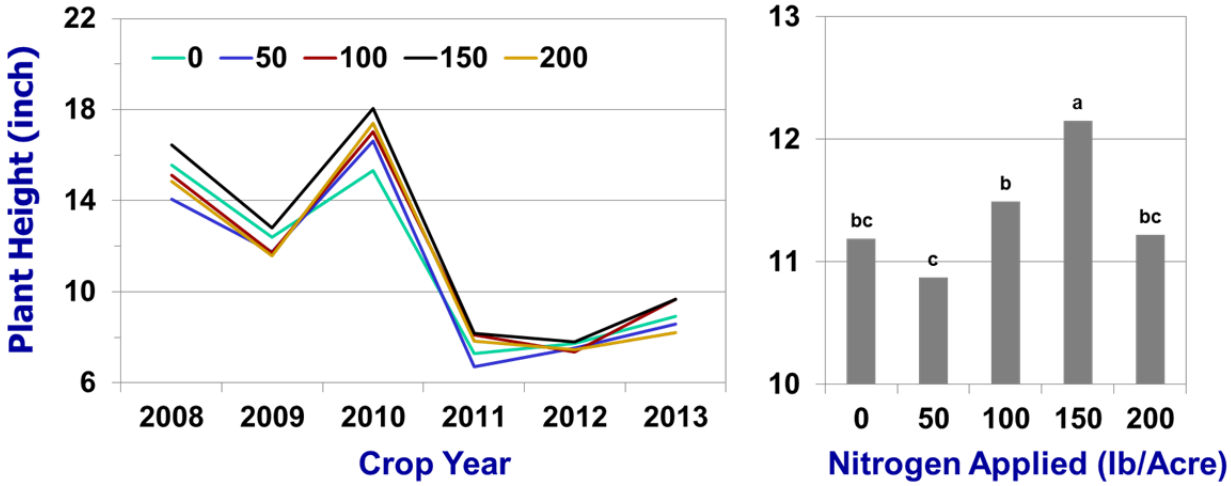

Figure 6. Effect of residual N from the previous crop year on plant height during the early crop growth period of each of the six study years (left) and average plant height over a six-year period (right).

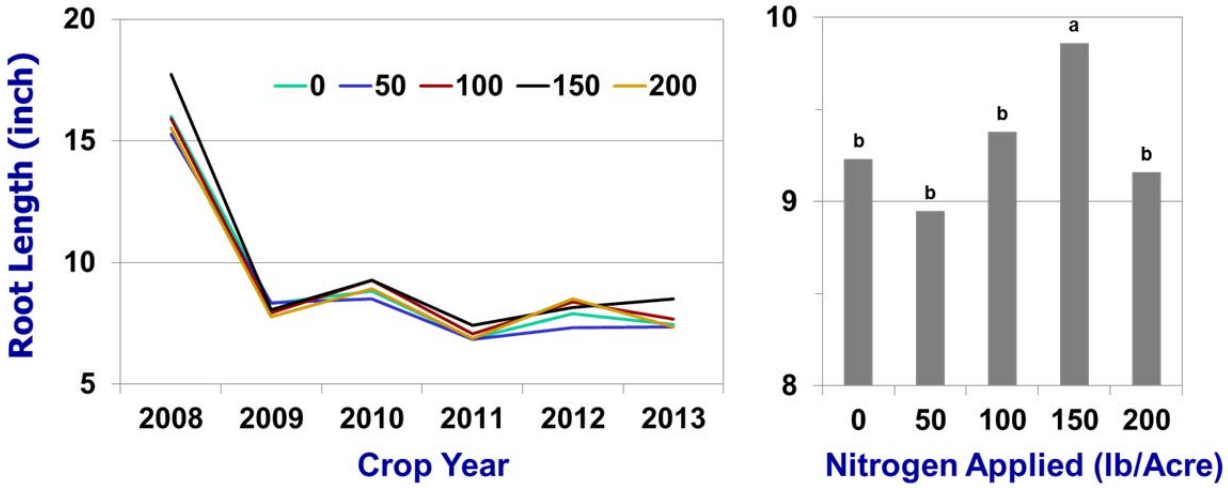

Figure 7. Effect of residual N from the previous crop year on root length during the early crop growth period of each of the six study years (left) and average root length over a six-year period (right).

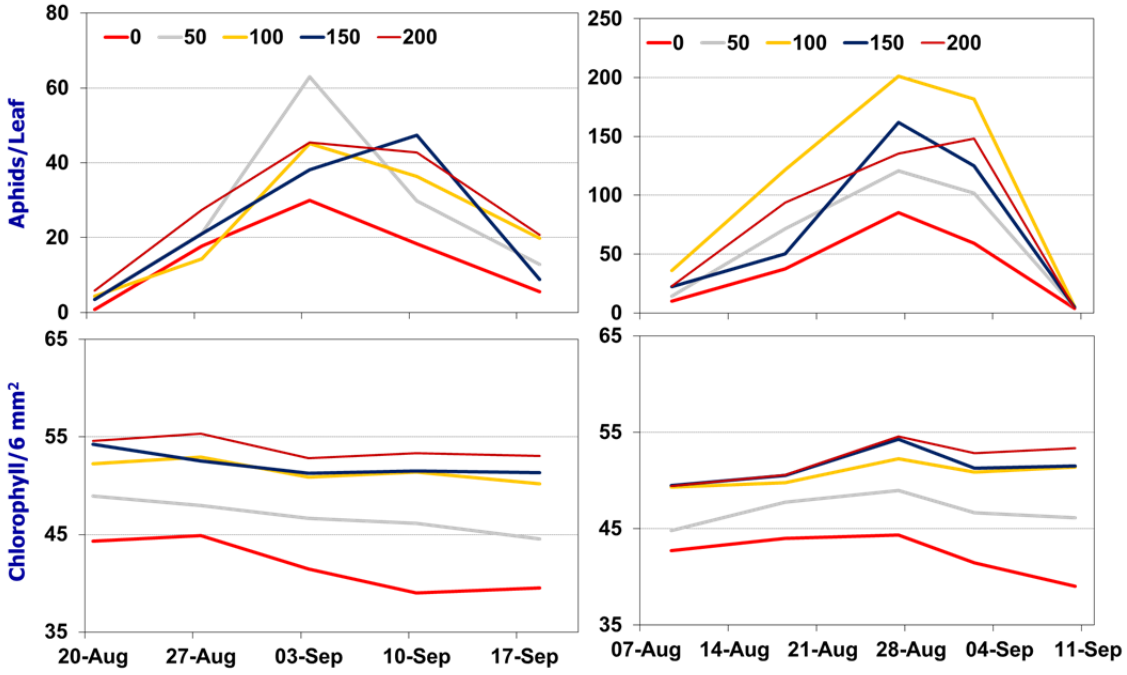

Figure 8. Temporal dynamics of cotton aphid abundance in relation to cotton leaf (5th main stem) chlorophyll content as affected by variable rates of nitrogen application (left chart – 2009, right chart – 2010).

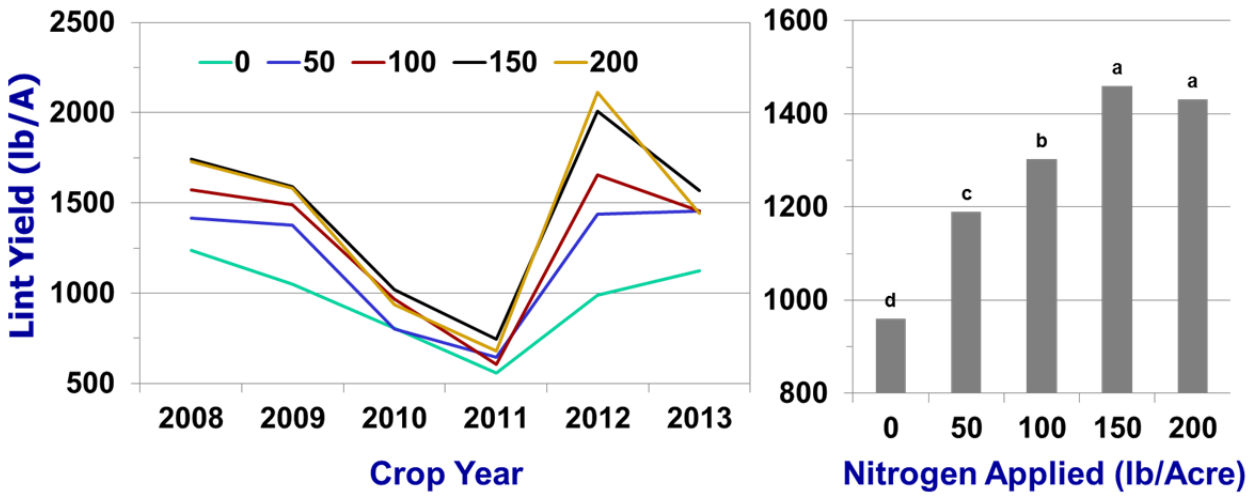

Figure 9. Year-to-year variation in the effect of nitrogen application rates on cotton lint yield (left) and average lint yield over a 6-year period (right), Helms Farm, Hale County, TX.

Acknowledgments

Cotton Incorporated Core Program and Plains Cotton Growers, Inc. have provided funding for this long-term nitrogen fertility study.

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MANAGING THRIPS IN ORGANIC COTTON WITH HOST PLANT RESISTANCE AND SPINOSAD INSECTICIDE

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Abstract

Thrips are a recurring problem to seedling cotton in the Texas High Plains. It has been estimated that thrips impact to the High Plains cotton industry in 2010 was in excess of $6 million. A replicated trial, evaluating 4 cotton cultivars, 2 experimental cultivars, a susceptible check, and a commercial standard was conducted near Muleshoe, TX. Plots were split into 2 foliar regimes, spinosad (Entrust®) at 2 oz/acre and unsprayed. In general thrips pressure was moderate. Spinosad insecticide reduced thrips pressure and subsequent applications appear to be additive. Cultivars did not differ in thrips colonization, but the experimental cultivars did have a significant impact on thrips damage. These data suggest that these cultivars do not express host plant resistance but may have more tolerance to thrips compared to commercial varieties.

Introduction

Thrips are a recurring problem to seedling cotton in the Texas High Plains where the dominant species is western flower thrips, Frankliniella occidentalis (Pergande). More acres of cotton were infested by thrips than any other pest in 2012; in addition more cotton acres were treated for thrips than all other pests combined. It has been estimated that thrips impact to the High Plains cotton industry in 2010 was in excess of $6 million. In irrigated cotton where thrips populations are historically high (usually areas where there is a significant acreage of wheat) many conventional growers may choose to utilize preventative insecticide seed treatments and/or foliar remedial insecticide treatments to suppress thrips. One of the most challenging factors facing organic cotton producers in the Texas High Plains is the effective management of early-season thrips in an organic production system. In 2011 we investigated the efficacy of 13 Organic Materials Review Institute (OMRI) approved insecticides at various rates and combinations for thrips suppression in cotton (Aza-Direct, Bugitol, Cedar Gard, Ecotec, Entrust, Pest Out, Pyganic, Saf-T-Side, SucraShield, and Surround). In 2012 we continued the study but reduced the treatment list to only those products which showed potential to provide significant thrips suppression in 2011 (Aza-Direct, Bugitol, Entrust, and Saf-T-Side+Ecotec). Entrust proved to be most effective in suppressing thrips in 2012 and was selected for continued testing in 2013 along with 3 cultivars with varying degrees of host plant resistance (tolerance) to thrips and a susceptible check. Organic Materials Review Institute (OMRI) provides organic certifiers, growers, manufacturers, and suppliers an independent review of products intended for use in certified organic production, handling, and processing.

Materials and Methods

This trial was conducted in commercial organic cotton field in Bailey County near Muleshoe, TX. Historically western flower thrips have been the dominant thrips species infesting cotton in this area. The trial was planted 13 May, 2013 on 30-inch rows with a John Deere MaxEmerge planter equipped with cone planting units and irrigated using a low elevation spray application (LESA) center pivot irrigation system. Plots were 4-rows wide × 55 ft long and were arranged in a split-plot design with 4 replicates. Treatments included 4 cotton cultivars, two experimental, (07-7-1407 and 07-7-1020), a susceptible check (AT Atlas), and the industry standard (FM 958). Each cultivar plot was split into untreated and treated plots; spinosad (Entrust®) was applied to treated plots at 2 oz/acre. The insecticide application was applied in accordance with label recommendations at 26.4 gallons/acre (GPA) total volume and included AgAid, an OMRI approved adjuvant, at 8oz/100 gallons of water. Three insecticide

applications were made weekly, beginning at near 100% emergence, 28 May. Treatments were applied in a 15 inch band directly over the top of the crop row with a CO2 pressurized backpack sprayer and hand held boom equipped with hollow cone nozzles. The crop stage was noted and thrips were counted at crop emergence and 7, 14, 17, and 21 days after emergence (DAE); all counts were made prior to insecticide applications. Thrips counts were made by collecting ten plants/plot and washing in an alcohol solution; adult and immature thrips collected in solution were filtered out and counted under a dissecting stereo scope. Thrips samples collected were also separated by life stage. Plant damage ratings were assessed at 14 and 21 DAE, the rating scale ranged from 1 to 5, where a rating of 1 indicates no damage and a rating of 5 indicates severe damage. Leaf area was estimated 7, 14, and 21 DAE by collecting 10 plants per plot and measuring the leaf area per plant using a LI-COR, Inc. LI-3100 laboratory area meter. Data were subjected to analysis of variance (ANOVA) and when a significant F test was observed, mean separation was performed using the least significant difference (LSD) at the 5% probability level. Thrips days were calculated by following the methodology described by Ruppel (1983; J. Econ. Entomol. 76:2, pp. 375-377).

Results and Discussion

Environmental conditions at the trial site were windy with temperatures near normal to slightly above normal (Figure 1). Three separate rain events occured June 3, 6, and 8; a nearby NOAA weather station recorded .38, .88 and .97 inches respectively. Thrips pressure, in general, was moderate. Much of the area wheat, which is an alternative host that normally supports and bridges thrips populations until cotton emergence, had desiccated prematurely due to extreme winter and early spring environmental conditions limiting early season populations.

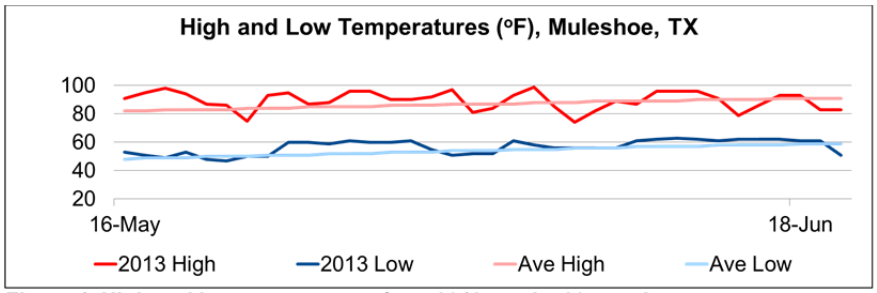

Figure 1. High and low temperatures from 2013 vs. the 30 year long term averages (1980-2010).

The cotton was slow to emerge, 15 days were required to attain near 100% emergence 28 May and an additional 7 days from emergence until a trial average of 1.5 true leaves had developed 4 June. Mean thrips numbers of untreated plots were less than 50% of action threshold when the initial insecticide application was applied (28 May, 100% emergence) but was over 2X the established action threshold of one thrips per true leaf by 7 DAE and maximum pressure, 8X action threshold, was reached 17 DAE 14 June (Figure 2). No differences in thrips densities were observed at any sample date when comparing cotton cultivars within insecticide treatments. A significant difference was only observed when comparing all treatments at the 4 true leaf stage 17 DAE (Figure 3). No statistical differences were noted in plant damage ratings 14 DAE (data not presented) but by 21 DAE significant differences were apparent (Figure 4). The untreated commercial cultivars exhibited the greatest thrips damage; injury was reduced in the experimental cultivars and plots treated with spinosad insecticide. Leaf area measurements revealed significant differences between treatments 21 DAE but no differences were observed on earlier sampling dates (Figure 5). The treated 7-07-1020 cultivar had most leaf area and the untreated 7-07-1020 cultivar had similar leaf area as treated commercial and 7-07-1407 cultivars.

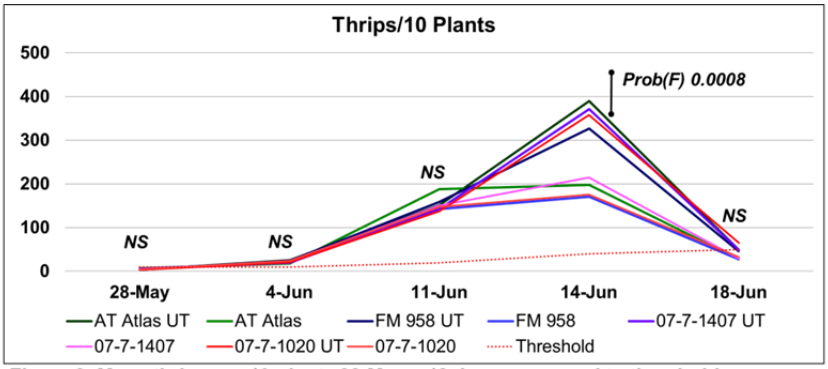

Figure 2. Mean thrips per 10 plants 28 May – 18 June compared to threshold.

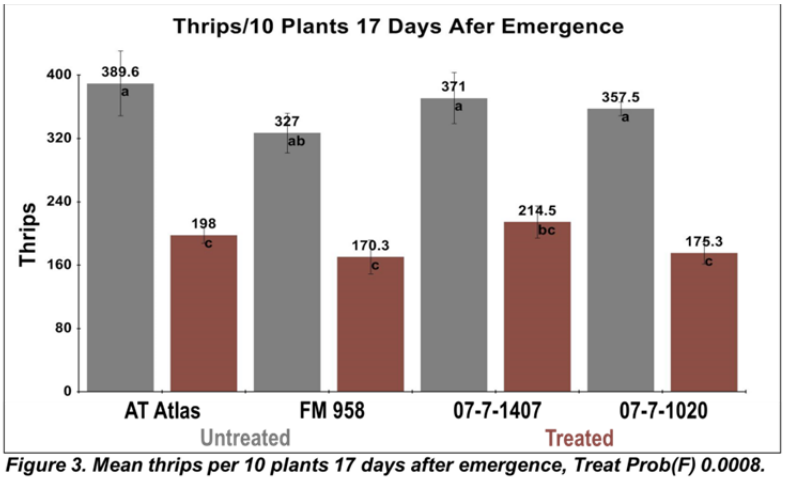

Figure 3. Mean thrips per 10 plants 17 days after emergence, Treat Prob(F) 0.0008.

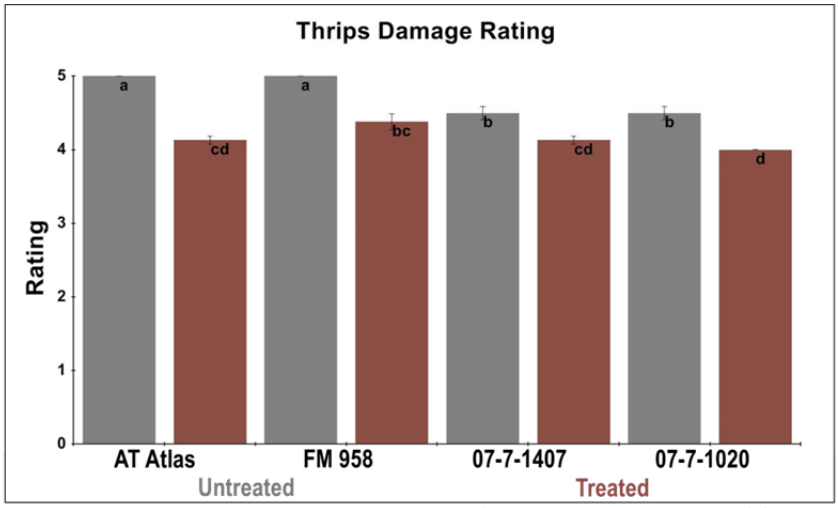

Figure 4. Mean thrips damage ratings 21 days after emergence, Treat Prob(F) 0.0005.

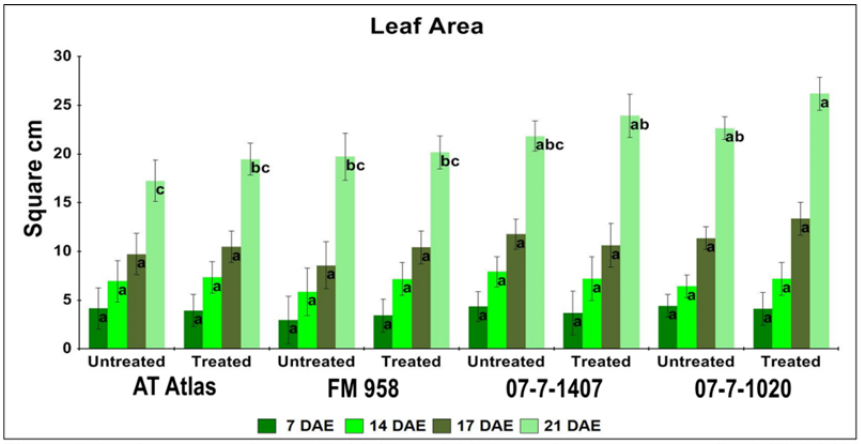

Figure 5. Leaf area per plant at 7, 14 17, and 21 days after emergence, Prob(F) 0.0166 21 DAE.

The percent of a thrips population which is immature is a good indicator of that population's ability to colonize; a higher percentage of immature thrips suggests a higher degree of colonization. When data from all post treatment sampling dates were combined and analyzed, cultivar had no impact on the percentage of the population which was immature (Figure 6). In 2 cultivars, Atlas and 07-7-1020, the Entrust insecticide significantly reduced the immature percentage but only provided slight numeric reductions in the other cultivars. Based on this data, Entrust appears to suppress colonization to a degree but cultivar did not have an impact.

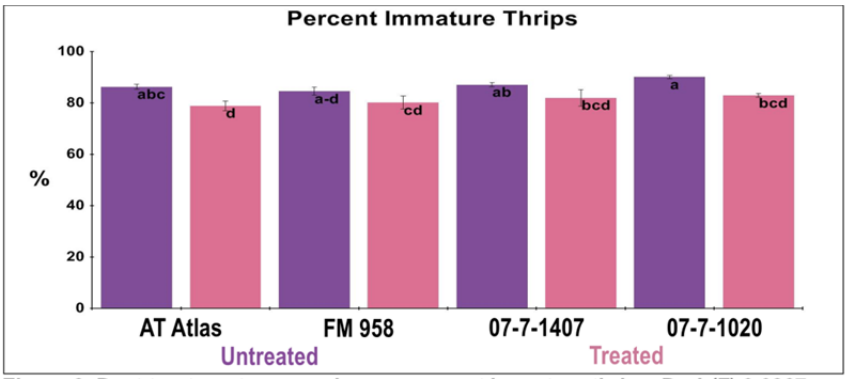

Figure 6. Post treatment seasonal mean percent immature thrips, Prob(F) 0.0367.

Cumulative thrips days can give an indication of thrips pressure over time. No differences in thrips days were observed when comparing cotton cultivars within insecticide treatments but a significant difference was observed when comparing all treatments (Figure 7). Spinosad reduced thrips days by 23.4% when comparing only insecticide treated vs untreated plots. This decrease is an indication of reduced overall thrips pressure and feeding duration.

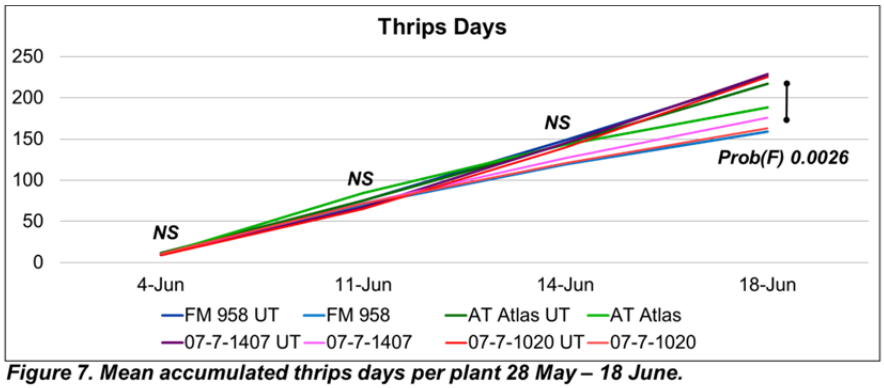

Figure 7. Mean accumulated thrips days per plant 28 May – 18 June.

Conclusions

Thrips pressure was moderate but exceeded action threshold throughout most of the seedling stage. Spinosad insecticide lowered the seasonal mean percent immature thrips, decreased thrips numbers 17 DAE, and reduced accumulated thrips days. Cultivars did not differ in thrips colonization but had a significant impact on thrips damage and leaf area. These data suggest that the new cultivars do not express host plant resistance but may have more tolerance to thrips compared to commercial varieties.

Acknowledgements

The project site was provided by Jimmy Wedel, Muleshoe, TX. This project was funded by the USDA National Institute of Food and Agriculture. We also acknowledge and thank Ray White, Hayden Hadley, Austin Mason, and Cole Miller for their contribution in collecting and processing thrips samples.

COTTON FLEAHOPPER DAMAGE ON WATER-STRESSED COTTON

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Introduction

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), has been documented to cause excessive loss of cotton squares in Texas and Oklahoma, resulting in reduced yield and harvest delays. Cotton fleahopper is also an occasional pest in New Mexico, Arkansas, Louisiana, and other mid-south states. Within Texas, regional average cotton fleahopper induced yield loss estimates vary, reaching up to 6% (Williams 2000). A challenge to management is that square loss and subsequent yield loss to individual fields varies considerably as populations build.

This variability has been partly associated with cultivar differences and other host plant factors (Holtzer and Sterling 1980, Knutson et al. 2009, Barman et al. 2011), with the stage of cotton development when movement into the field occurs (Parajulee et al. 2006), and with environmental stressors in particular plant water stress (Stewart and Sterling 1989). Even though foliar insecticide application may control the population, benefits to control may depend on these factors.

Understanding the degree to which these factors contribute to cotton fleahopper fluctuations and subsequent plant damage may allow better estimation of cotton risk from cotton fleahopper leading to improved in-season management (i.e., insecticides).



From left to right: cotton fleahopper, a blasted square (damage), and a healthy square. Photos provided by authors and Texas AgriLife Research, Lubbock and Corpus Christi.

Experimental Approach

We hypothesize that plant water stress and plant vigor, and plant development at the time of infestation are main factors that affect cotton fleahopper population fluctuation and plant response/yield loss. These factors were considered in two studies, one in South Texas, and the second in the Texas High Plains.

Field testing in 2013 during drought conditions provided opportunity to assess insect activity in a high contrast of dryland (with supplemental irrigation due to severe drought) and irrigated (irrigation targeting 90% crop ET replacement) water regimes. The South Texas location focused on following a natural cotton fleahopper population

and subsequent yield in a plot with two water regimes, two planting dates, two cultivars, and controlled with insecticide or not. The Texas High Plains location focused on plant response using an augmented population of cotton fleahopper under two water regimes. Details of the experimental layout at each location follow:

South Texas: Corpus Christi: Texas A&M AgriLife Research & Extension Center

- **A. Plot Design:** A split-split-split plot design was implemented with 5 replications. The main plot was two water regimes, 1) low irrigation during drought (6.1 acre-inch for the earlier planting, 7.9 acre-inch for the later planting) and 2) high irrigation during drought (10.4 acre-inch for the earlier planting, 13.8 acre-inch for the later planting). The 1st split was two planting dates; Earlier (May 6) and Later (May 31), with both planting dates being agronomically late for the region. The 2nd split was two cotton cultivars; PhytoGen 367 WRF (Dow AgroSciences) and Stoneville 5458 B2RF (Bayer CropScience). The 3rd split was insecticide treatment using Centric 40 WG (thiamethoxam, Syngenta Crop Protection) at a rate of 1.25 oz/acre on June 11, 1, July 3, and 15. Irrigation was delivered by above ground drip.
- **B. Insect Measurements:** Insect counts were made on a weekly basis for 9 weeks after fleahopper numbers exceeded 10 bugs per 100 plants using a beat bucket technique. A total of 20 plants were sampled per plot.
- **C. Plant measurements:** Plant data included yield (lbs. lint/A) as well as boll load and plant height for the unsprayed plots.

Texas High Plains: Lamesa

- **A. Plot Design:** The plot design was a 2 by 2 factorial with 3 replications. The 1st factor was irrigation at 2 levels: a low rate in drought (4.5 acre-inch) and a high rate in drought (9.0 acre-inch). The 2nd factor was infestation rate: a control (no infestation) and 5 nymphs/plant at the 3rd week of squaring. Infestations were applied to uniform-sized plants. Plot size was 45 ft by 4 rows, and irrigation was by center pivot.
- **B. Insect Measurements:** Because cotton fleahopper populations were very low the infestation was augmented with a specific and acute insect feeding pressure of 5 nymphs/plant at the 3rd week of squaring.
- **C. Plant measurements:** Plant data included yield (lbs. of lint/A) and boll load (bolls/plant).

All measurements were analyzed with ANOVA, conforming to a split-split-split plot design in Corpus Christi, and a 2 by 2 factorial in Lamesa. Count data were transformed by the square root of the count + 0.5.

Results

South Texas: Insect Measurements. Fleahoppers exceeded an ET of 15% of plants infested. More cotton fleahoppers were seen on earlier planted cotton ($P < 0.0001$), especially early in the infestation (June 27 when the earlier planted cotton was at 3rd week of squaring and the later planted cotton was at the 1st week of squaring). Cotton fleahopper density did not differ between dryland and irrigated plots at the beginning of the infestation (June 27, $P = 0.24$) (Fig. 1), but as the infestation progressed more fleahoppers were detected in irrigated plots on July 3 ($P = 0.04$) (Fig. 2) and on irrigated plots of the earlier planted cotton on July 11 ($P = 0.009$) (Fig. 3). Cultivar differences were also detected, supporting historical claims of cultivar effects ($P = 0.005$) (Figs. 1-3). The insecticide Centric controlled fleahopper well across most conditions ($P < 0.0001$) (Figs. 1-3), including the very high populations found on June 27 in the earlier planting during the 3rd week of squaring (Fig. 1).

Plant Measurements. There was a good yield response with the best yields seen under irrigation for both cultivars, planting dates, and with or without insecticide protection ($P = 0.0008$) (Fig. 4). The benefits of good soil moisture were seen on unsprayed plots, which had higher bolls loads (Fig. 5) on taller plants (Fig. 6). Yield also increased when plots were sprayed, but to a much smaller degree ($P = 0.05$), and the later planted cotton (which had fewer cotton fleahoppers) had higher yield than earlier planted cotton ($P = 0.006$) (Fig. 4).

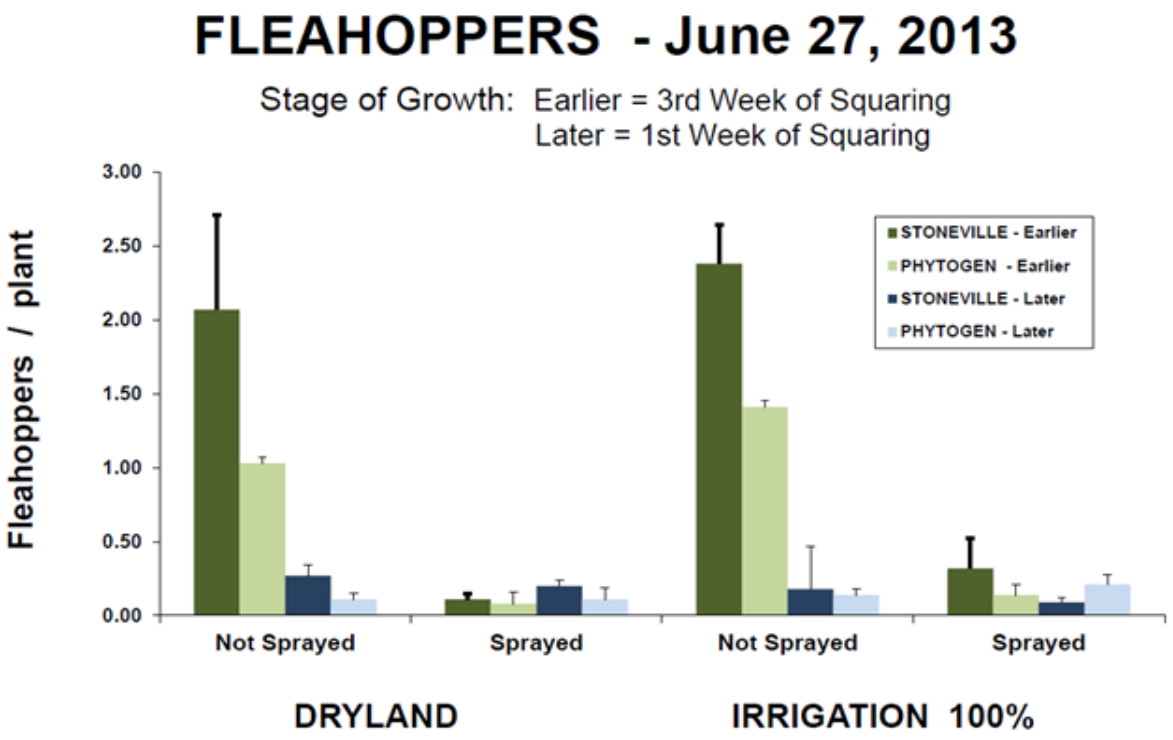

Figure 1. Number of cotton fleahoppers per plant for two sprayed and not sprayed cotton cutivars under two water regimes and two planting dates on June 27, 2013, Texas A&M AgriLife Research and Extension Center, Corpus Christi, Texas, 2013.

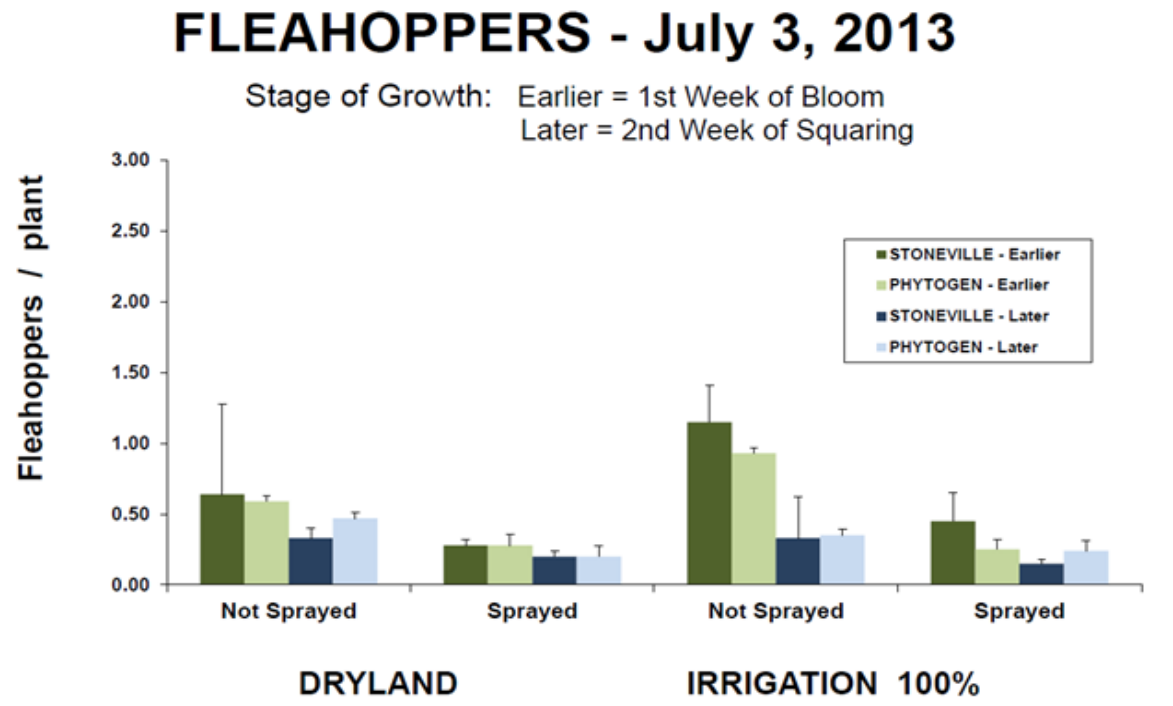

Figure 2. Number of cotton fleahoppers per plant for two sprayed and not sprayed cotton cutivars under two water regimes and two planting dates on July 3, 2013, Texas A&M AgriLife Research and Extension Center, Corpus Christi, Texas, 2013.

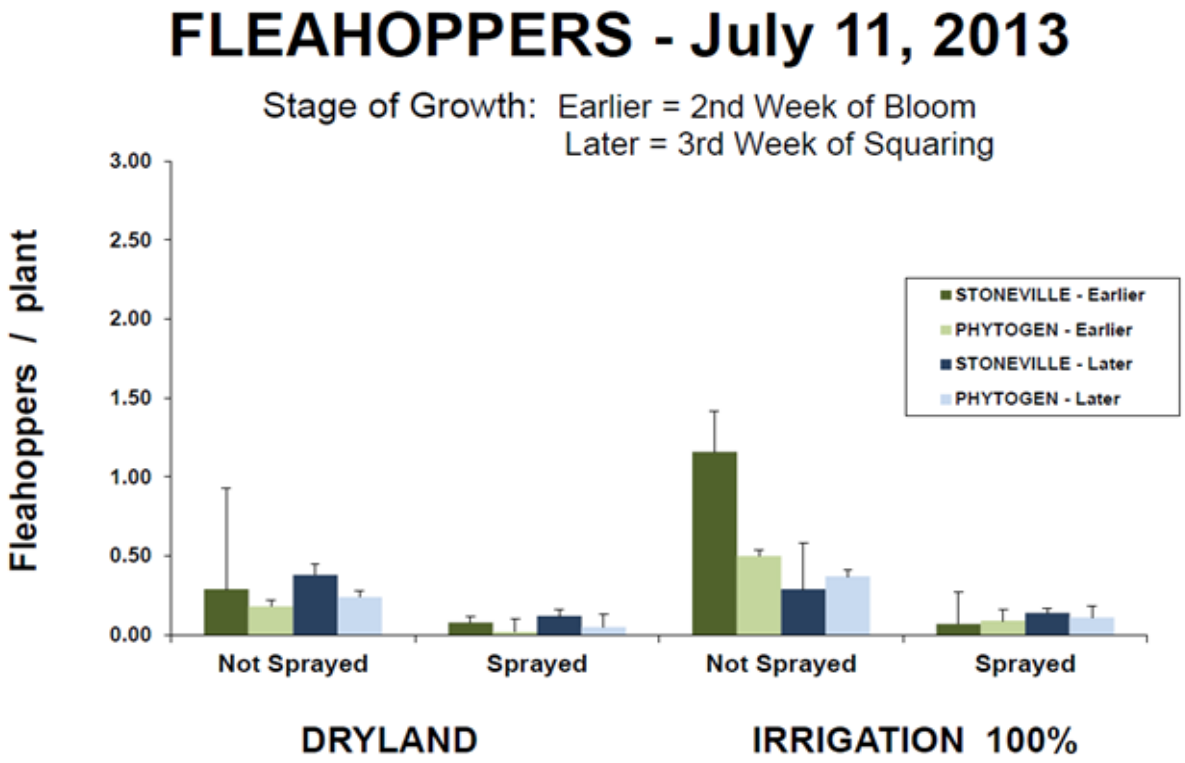

Figure 3. Number of cotton fleahoppers per plant for two sprayed and not sprayed cotton cutivars under two water regimes and two planting dates on July 11, 2013, Texas A&M AgriLife Research and Extension Center, Corpus Christi, Texas, 2013.

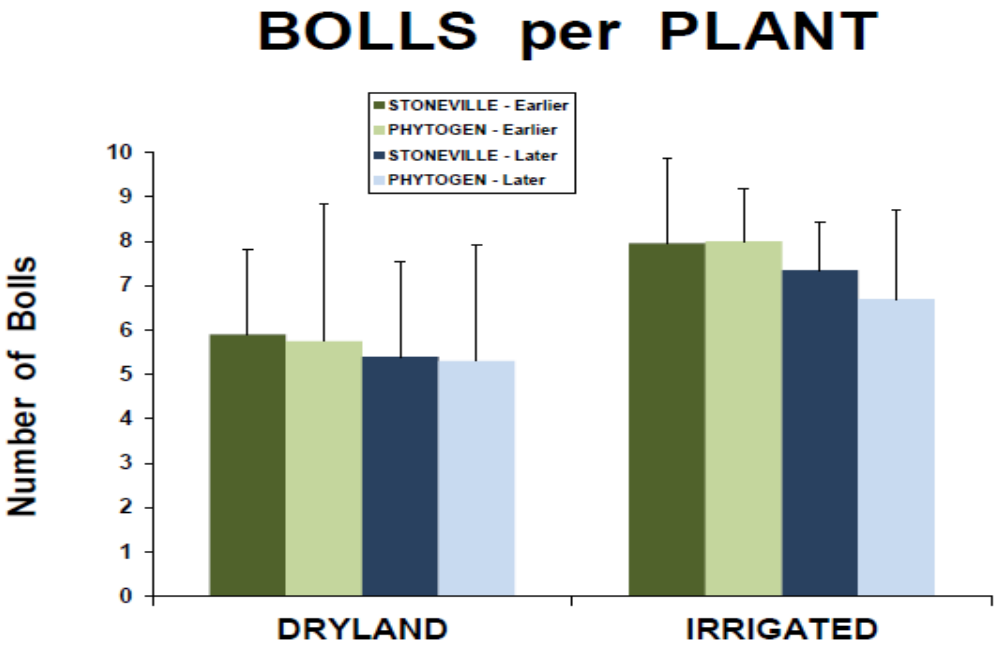

Figure 4. Number of bolls per plant for cotton cutivars under two water regimes, two planting dates, and not sprayed with insecticide, Texas A&M AgriLife Research and Extension Center, Corpus Christi, Texas, 2013.

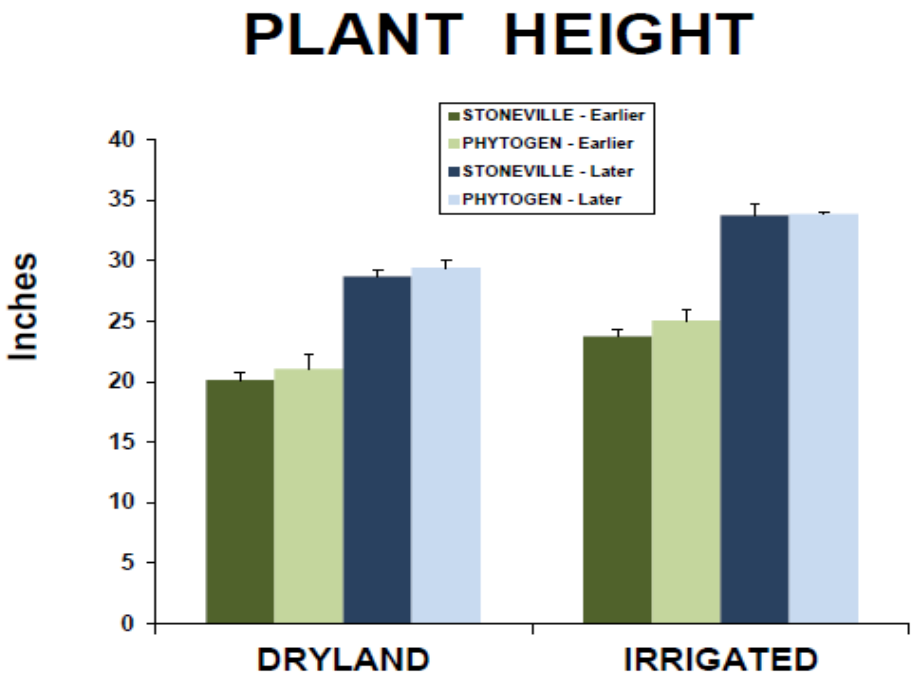

Figure 5. Plant height of two cotton cutivars under two water regimes and two planting dates, and not sprayed with insecticide, Texas A&M AgriLife Research and Extension Center, Corpus Christi, Texas, 2013.

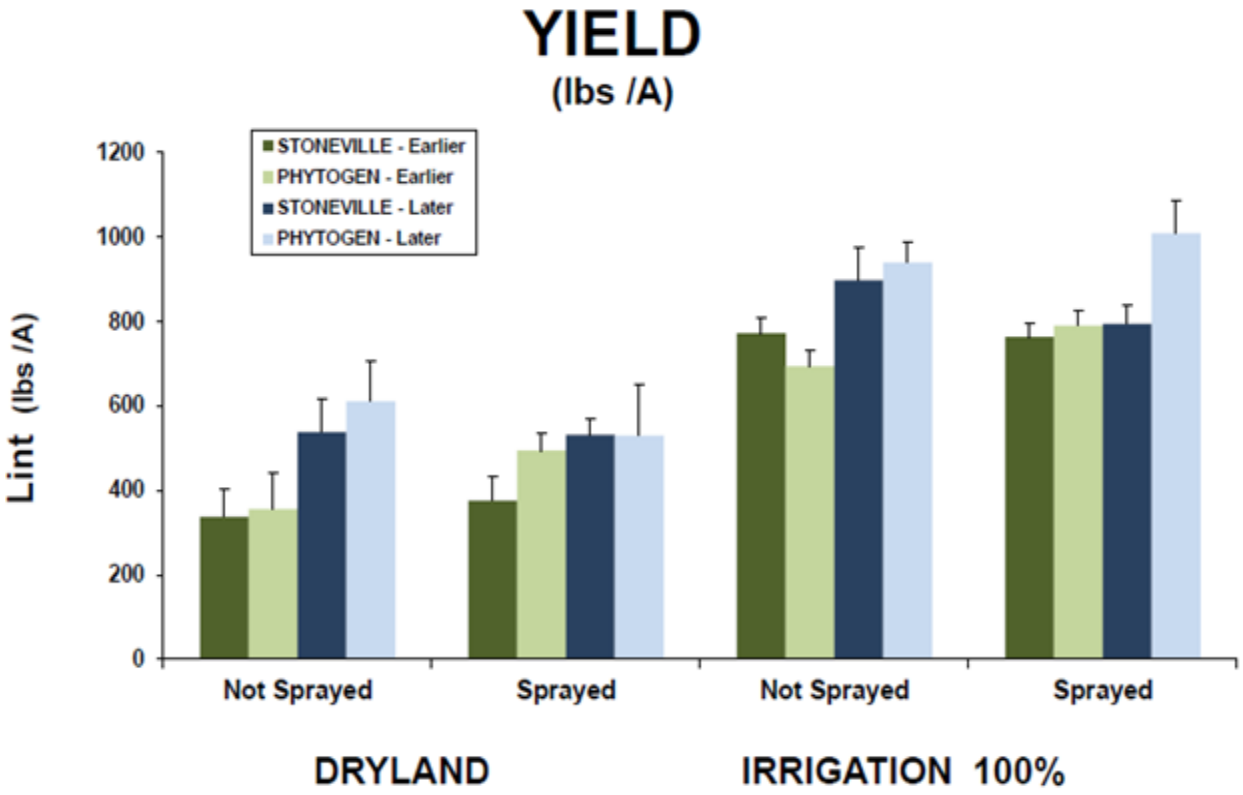

Figure 6. Yield (lbs. of lint/acre) for two sprayed and not sprayed cotton cutivars under two water regimes and two planting dates, Texas A&M AgriLife Research and Extension Center, Corpus Christi, Texas, 2013.

Texas High Plains: Plant Measurements. Natural populations of cotton fleahopper were low at this site which allowed field comparison of plant response to a specific and acute cotton fleahopper insect feeding pressure of 5 nymphs/plant at the 3rd week of squaring (fleahopper augmented) and a control (no augmentation of cotton fleahopper). This plant growth stage has been shown to host cotton fleahopper well. When plants were not water stressed (high irrigation), there was no effect of cotton fleahopper pressure looking at boll load (Fig. 7) and lint yield (Fig. 8). But under water stress (low irrigation during a drought year), there was yield loss due to cotton fleahopper pressure ($P < 0.05$) (Fig. 8), which was also reflected in reduced boll load (although not significantly different) (Fig. 7).

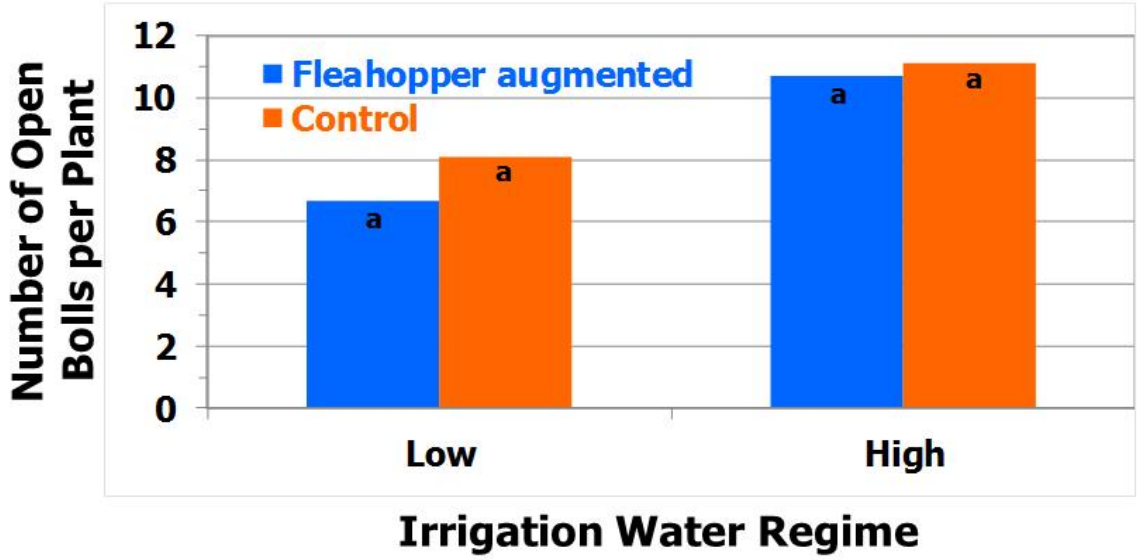

Figure 7. Number of open bolls per plant under low and high irrigation with and without (control) an augmented population of cotton fleahopper (fleahopper augmented) of 5 nymphs/plant released at the 3rd week of squaring, Texas A&M AgiLife Research, Lamesa, Texas, 2013.

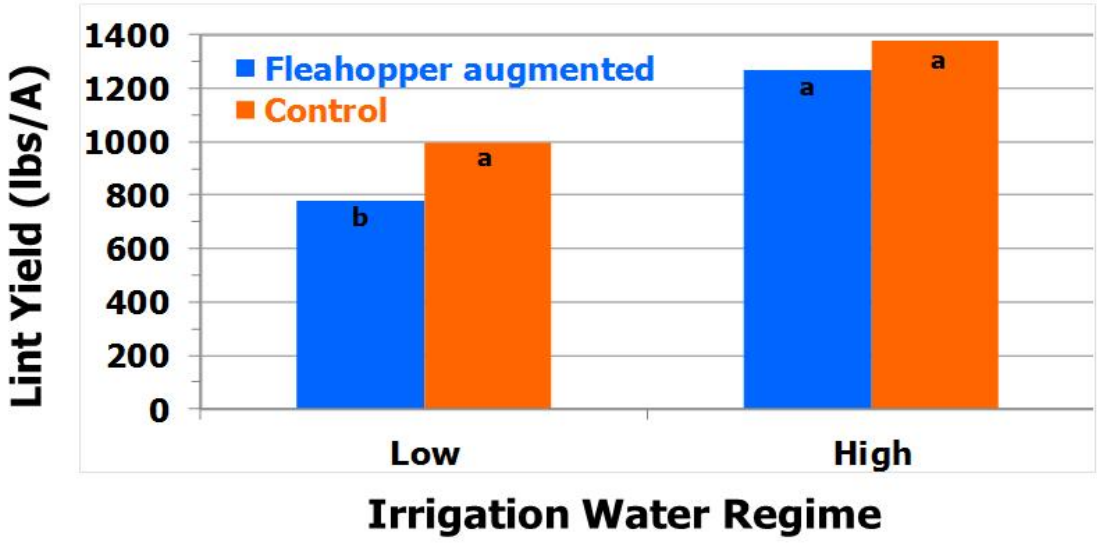

Figure 8. Yield (lbs. of lint/acre) under low and high irrigation with and without (control) an augmented fleahopper population (fleahopper augmented) of 5 nymphs/plant released at 3rd week of squaring, Texas A&M AgiLife Research, Lamesa, Texas, 2013.

Summary Interpretation

We live in a climate that produces highly variable weather, as seen in drought conditions in Texas the last two years. Plant water stress affects natural cotton fleahopper populations (South Texas study: increasing more in irrigated plots) and water stressed plants are more sensitive to equal cotton fleahopper pressure (High Plains study: lint loss and possibly boll load decreasing more in low irrigation plots). As seen last year, plant development stage at the time of initial cotton fleahopper infestation is crucial, with early squaring cotton having higher densities than cotton at early bloom in the infestation (South Texas study). For field application, detection of fleahoppers in early planted cotton may serve as early warning of cotton fleahoppers in later-planted cotton. As the infestation progresses, fleahoppers may persist better in cotton with low water stress. But the greatest potential for yield decline from cotton fleahopper was when cotton was water stressed and infestations occurred during pre-bloom squaring.

Acknowledgements

Many thanks to L. Pruter, J. Glover, C. Farias, and A. Cartwright for assistance in field data collection. We thank R. Kurtz for discussions as we developed this study. Cotton Inc. Core Program funds (project 11-952) were critical in launching this project.

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FIELD PERFORMANCE AND HERITABILITY OF THRIPS RESISTANCE FOR COTTON VARIETY DEVELOPMENT

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Abstract

In the absence of synthetic pesticide applications, thrips (Thysanoptera: Thripidae) management can be more problematic in organic production systems than conventional cotton systems. Additionally, nearly all organic cotton acreage on the Texas High Plains (THP) is planted with one or two conventional cultivars and seed-saving is near-ubiquitous, as these cultivars are no longer commercially available. Therefore, development of new thrips-tolerant, non-transgenic cultivars has the potential to greatly improve the availability and diversity of viable cultivars and overall production of organic cotton on the THP. Fifteen advanced breeding lines, 4 cultivars, and 1 newly-released germplasm line were planted at 2 field locations in 2013. Each genotype was evaluated for thrips resistance potential and overall field performance under organic management. Thrips resistance was assessed using visual injury ratings at both study sites. Yield and fiber quality data were collected to evaluate overall field performance of each genotype. Breeding lines '07-7-519CT', '07-7-1407CT', and '11-2-802GD' exhibited high field tolerance to thrips feeding. Lines '07-14-510FS' and 11-2-802GD and cultivars FiberMax® 'FM 958' and 'Tamcot 73' displayed the greatest lint yields among all evaluated genotypes. Both 07-7-519CT and 11-2-802GD exhibited a desirable combination of high thrips tolerance and yield potential, and would therefore be candidates for release as cultivars or parent material.

In addition, two broad-sense heritability trials were conducted to evaluate the inheritance of the thrips resistance trait and potential utility in variety development. Two separate families were evaluated, each originating from different interspecific *Gossypium hirsutum* L. and *Gossypium barbadense* L. crosses. The first family was derived from cold-tolerant *G. hirsutum* breeding line 07-7-1407CT and *G. barbadense* 'Cobalt'. Parents and the F_1 and F_2 generations were evaluated in a field study in 2012, and visual thrips injury ratings were conducted on individual plants for each genotype at 4-5 true leaves. The H^2 value for thrips resistance in this trial was 26.1%. The second family was derived from a CA 2266 (*G. hirsutum*) x TX 110 (*G. barbadense*) cross, and parents and F_1, F_2, and F_3 generations were evaluated in a greenhouse trial under elevated thrips pressure in 2013. H^2 values for F_2 CA 2266 x TX 110 and F_3 CA 2266 x TX 110 generations were 22.9% and 28.4%, respectively. These values support previous assumptions regarding the quantitative nature of thrips resistance. While these values were relatively low, they indicate that visual phenotyping for thrips resistance and subsequent selection is consistent between the field and greenhouse. More work is necessary to further validate these data at both the greenhouse and field level.

2013 ANNUAL REPORT

Cotton Incorporated Core Program

Project Number: 12-364

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

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Introduction

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

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

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

producers, the outcome of this study will aid crop consultants and county IPM agents in making recommendations to improve thrips management in Texas High Plains cotton.

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

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

Materials and Methods

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

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

Cotton germination was delayed due to cooler soil temperatures, but the plant emergence was satisfactory in most plots. Poor crop stand on some experimental plots may be attributed to variations in cultivar seed vigor rather than to the soil conditions. Plant stand counts were performed on May 23 and June 3 by counting all plants in 3 row-ft per row in all 48 plots. Thrips densities were monitored in all 48 plots using a ten-plant thrips washing technique. Thrips sampling dates were May 23, May 25, June 3, June 10, and June 17. An insecticide (Orthene® 97S @ 3.0 oz/acre) was sprayed in all 24 'sprayed' treatment plots after each thrips sampling event on May 24, May 30, June 11, and June 18, and the entire test (all 48 plots) was sprayed

with this insecticide on June 26. Insecticide treatment application was skipped after the thrips sampling event on June 3 due to spray logistic issues, but the residual insecticides from previous week's application kept the thrips populations suppressed until the insecticide application on June 11. Plant response to thrips injury was monitored by measuring plant height, shoot length, root length, total leaf area, and total dry biomass of cotton seedlings from each plot on June 24. A 10-ft section was marked on each of the two center rows within each plot and the flowering profile was monitored 2-3 times per week. This type of phenological monitoring began prior to the initiation of flowering and continued until crop cut-out. Flowering profiles were monitored on July 10, 12, 16, 19, 22, 24, 26, 30, August 13, and 30. The two 10-ft sections from the middle two rows (20 total row-ft/plot) that were designated for plant fruiting response were harvested to estimate the cotton lint and seed yields from each experimental plot.

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

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

A greenhouse study was conducted to determine the maximum potential effect of different densities of thrips on seedling cotton. Six cotton varieties (07-7-1001 CT-1206, 07-7-1407 CT-1205, PHY367 WRF, SSG HQ212 NCT, FM 1740 B2 RF and ST 5458 B2RF) were planted in 16-oz Styrofoam cups on October 8, 2013. At the bottom of Styrofoam cups, 1-3 small holes were made to allow for drainage of the potting soil. The study was deployed in a completely randomized block design with four replications, six cultivars, and four thrips densities. Each experimental unit contained 6 plants. Thrips were field-collected from cotton and reared on green beans in the laboratory. Immature thrips were transported to the greenhouse in containers with green beans. A brush was used to dislodge thrips from the green beans onto the cotton seedlings. Every effort was made to release only immature thrips to avoid unintentional movement of thrips between treatments. Thrips densities released included: no thrips (control), ½ thrips per plant (.e., one thrips per two plants), one thrips per plant, and two thrips per plant at the 1- to 2-true leaf stage. Automatic sprinkler system was programmed to water the plants three times per week for 8 minutes. In addition, supplemental water was manually applied as needed.

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

Thrips were allowed to feed and reproduce for three weeks (the duration that is equivalent to the western flower thrips lifecycle) before plants were clipped near the soil surface and placed into denatured ethyl alcohol. Later, the adult and juvenile thrips were quantified via a plant washing

technique as follows: All six plants per unit were placed on a fine sieve and rinsed in water until all thrips could be dislodged from the leaves onto a very fine sieve (No. 150)**,** then thrips were washed in a salt solution. Sand and heavy materials were removed from the bottom opening of the separatory funnel and thrips were placed on a filter paper. A vacuum system was used to remove extra water. Adults and juveniles were counted using a microscope at a 10X or higher magnification. Number of thrips from each treatment and variety were recorded and used in the analysis. Analysis of variance was used to determine the effect of thrips densities on cultivars.

Objective 3. Design a field cage prototype to determine the cotton crop damage potential of the western flower thrips for developing economic threshold

Despite unpredictable weather and lack of prior thrips research in the greenhouse and in the field cages at our setting, we have made significant progress toward developing techniques and protocols for conducting thrips field trials with known thrips densities in No-Thrips® cages. We are excited about opportunities to examine thrips behavior and biology in relation to selected cotton cultivars.

Previously, we have evaluated several types of fabrics and cage designs to study thrips, but the efforts failed due to increased temperatures inside the cages, resulting in high thrips mortality or escapes. These failures might have been due to: 1) inappropriate cage material (fabric), 2) size of the cage, 3) supporting frame of the cage (plastic or glass), and 4) number of plants used in each unit (single plant). We now have developed a field cage prototype which should allow us to conduct the thrips density studies in the field for developing economic thresholds.

Results and Discussion

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

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

During this study, we observed that field colonization of thrips was low during the study period, varied with cultivars, with DP353 attracting the most adult thrips and lowest densities observed in FM1740 and SSGHQ. However, drastic varietal difference in plant growth and yield masked the subtle difference in thrips tolerance across these tested varieties.

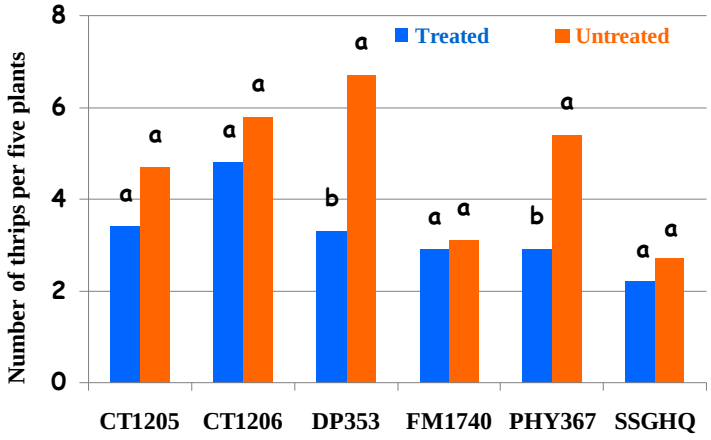

Figure 2. Thrips densities recovered using whole-plant washing procedure.

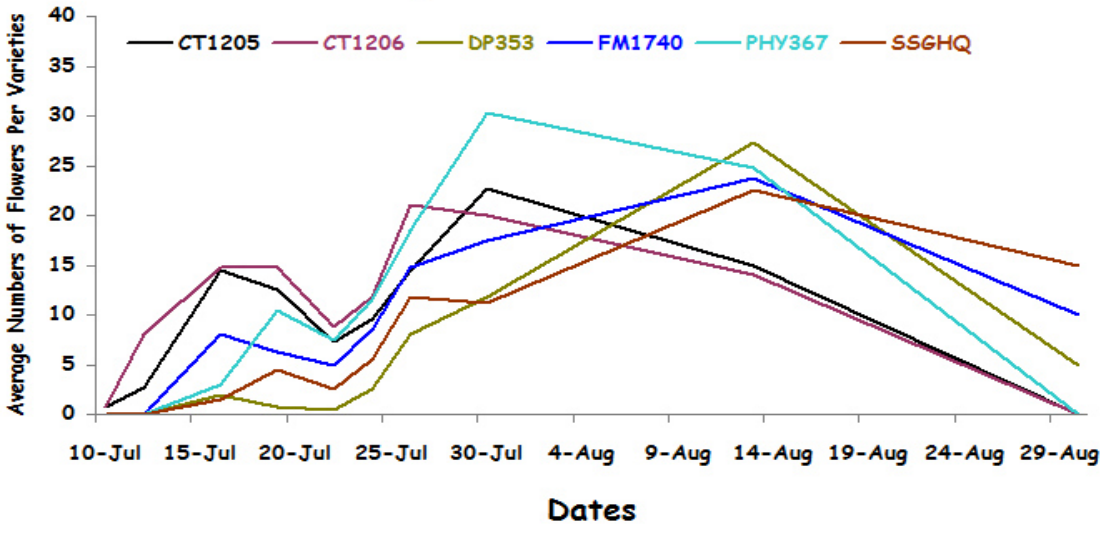

Figure 3. Flowering profile of cotton cultivars in untreated control plots.

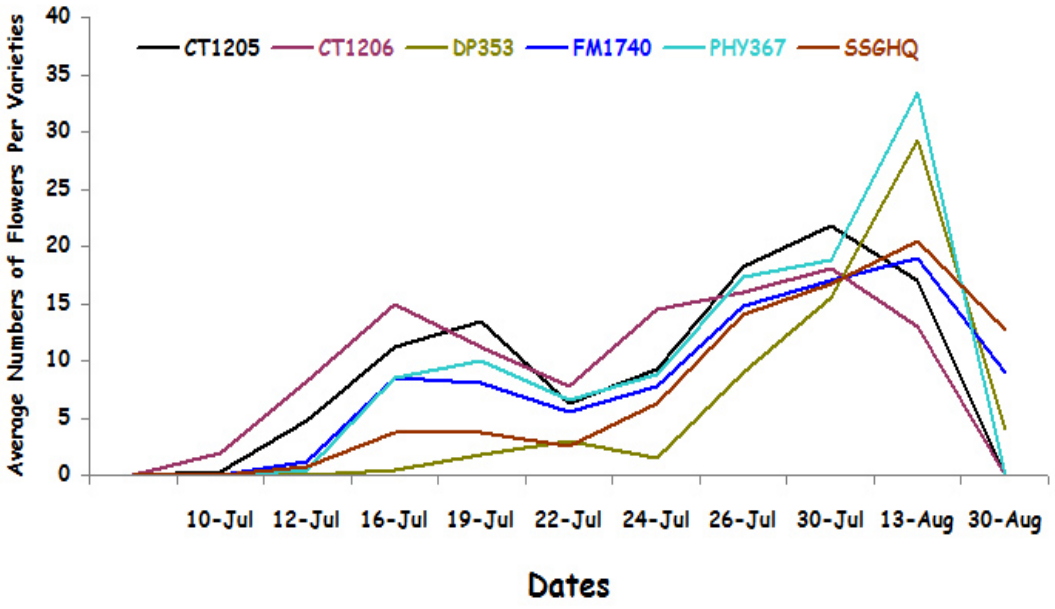

Figure 4. Flowering profile of cotton cultivars in insecticide sprayed plots.

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

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

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

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

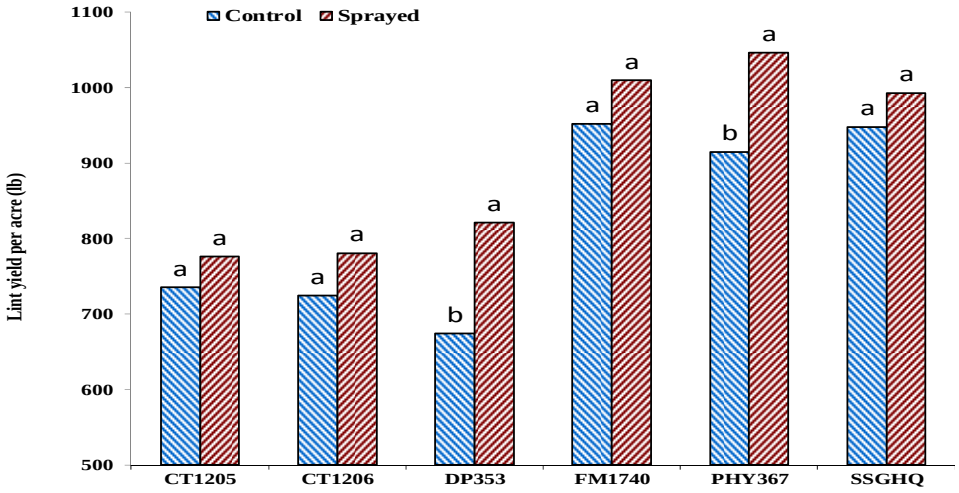

Figure 5. Lint yield (lb per acre) across tested cultivars and breeding lines.

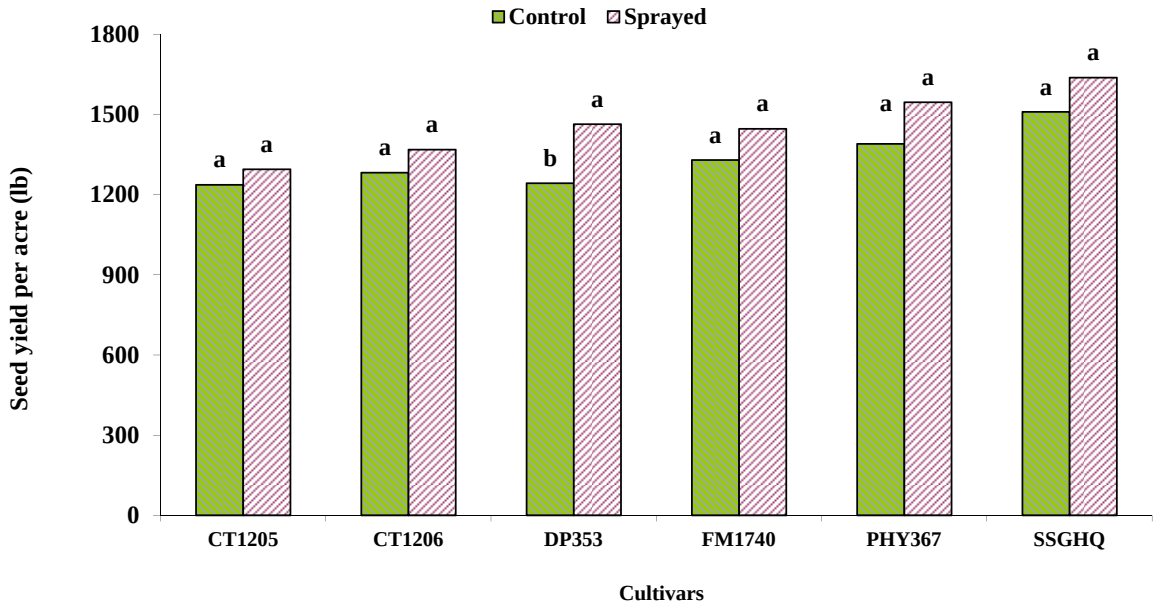

Figure 6. Seed yield (lb per acre) across tested cultivars and breeding lines.

During this study, we observed that natural thrips infestations (field colonization) was very light during the study period, varied with cultivars, with DP353 attracting the most adult thrips and lowest densities observed in FM1740 and SSGHQ. However, drastic varietal difference in plant growth and yield masked the subtle difference in thrips tolerance across these tested varieties. We plan to repeat this experiment at hopefully higher thrips pressure in 2014.

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

Thrips washing. Several factors were significant between released thrips densities and thrips numbers recovered. A significant number of thrips (adults + immatures) were recovered between densities 0, 0.5, 1 and 2 (Fig. 7). For both adult and immature thrips numbers, thrips release density 0 was significantly different than densities 0.5 and 1. However, no significant differences in the number of retrieved thrips were observed between densities 1 and 0.5; and densities 1 and 2 (Figs. 8 and 9). However, no significant differences were found between cultivars and recovered total number of thrips (adults + immatures), immatures only or adults only.

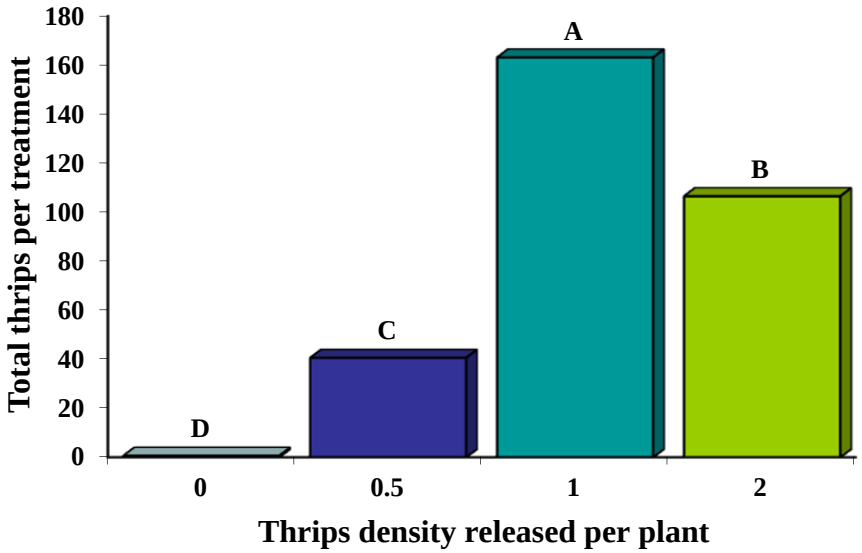

Figure 7. Recovery of total thrips (adult and immature) from seedling cotton using a plant washing technique in a greenhouse study, 2013.

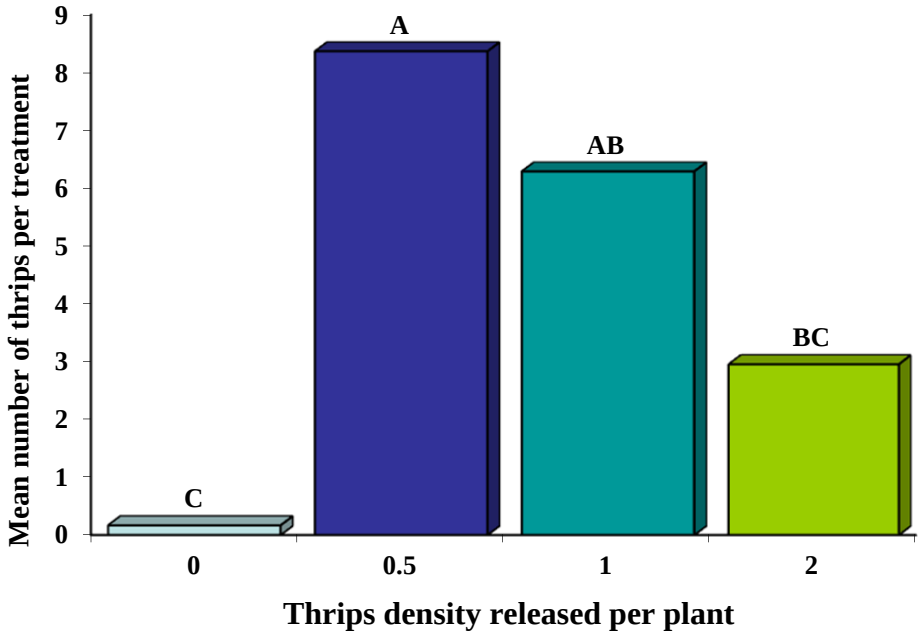

Figure 8. Recovery of adult thrips (22 days after initial thrips releases) from seedling cotton using a planting washing technique in a greenhouse study, 2013.

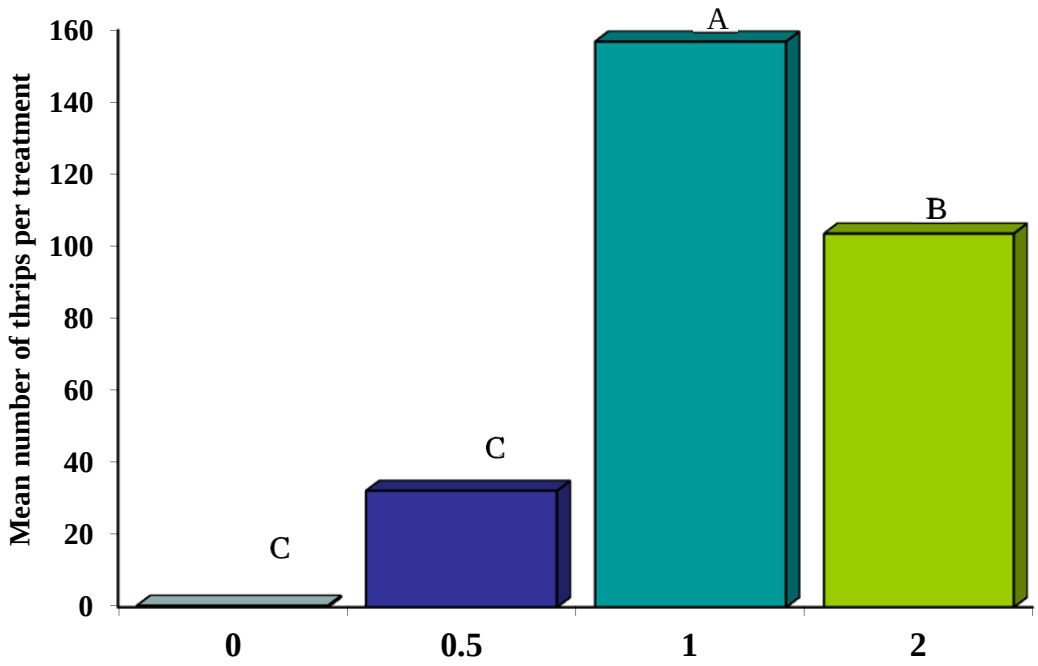

Figure 9. Recovery of immature thrips from seedling cotton using a washing technique in a greenhouse study, 2013.

Leaf area. Leaf surface area measurements were significant between thrips densities 0, 0.5 and 2; however, no significant differences in leaf area were recorded between thrips release densities of 0 and 1 per plant; and densities 1 and 2 (Fig. 10). Additionally, no significant differences were found in leaf area reduced by thrips among the cultivars tested. There was a clear indication that thrips infestations, regardless of the densities, tended to reduce the leaf surface area in seedling cotton.

Visual ranking. Significant differences were observed in visual ranking of the cotton seedlings between thrips densities released (P = 0.0001); however, no significant differences (P>0.05) were recorded in visual ranking between cultivars. Visual injury ranking was significantly lower (significantly less injury) in thrips densities 0 and 0.5 compared with that in thrips densities 1 and 2; however, no significant differences (P>0.05) were recorded in visual ranking between thrips densities 1 and 2 (Fig. 11). It is noteworthy that 0.5 thrips per plant exerted significantly higher injury, based on visual ranking, compared with that in no-thrips control plants.

Chlorophyll readings. No significant differences were observed in chlorophyll readings of the indicator leaf on seedlings between thrips densities released (P>0.05) but various significant

differences (P<0.05) were recorded in chlorophyll readings between cultivars tested. Cultivar CT-1206 showed the highest chlorophyll readings, which were significantly different from ST 5458 B2RF, PHY 367 WRF and HQ212NCT. No significant differences (P>0.05) in chlorophyll levels were recorded among cultivars CT-1205, CT-1206 and 1740B2RF. Also, no significant differences (P>0.05) in chlorophyll levels were recorded among ST 5458 B2RF, PHY 367 WRF and HQ212NCT (Fig. 12).

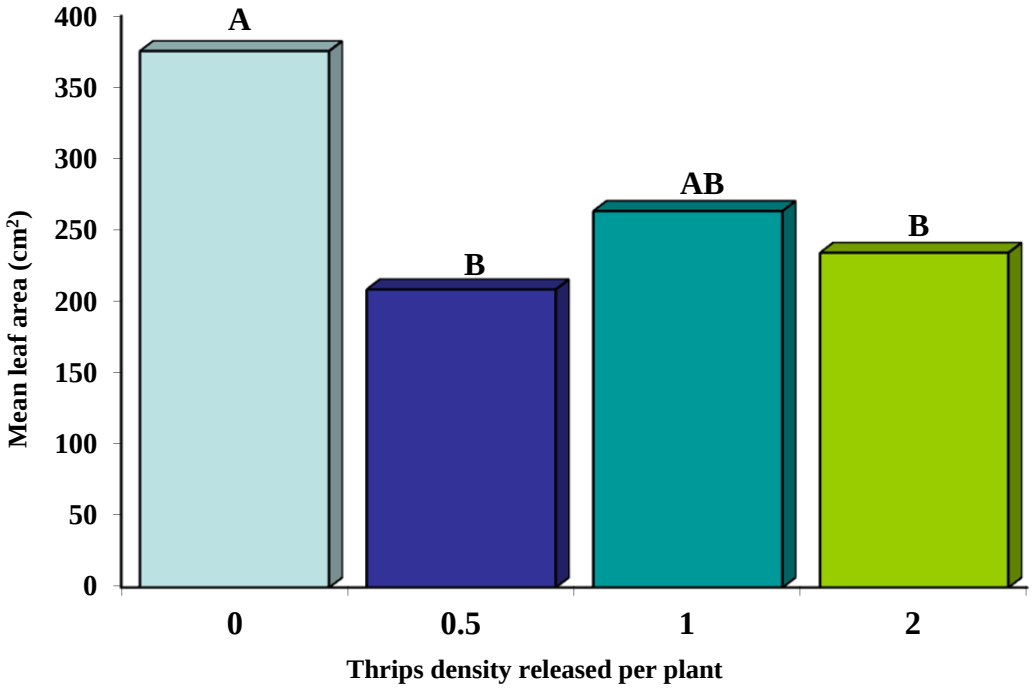

Figure 10. Effect of western flower thrips injury on leaf surface area of the cotton seedlings at various thrips densities in a greenhouse study, 2013.

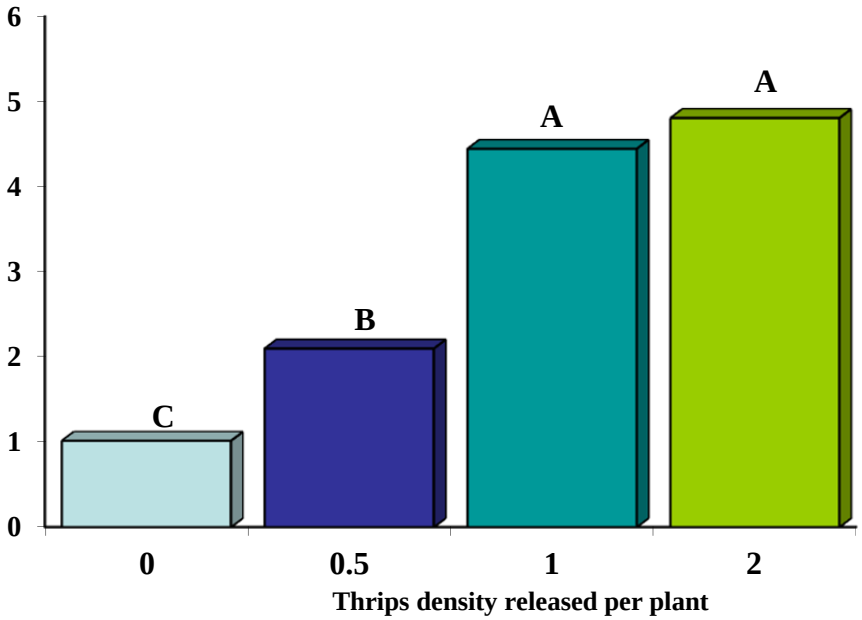

Figure 11. Effect of western flower thrips injury on visual leaf damage ranking of the cotton seedlings at various thrips densities in a greenhouse study, 2013.

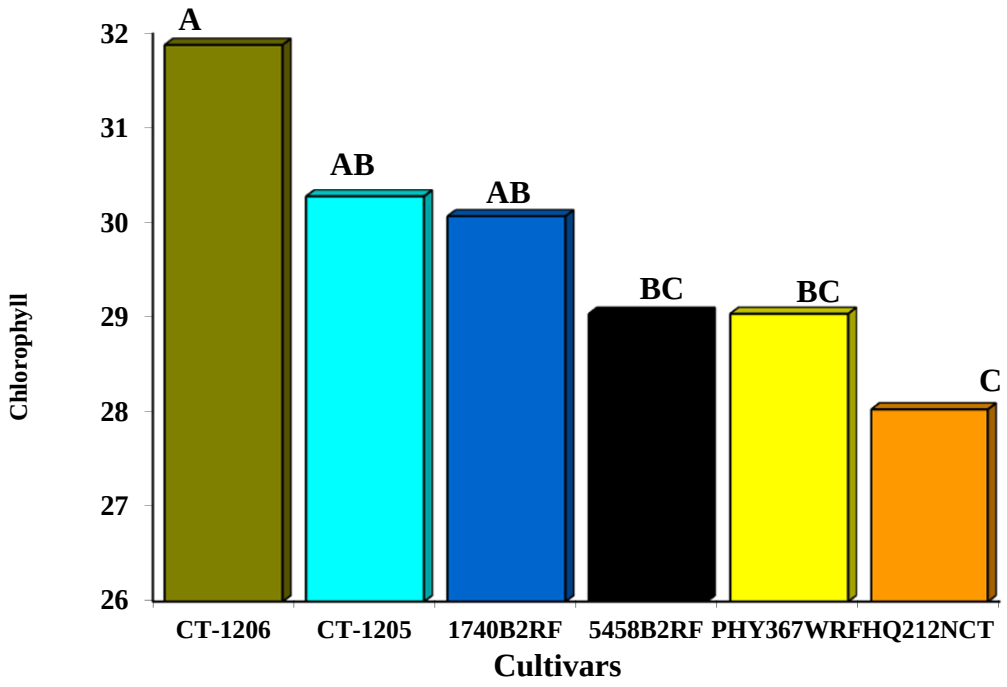

Figure 12. Effect of western flower thrips injury on chlorophyll readings of the cotton seedlings of selected cultivars in a greenhouse study, 2013.

Objective 3. Design a field cage prototype to determine the cotton crop damage potential of the western flower thrips for developing economic threshold

In 2012, six different types of single-plant potential thrips rearing field cages were developed (Fig. 13), but none proved useful for effective thrips research in the west Texas environment. On the basis of our previous experiments, we have made some important changes to our strategy. These strategies include 1) designing a bigger cage which can hold 10+ plants. More plants in a cage might provide a better environment for thrips to feed and survive hot weather conditions. 2) acquired a No-Thrips® cage material which will hold thrips inside the cage. We are excited and cautiously optimistic that the new cage design and material will facilitate a quality study which was previously not possible due to above mentioned issues.

Figure 13. Cage types evaluated previously: 1) transparent plastic cup cage, 2) wire mesh sleeve cage, 3) opaque plastic cylinder, 4) transparent plastic jar without ventilation, and 5) transparent plastic jar with ventilation, 6) utilization of thrips cages in a cotton field for thrips survival pilot studies (from CI Grant 12-364 Report 2012).

The new thrips cage prototype is a rectangular wooden-frame cage with No-Thrips® fabric covering which is expected to hold thrips inside the cage. We will be constructing 60-80 such cages for the threshold study in 2014.

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PROJECT FINAL REPORT
2008-2013

Cotton Incorporated Core Program

Project Number: 08-451

COTMAN Monitoring of Agronomic and Entomological Parameters in the Evaluation of Nitrogen Fertility Rate in Drip Irrigated Cotton

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COTMAN Monitoring of Agronomic and Entomological Parameters in the Evaluation of Nitrogen Fertility Rate in Drip Irrigated Cotton

Project Summary

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, particularly in a high yield potential subsurface drip irrigation production system. 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 project 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 or before treatment deployment. Rates of applied N exceeding 100 lb/acre resulted in higher residual nitrogen detection during the following season. Variation in residual nitrogen showed varied response to early plant growth (plant height, root length, or leaf area). Increased N levels corresponded to increased leaf chlorophyll content, but leaf chlorophyll content was generally consistent across nitrogen levels exceeding 100 lb/acre. Leaf N generally followed the trend that was observed for leaf chlorophyll content. Aphid abundance was significantly lower in zero N plots versus other plots every year when cotton aphids were present. In 2010, aphid populations surpassed economic threshold in all N-augmented plots, whereas aphids remained below 50/per leaf, except for 1 week, in zero-N plots. Higher rates of applied N (>100 lbs/A) resulted in significantly higher leaf chlorophyll content compared to that in lower or zero N plots. Nitrogen fertility level influenced fruiting profile and boll maturity. Plants ceased setting additional squares in zero and 50-lb N plots 2 wk into flowering while higher N plots were actively producing squares. Averaged over six years, 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. The N rates exceeding 100 lb/acre also reduced the micronaire values to a discount range.

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.

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. 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.

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. 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.

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.

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), which were reflected on temporal chlorophyll contents of the fifth leaf (Fig. 9). Chlorophyll contents were always lower in zero-N plots compared to that in N augmented plots at or beyond crop cut-out (Fig. 9). Temporal leaf N profile generally followed the trend of the leaf chlorophyll content; that is, zero-N plots had lower leaf N content compared to that in N augmented plots (Fig. 10).

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. 11). 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. Lint maturity, measured in terms of micronaire values, also varied with N treatments (Fig. 12). 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 (<3.4).

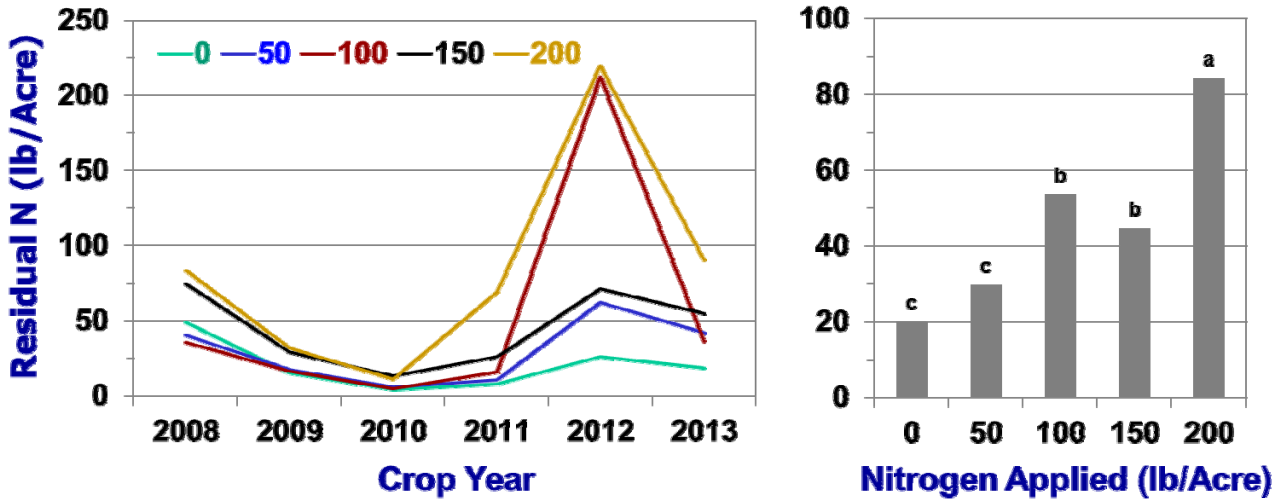

Figure 4. Effect of prior year's N application (0, 50, 100, 150, and 200 lb per acre) on residual N accumulation for the current crop year (left) and average residual N over a six-year period (right).

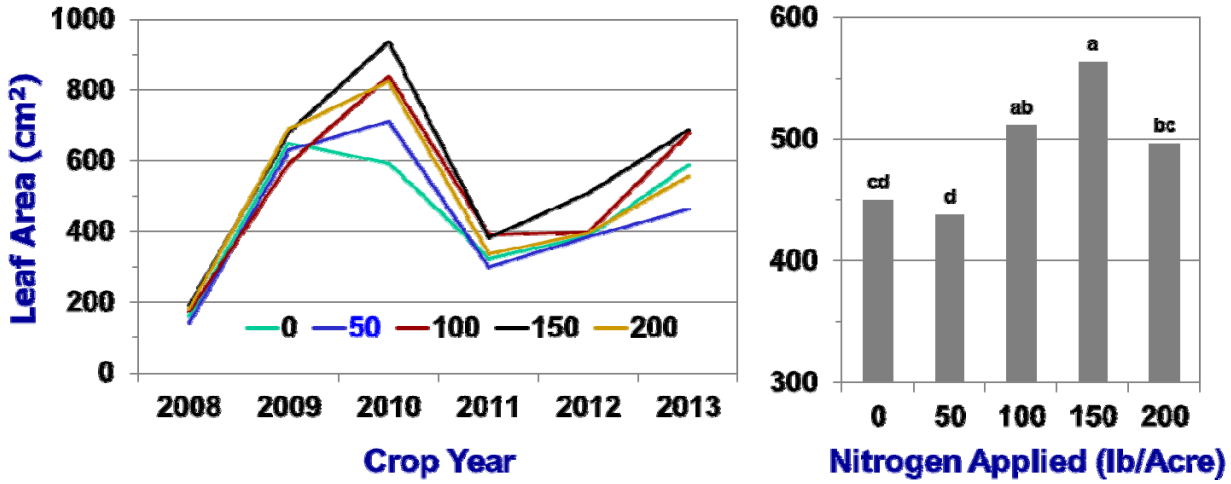

Figure 5. Year-to-year variation in total leaf surface area per plant as affected by N treatments (left) and average leaf area per plant over a six-year period (right).

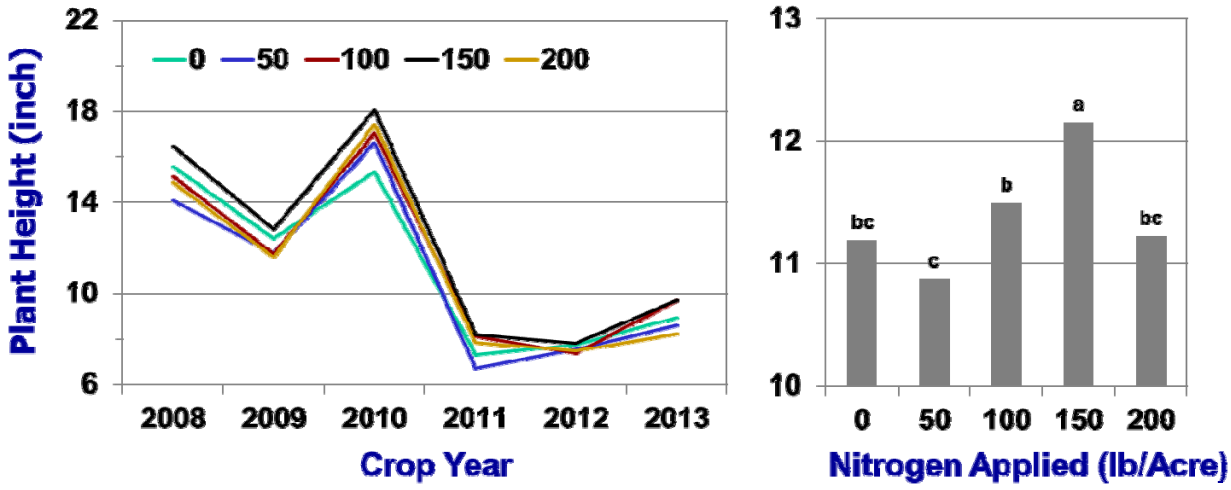

Figure 6. Effect of residual N from the previous crop year on plant height during the early crop growth period of each of the six study years (left) and average plant height over a six-year period (right).

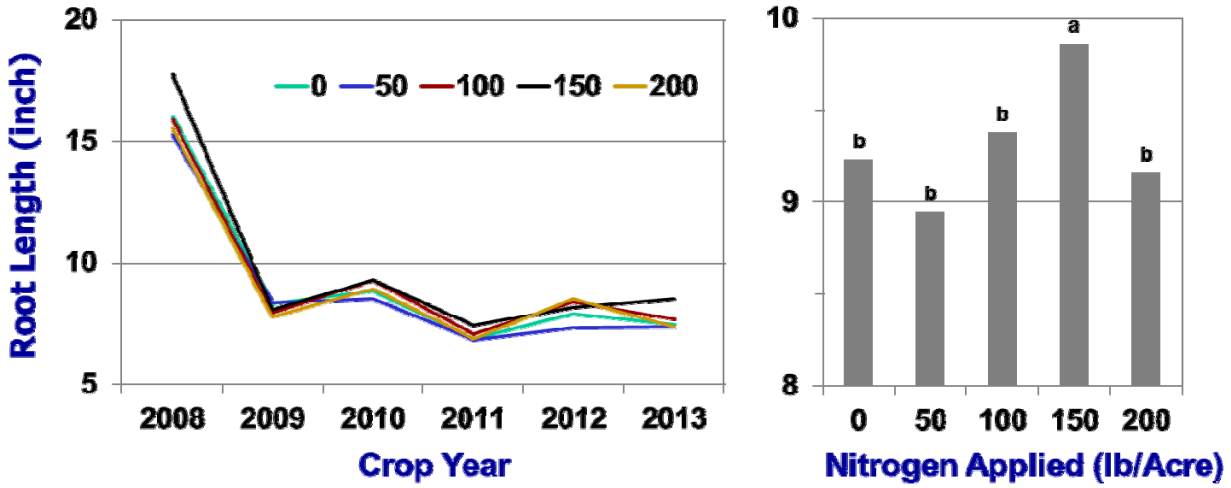

Figure 7. Effect of residual N from the previous crop year on root length during the early crop growth period of each of the six study years (left) and average root length over a six-year period (right).

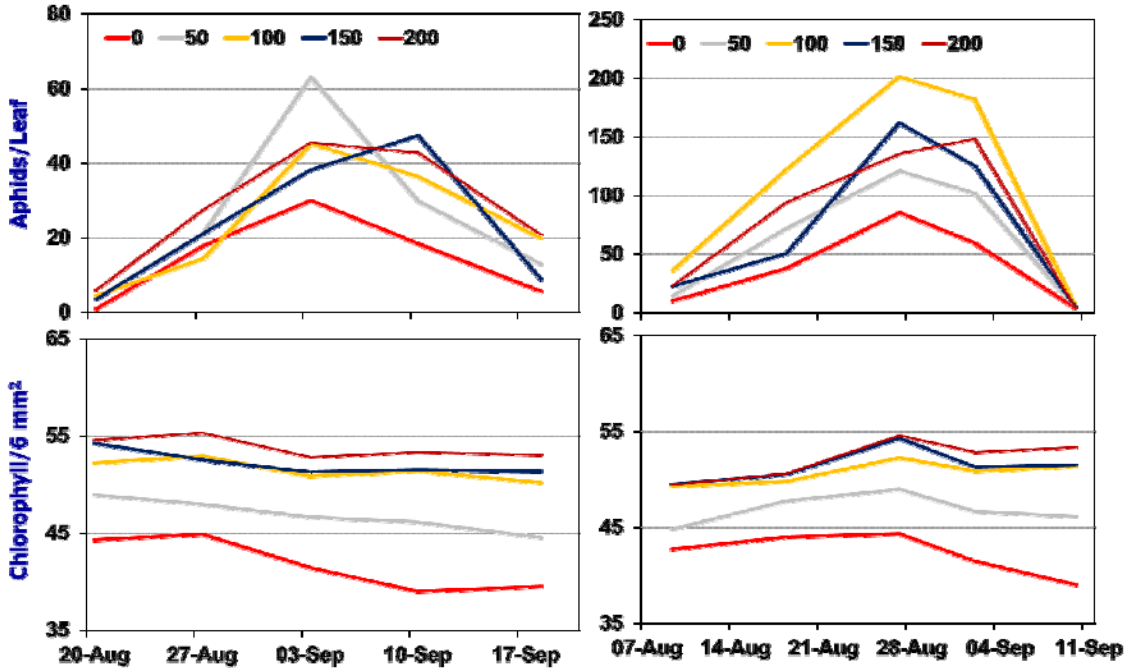

Figure 8. Temporal dynamics of cotton aphid abundance in relation to cotton leaf (5th main stem) chlorophyll content as affected by variable rates of nitrogen application (left chart – 2009, right chart – 2010).

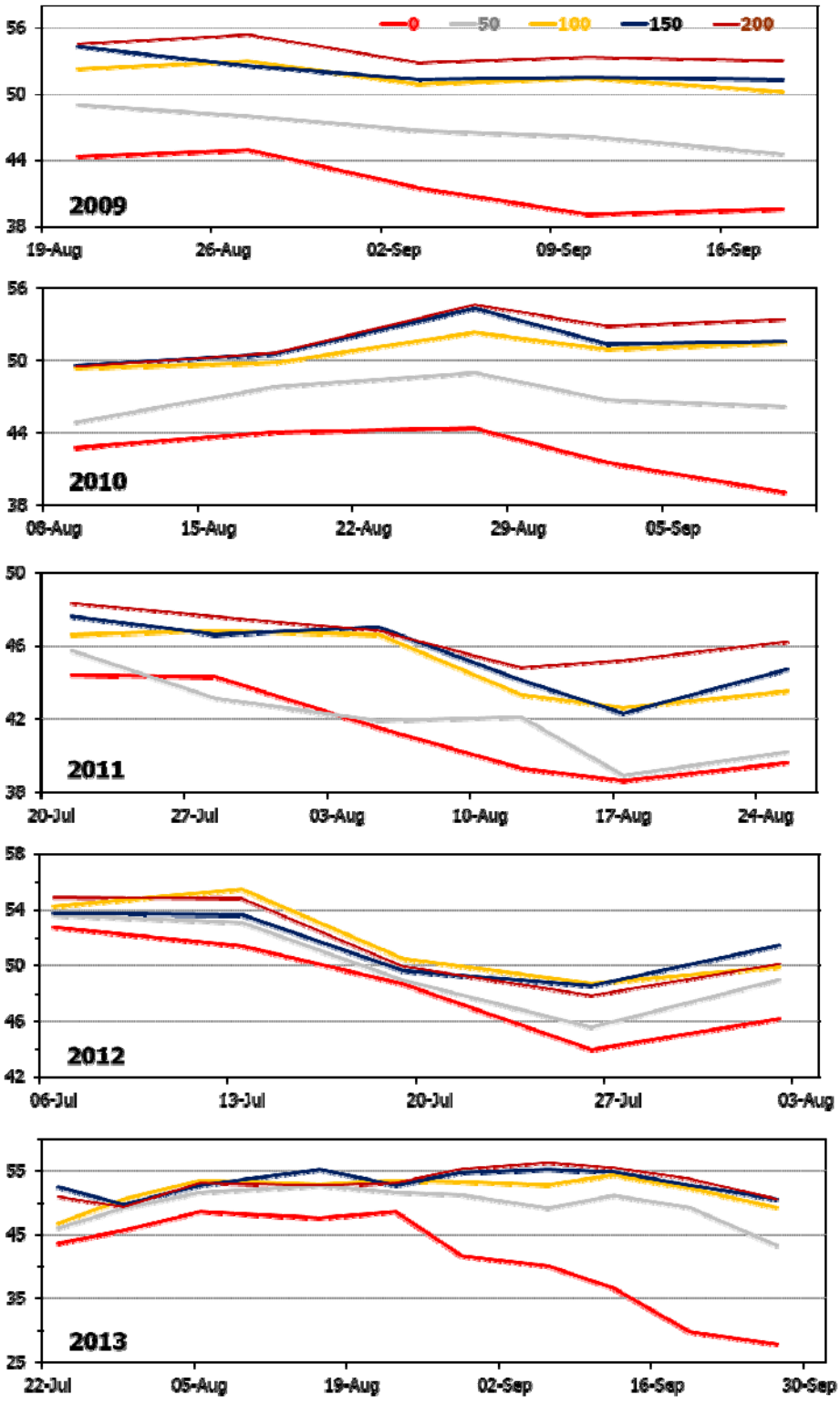

Figure 9. Effect of nitrogen rates (0, 50, 100, 150, and 200 lb per acre) on temporal dynamics of leaf chlorophyll content measured on 5th mainstem leaf, 2009-2013, Hale County, TX.

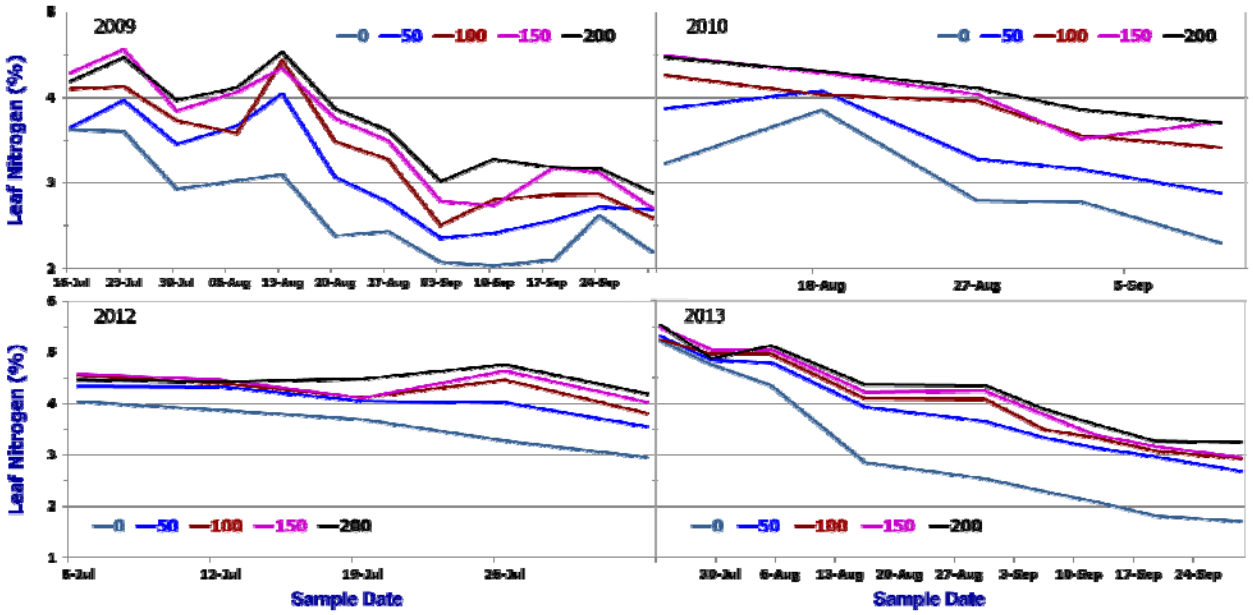

Figure 10. Effect of nitrogen augmentation rates (0, 50, 100, 150, and 200 lb per acre) on temporal dynamics of leaf nitrogen content measured on 5th mainstem leaf from the terminal of the plant, 2009-2013, Hale County, TX.

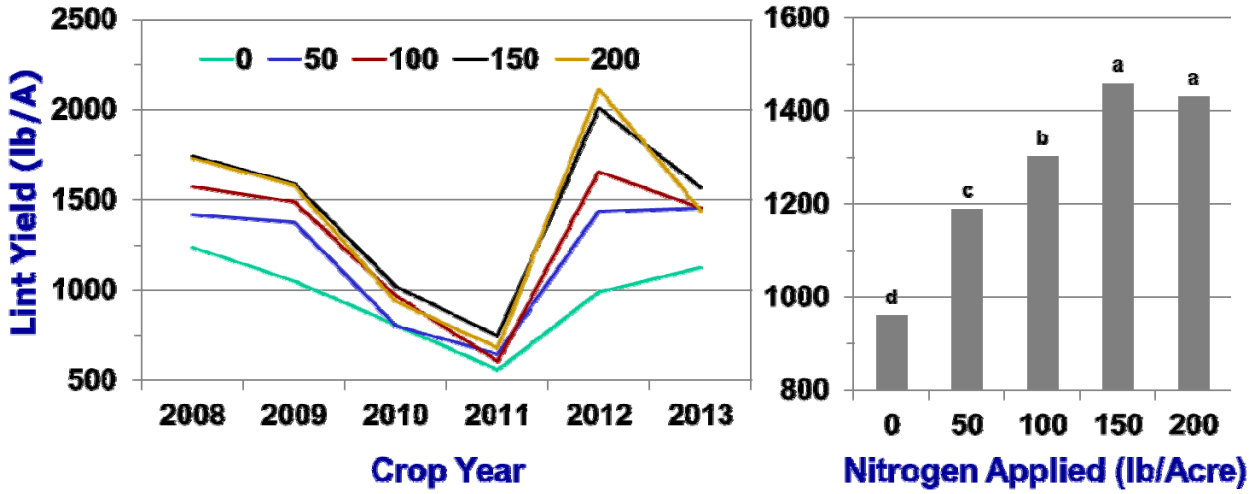

Figure 11. Year-to-year variation in the effect of nitrogen application rates on cotton lint yield (left) and average lint yield over a 6-year period (right), Helms Farm, Hale County, TX.

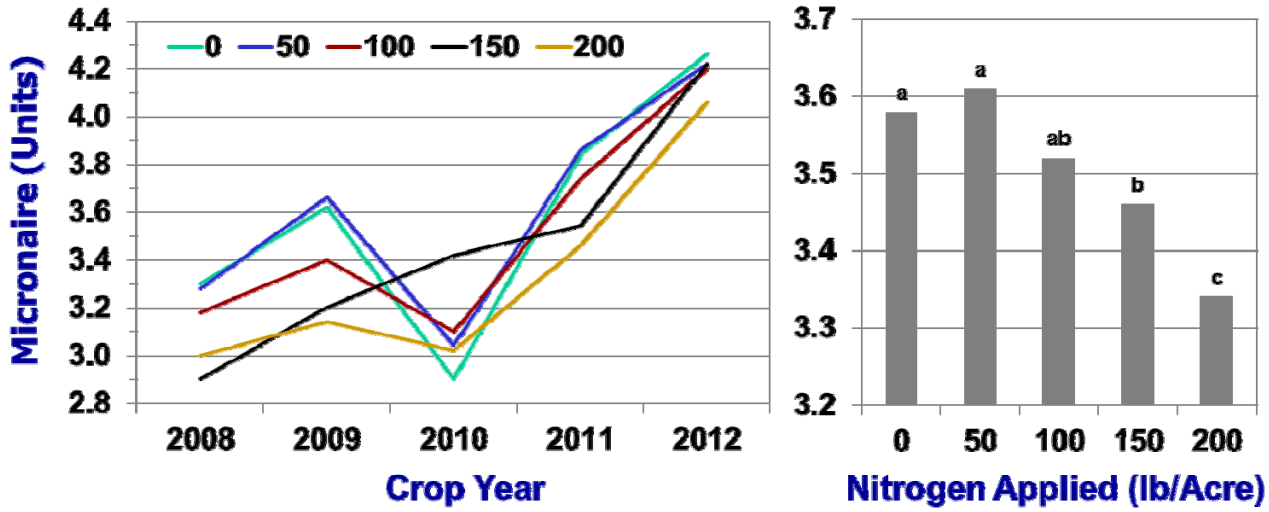

Figure 12. Year-to-year variation in the effect of nitrogen application rates on cotton lint micronaire (left) and average micronaire over a 6-year period (right), Hale County, TX.

Acknowledgments

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2013 ANNUAL REPORT (YEAR 2)

Cotton Incorporated/Texas State Support Program

Project Number: 12-112TX

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

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

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PROJECT SUMMARY

Western tarnished plant bug, *Lygus hesperus,* is the primary *Lygus* species inhabiting cotton and several other hosts in the Texas High Plains. In Texas High Plains cotton, *Lygus* is generally more pestiferous in the boll development stage than in early squaring stage. Our recent study on boll damage assessment based on heat unit-delineated maturity provided a boll-safe cutoff value of 350 heat units (~2-3 weeks from flowering), although *Lygus* adults and nymphs both cause external lesions on bolls throughout boll development and may give farmers a false impression of *Lygus* damage. A four-year State Support funded project revealed that late-instar nymphs caused significantly more damage to maturing bolls than adults, and inflicted 23, 29, and 15% more loss in lint yield, seed weight, and seed counts per boll, respectively, versus adults. Nevertheless, no economic threshold for *Lygus* boll management has been developed for Texas cotton. This project aims to conduct a comprehensive threshold study for *Lygus* in Texas cotton.

The major goal of this project was to develop economic threshold-based management recommendations for *Lygus* in Texas High Plains cotton, thereby aiming to minimize economic losses to producers. Specific objectives were to: 1) determine the maximum potential for *Lygus* to inflict damage to cotton bolls at various boll maturity levels (ages), 2) characterize the cotton boll preference behavior of *Lygus*, and 3) establish the *Lygus* economic threshold for Texas cotton. In both 2012 and 2013, boll damage potential of *Lygus hesperus* was determined in a no-choice cup-cage study. Ten cohorts of cup-caged single bolls (1-20 days old) were each exposed to a *Lygus* adult for 48 hours and the boll damages were quantified. After bolls reached 13 days of age, *Lygus* caused very little seed damage, which as expected, also did not result in significant lint yield loss. Cotton bolls were safe from *Lygus* damage when they reached >28 mm diameter or their carpel wall hardness was 0.7 lb per square foot or greater. Cotton boll feeding preferences of *Lygus hesperus*, within-plant boll distribution profile, and *Lygus* damage to cotton bolls at various *Lygus* densities were determined in a whole-plant cage field study. Individually caged cotton plants were exposed to 4 levels of *Lygus* (0, 1, 2 and 4 adults per cage) for one week when plants were at two selected boll development stages (350 and 550 HU after first flower). When the crop matured from 350 HU to 550 HU after first flower, the percentage of bolls vulnerable to *Lygus* feeding damage was reduced from 50% to 30%. Internal warts were mostly limited to the bolls measuring <35 mm in diameter. In this open-choice boll feeding situation, *Lygus* preferred to feed on bolls that were 10-30 mm in diameter. There were no significant yield differences between control plants and *Lygus* infested plants when plants were first infested with *Lygus* bugs at 550 HU after first flower, but the *Lygus* augmentation at 350 HU reduced lint and seed yield. Overall, 8-9% lower lint yield was observed in *Lygus* infested plots compared to that in control plots at both HU. We plan to increase the augmentation densities for 2014 study to generate required data for threshold calculation. A detailed understanding of *Lygus* boll feeding biology and behavior will be highly valuable in improving *Lygus* management decisions during the different boll developmental stages. With these series of multi-year field studies, we hope to characterize the relationships between cotton boll maturity and *Lygus hesperus* infestations as well as to develop a *Lygus* economic threshold for Texas High Plains cotton.

Introduction

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

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

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

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

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

Materials and Methods

Estimating *Lygus* Boll Damage Potential

A field study to quantify adult *Lygus hesperus* cotton boll damage potential was conducted at the Texas A&M AgriLife Research and Extension Center farm in Lubbock, Texas. On May 18, 2012, cotton cultivar ST 5458B2RF was planted on May 18 (2012) and May 22 (2013) on 40-inch spaced rows of a furrow-irrigated field. The targeted seeding rate was 56,000 seeds per acre. On June 2, 2012, the entire test was treated with Orthene® 97S for thrips at the rate of 3.0 oz per acre and with Cornerstone Plus® herbicide (41% glyphosate) at 32 oz per acre for weed management. No insecticide interventions were necessary for thrips control in 2013.

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

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

infestation levels x ten boll age cohorts x eight subsamples).

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

Figure 1. Deployment of cup-cages to enclose age-specific bolls for *Lygus* damage potential study, Lubbock, TX, 2012-2013.

Determination of Boll Maturation Profile, Feeding Preference and Economic Threshold

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

2012 Study. The field study was laid out in a split-plot randomized block design with three replications, two main plot factors (two cotton boll developmental stages [early boll development and late boll development]), and four subplot factors (four levels of *Lygus* infestation [control or zero bugs, one bug/plant, two bugs/plant, and four bugs/plant]). There were a total of 24 experimental units. Each experimental unit had 8 cotton plants as subsamples (4 used for damage assessment and 4 for yield and quality assessment). A total of 192 whole-plant sleeve-caged cotton plants (three blocks x two cotton boll stages x four *Lygus* densities x eight subsamples) were used for this study (Fig. 2).

The cotton field study site was closely monitored and kept virtually arthropod pest-free until cages were deployed on July 24, 2012. When the cotton plants reached the target maturity level (350 HU after first flower on August 7, and 550 HU after first flower on August 21), lab-reared *Lygus* were released into the whole-plant sleeve-cages at the rates of 0, 1, 2, and 4 bugs/plant. Again, the *Lygus* adults were initially reared on artificial diet, yet "trained" on fresh green beans and cotton squares for a week before using them for the boll feeding experiment. Cotton plants were exposed to the *Lygus* adults for 6-7 days, after which time, the insects were killed via a pesticide application. Four randomly selected cotton plants from each plot were cut and brought to the laboratory on August 13 and August 21 for the 350 HU and 550 HU plots, respectively. Boll positions, internal and external *Lygus* damage, boll weights, boll diameters, and boll hardness were recorded for all plants from Block 1. For plants from the other blocks, external boll damage, boll weight, and size were recorded. The cotton crop was defoliated by spraying FOLEX® 6EC (12 oz per acre) and a boll opener (Ethephon® 6; 32 oz per acre) in a tank mix on October 3, 2012. After the crop was ready to harvest, the remaining 4 caged plants from each plot, which had been maintained pest-free, were harvested manually to evaluate the lint yields and fiber quality. Data from the whole-plant cage study were summarized by calculating average and standard errors. ANOVA, GLM model in SAS, 2010 were used to evaluate the treatment effects (α=0.1) and treatment means were compared by LSMEAN procedure.

2013 Study. The 2013 study was conducted in the same field as for 2012 study to quantify the effect of *Lygus* density and infestation timing on cotton yield and quality. The study design and treatments for 2013 were similar to that in 2012 study as outlined above. Each experimental unit consisted of 8 cotton plants as subsamples (3 used for damage assessment and 5 for yield and quality assessment). A total of **192 sleeve-caged cotton plants** (3 blocks x 2 cotton boll stages x 4 *Lygus* densities x 8 subsamples) were used for this study (Fig. 2). Approximately 400 plants with first white flower were tagged on July 29 and the daily heat unit accumulations (>60 ^{o}F) were monitored from that point forward. On August 12, the heat unit accumulation reached about 300, a general plant phenological stage indicating "early boll developmental stage. On August 13, *Lygus* density augmentation treatments were deployed in 96 individually caged "early boll maturing" plants (3 blocks x 4 *Lygus* densities x 8 subsamples). A second set of 96 caged plants attained 500 HU (late boll maturation stage) on August 29, so the *Lygus* density augmentation treatments on "late boll maturing stage" cotton were deployed on this date. *Lygus* bugs were allowed to infest the caged plants for 7 days and all cages were removed. Three plants per treatment were removed from the field (August 19 for 300 HU and September 2 for 500 HU), brought to the laboratory, and processed for boll positions, boll injury (external and internal), and boll weight and diameter. Remaining five plants per treatment were sprayed with acephate and maintained relatively insect-free for the remainder of the growing season. Test plants were harvested manually to evaluate yield and quality. Harvested single-plant samples were ginned individually via table-top gin and samples have been sent to Cotton Incorporated for fiber quality analysis.

Figure 2. Field deployment of whole-plant cages for threshold study, Lubbock, TX, 2012-2013.

Results and Discussion

Boll Development vs. *Lygus* Damage Potential

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

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

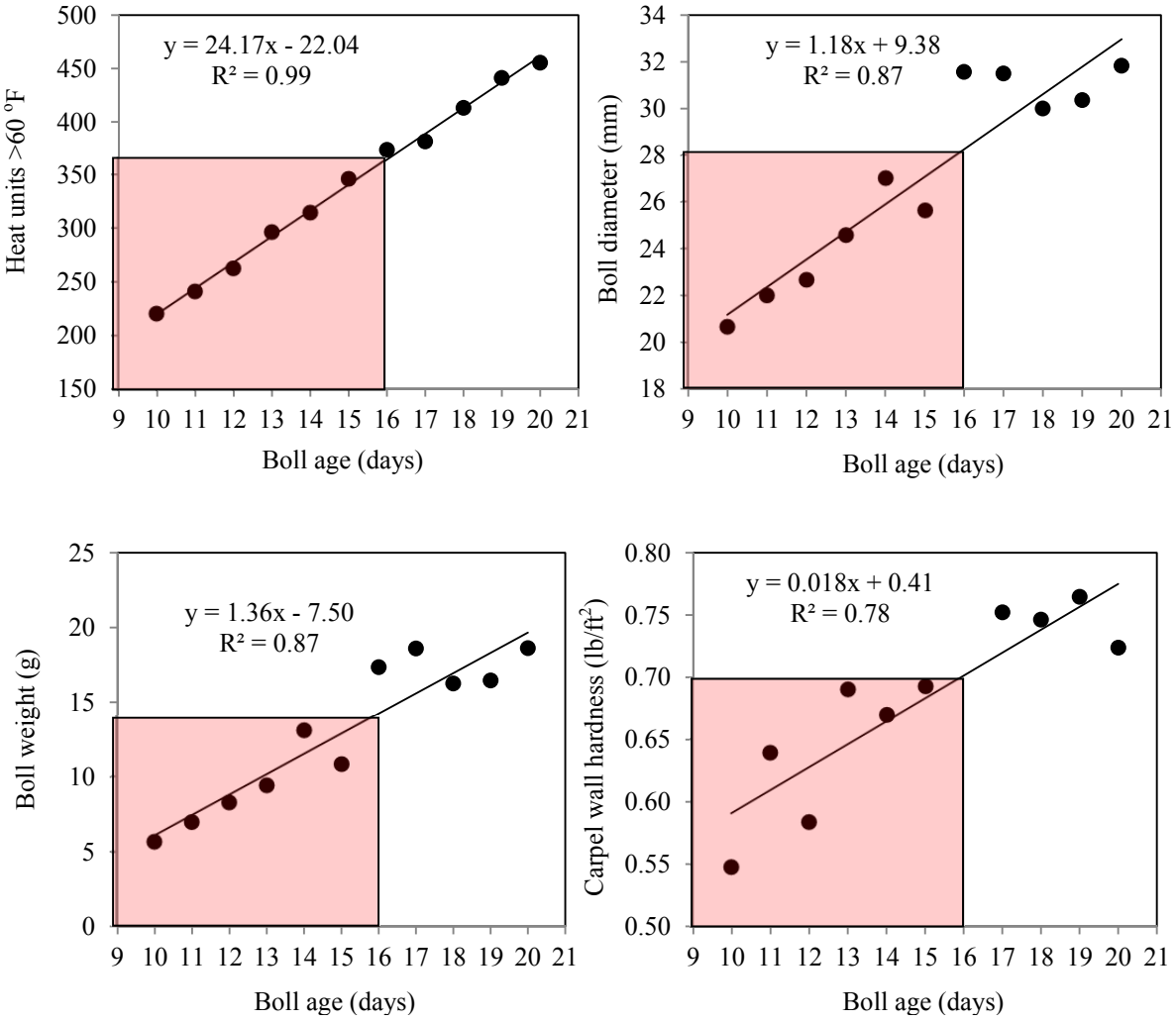

Figure 3. Cotton boll age relationships as associated to heat unit accumulations, boll size, boll weight, and carpel wall hardness, Lubbock, Texas, 2012.

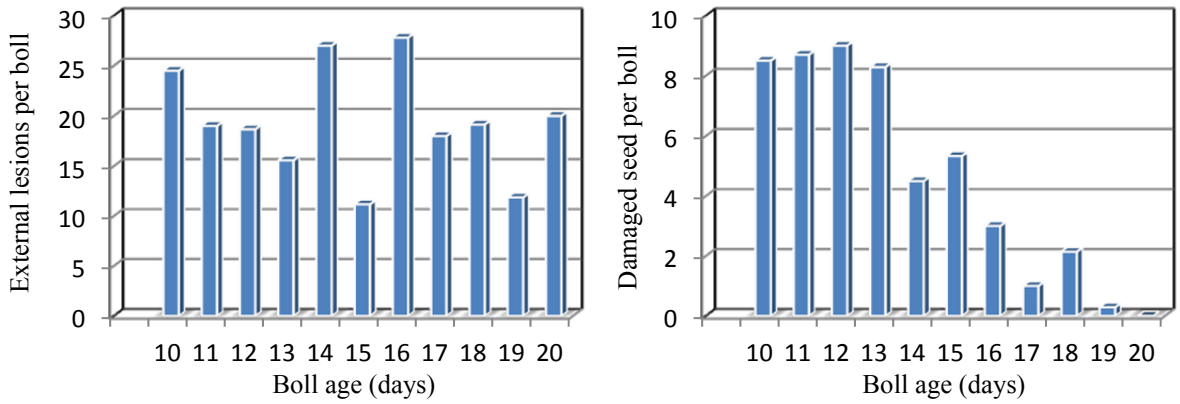

Figure 4. Cotton boll injury (external lesions and damaged seeds) at various boll ages following a 48-h feeding of a single *Lygus* adult, Lubbock, TX, 2012.

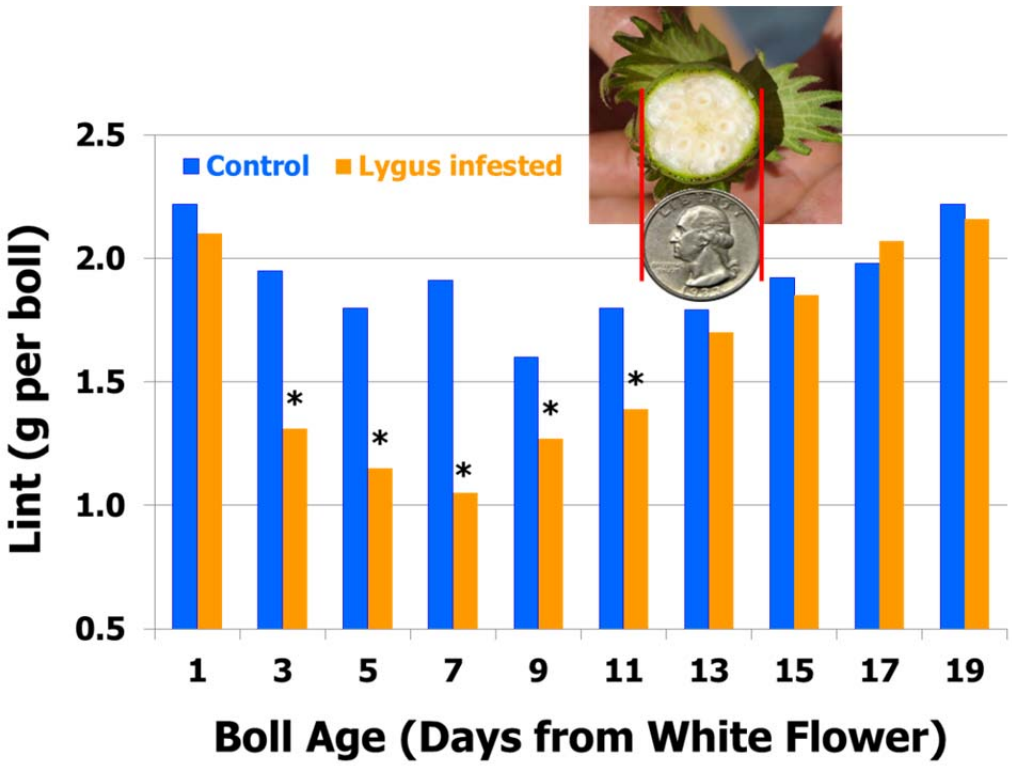

Figure 5. Single-boll lint yield (gram per boll) following 48 hours of feeding by a single *Lygus* adult versus uninfested boll at boll ages ranging from Day to Day 19, Lubbock, TX, 2013.

Fruiting Profile

At 350 HU after first flower, average of 57% fruit retention was observed, but fruit retention was decreased to 37% when cotton reached 550 HU after first flower. Cotton plants at 350 HU were observed to have 84% bolls, 14% squares and 2% flowers, while at 550 HU, the cotton plants had 99% bolls, 1% squares, and no flowers. Although there were a higher percentage of cotton bolls on 550 HU plants, the actual number of bolls per plant decreased from an average of 8.8 bolls per plant at 350 HU to 6.3 bolls at 550 HU. Approximately 28.4% of the bolls were naturally aborted from the plants as they matured from the 350 HU to 550 HU stage (Fig. 6).

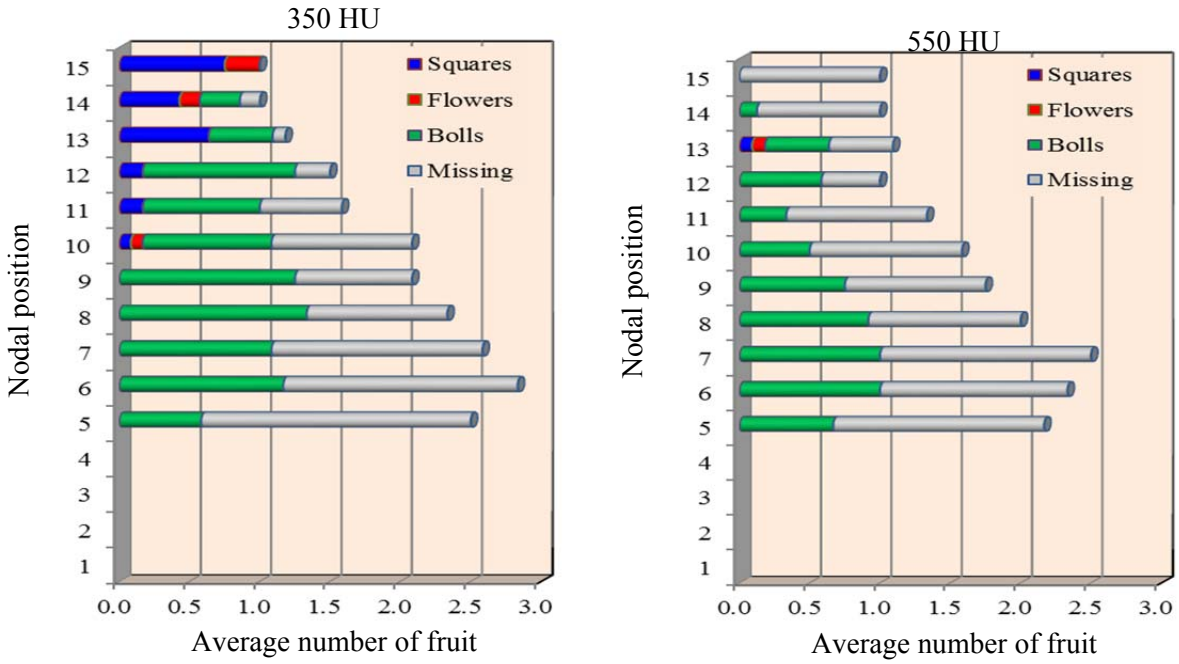

Figure 6. Cotton fruiting profile at 350 and 550 HU after first flower, Lubbock, TX, 2012.

Most of the bolls were from first fruiting positions of the sympodial branches. At 350 HU, 66%, 24%, 8%, and 2% bolls were from the first, second, third and fourth sympodial branch fruiting positions, respectively; while at 550 HU, 81%, 16%, 3%, and 0% bolls were from the first, second, third and fourth sympodial branch fruiting positions, respectively (Fig. 7). When the cotton plants matured from 350 HU to 550 HU, they dropped all of the 4th fruiting position and most of the 3rd fruiting position bolls. Since 97% of the bolls were on first and second fruiting positions on the cotton plants at the 550 HU stage, our sampling and crop protection efforts should be focused on protecting primarily the first and second position bolls at this stage. However, fruiting profiles may vary with cotton cultivar, cotton growing region, and crop management practices and input use patterns.

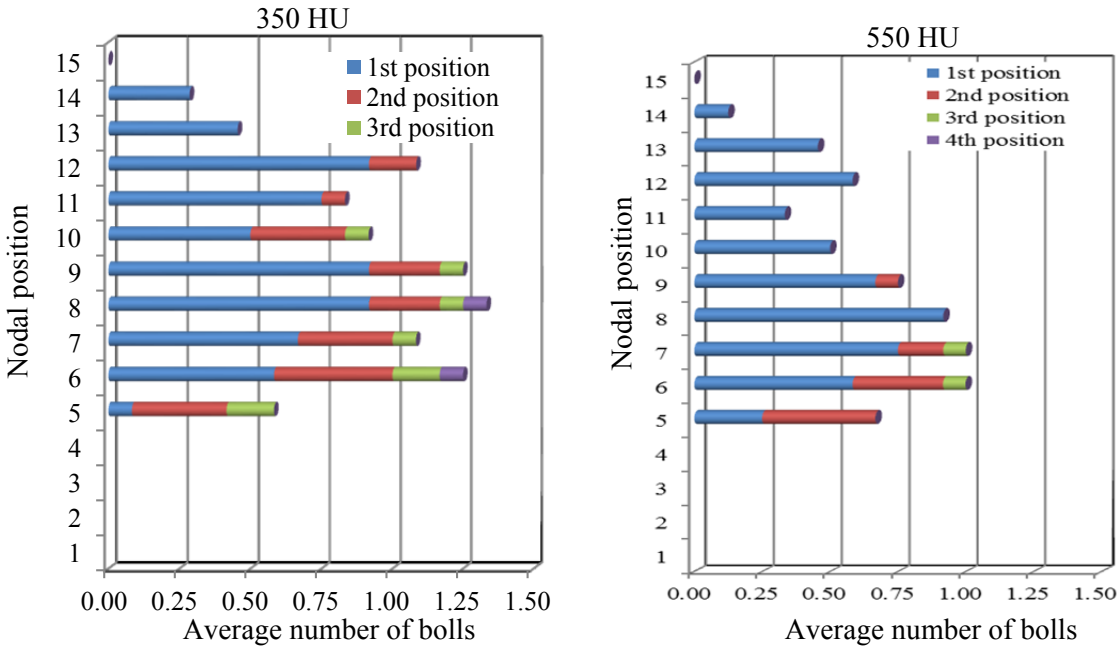

Figure 7. Boll distribution on sympodial branches at 350 and 550 HU AFF, Lubbock, TX, 2012.

Boll Maturation Profile

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

For our 2012 cotton crop, within-plant cotton boll maturation profile shows that bolls distributed from the 5th to 13th nodes (Fig. 8). At the 350 HU stage, the top 4 bolls (from 10-13th node) were <25 mm diameter size and were vulnerable to *Lygus* damage if bugs were present. When the cotton reached 550 HU, only the top 3 bolls (nodes 11-13) were <25 mm diameter size and therefore vulnerable to *Lygus* damage, if present. Bolls from the 5th to 9th nodes were larger and less vulnerable to *Lygus* feeding damage. There was a very strong positive relationship between boll size (diameter) and the hardness of the boll carpel wall. As we move from the top to bottom nodes of a cotton plant, as expected, we found larger bolls with harder carpel walls (Fig. 8). The vertical boll profile suggests that cotton growers or crop consultants need to focus their *Lygus* damage evaluations primarily during the 350-550 HU, and mostly on the top 3-4 bolls, since they are the most vulnerable to *Lygus* feeding injury. The 2013 data also showed similar trend in terms of within-plant boll maturation distribution.

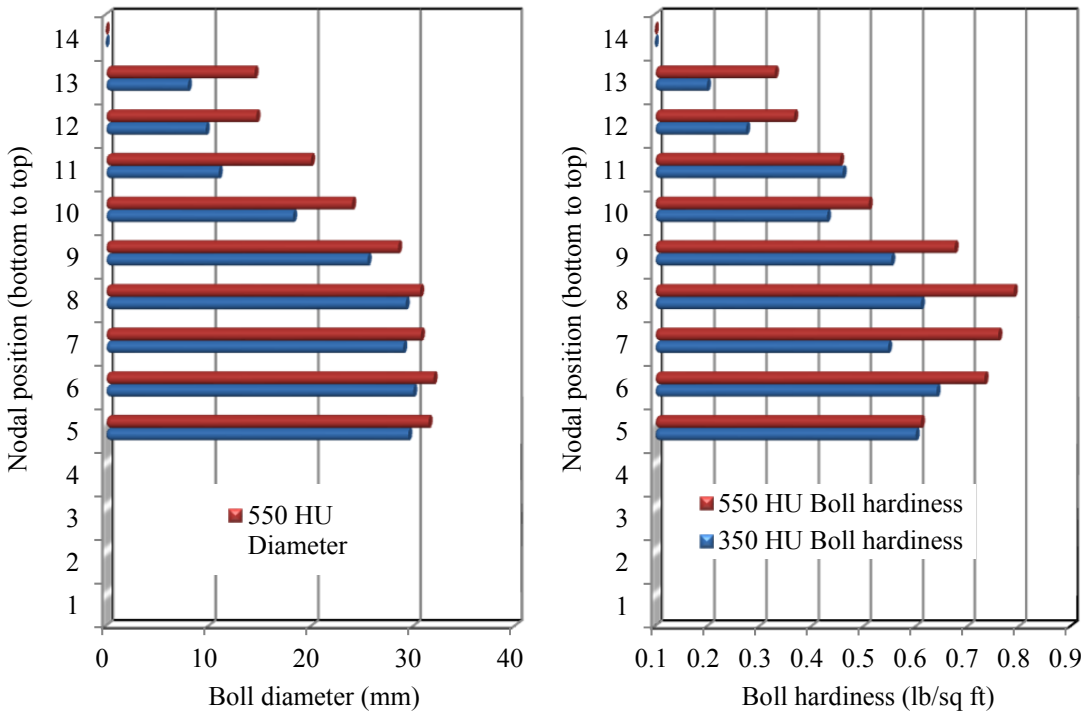

Figure 8. First position boll size profiles of 350 and 550 HU (after first flower) cotton. Lubbock, TX, 2012.

Lygus Boll Feeding Preference and Boll Damage

In the whole-plant caging study, *Lygus* external feeding lesions were found in bolls of all sizes, indicating *Lygus* attempted to feed on cotton bolls irrespective of boll size. Nevertheless, successful punctures and the resulting internal warts were limited to the bolls <35 mm in diameter. A significantly higher proportion of bolls had internal warts (>20% of bolls) for <30 mm bolls, indicating that in an open-choice situation, *Lygus* preferred to feed on bolls that were <30 mm in diameter (Fig. 9). Cotton plants at the 350 HU had 90% of the bolls measuring <30 mm in diameter, whereas plants at the 550 HU had 78% of the bolls at <30 mm diameter (Fig. 9). The no-choice cup-cage study showed bolls that are >25 mm diameter were safe from *Lygus* damage, whereas in the open-choice whole-plant caging study, *Lygus* preferred to feed on bolls up to 30 mm in diameter. This slight discrepancy might be due to difference in cotton boll development inside cup-cages versus whole-plant cages, or due to differences in *Lygus* behavior in the presence of different boll size options and containments. Evaluation of internal lesions and internal warts suggests there is not a significant relationship between external *Lygus* feeding lesions and actual seed damage due to *Lygus* feeding (Fig. 10), but there were strong relationships between the number of internal warts and number of *Lygus* damaged seed. It clearly indicates that estimating *Lygus* damage by using external lesions can be misleading; therefore, it is best to use the number of internal warts to estimate the degree of *Lygus* crop damage.

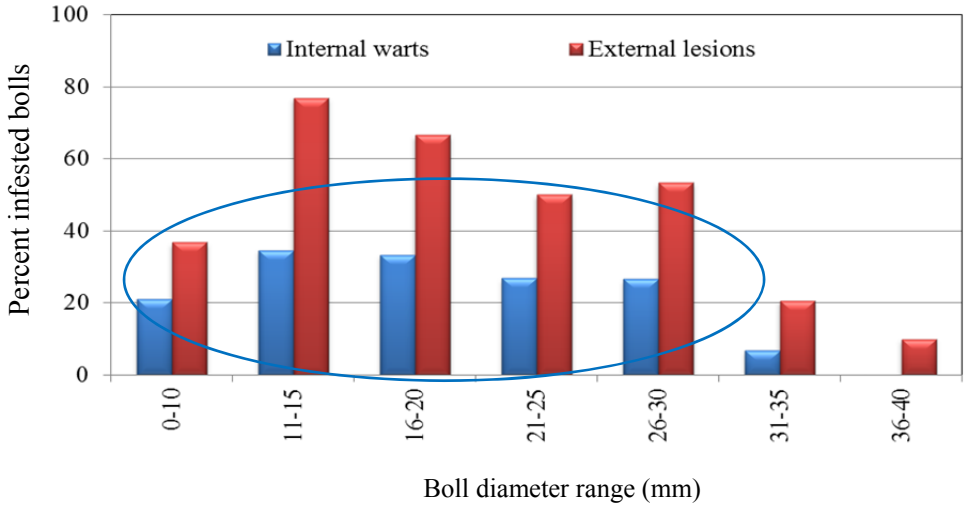

Figure 9. Boll feeding preference of *Lygus* in whole-plant cages based upon the proportion of external and internal boll damage. Lubbock County, TX, 2012.

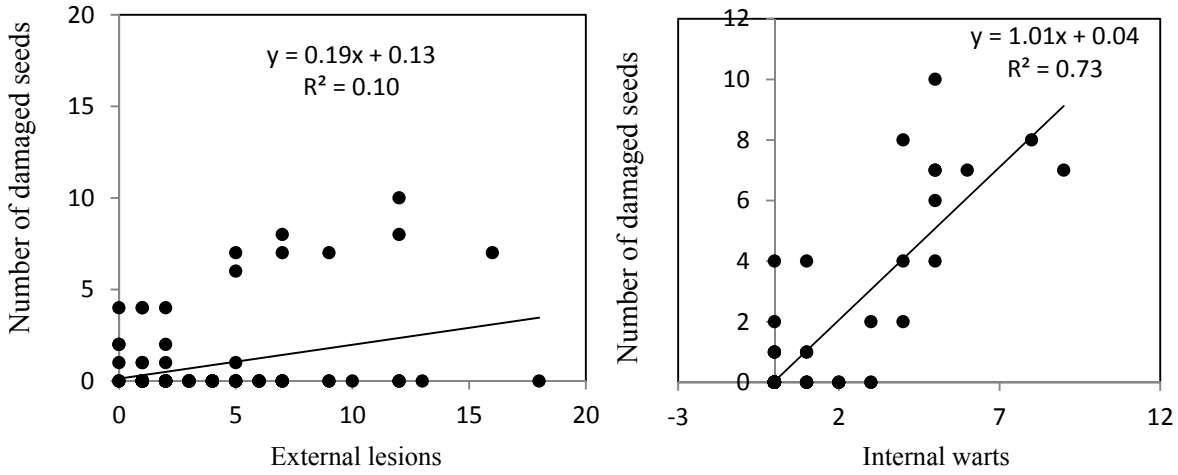

Figure 10. Relationships between the number of damaged seeds per boll and the number of external lesions or internal warts, Lubbock, TX, 2012.

Yield Loss

In 2012, artificial augmentation of 2-4 *Lygus* bugs per plant at 350 HU after first flower significantly reduced the cotton lint yield, but the same level of *Lygus* infestation at 550 HU did not result in significant lint yield reduction compared with that in uninfested control plants (Fig. 11). These data suggest that the maturing bolls are much more tolerant to *Lygus* injury when the plant attains 550 HU from first flower. It is also possible that *Lygus* bugs may choose to feed on superfluous bolls or squares and the yield contributing fruits may not be significantly impacted by such late infestation. Because potential yield loss risks due to certain *Lygus* density infestations vary with boll maturation profile, the *Lygus* management economic threshold should be optimized for a dynamic ET to accommodate for within-plant fruit maturity profiles.

In 2013, the lint yield values in *Lygus* augmented plots for 350 HU were lower compared to that in untreated control plots, but the values were not statistically significant (Fig. 12). The seed weight followed the identical trend to that for the lint weight. While we were surprised that the *Lygus* augmented plots did not show significantly reduced yield compared to that in untreated control plots, the trend is convincingly supportive of a clear influence of *Lygus* augmentation on yield reduction and the data trend is similar to what we observed in 2012. The total number of seeds in the most severe infestation (4 *Lygus* per plot) was much lower than in other treatments.

The 550 HU displayed the similar trend in lint and seed yield to that for 350 HU, but the yield reduction due to *Lygus* was somewhat weaker than that for 350 HU, which is similar to 2012 results (Fig. 13). It is somewhat surprising to observe that the seed reduction in 550 HU augmentation was much more pronounced than that for 350 HU (Figs. 12-13). More detailed research is needed to characterize the interaction between crop phenology and *Lygus*-induced yield loss. Our continuing project is expected to address some of these issues.

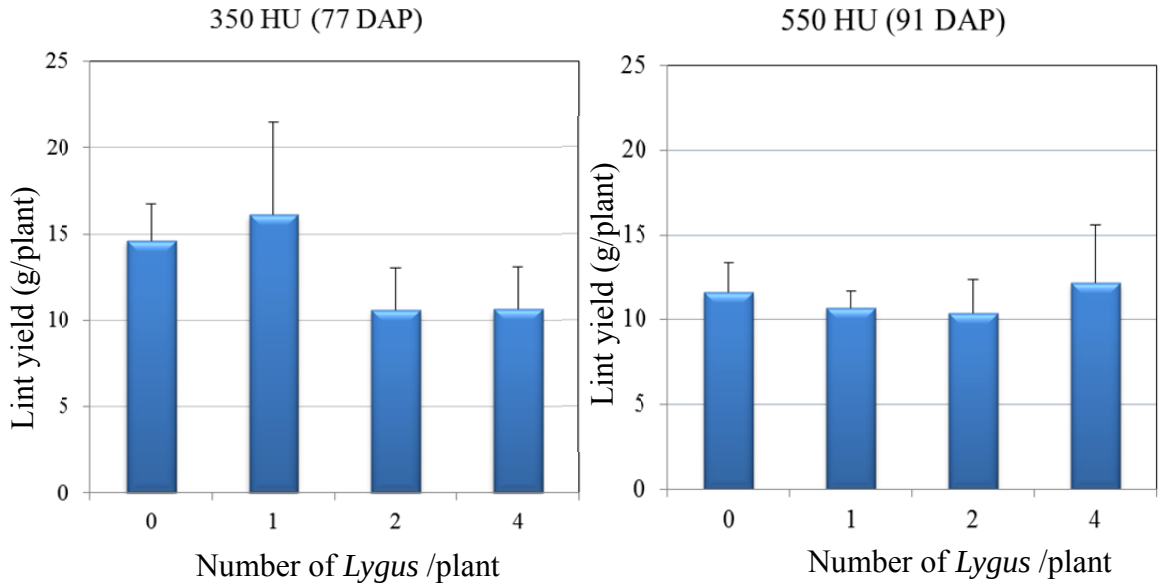

Figure 11. Influence of varying levels of *Lygus* infestations on lint yields at two crop phenological stages. Lubbock County, TX, 2012.

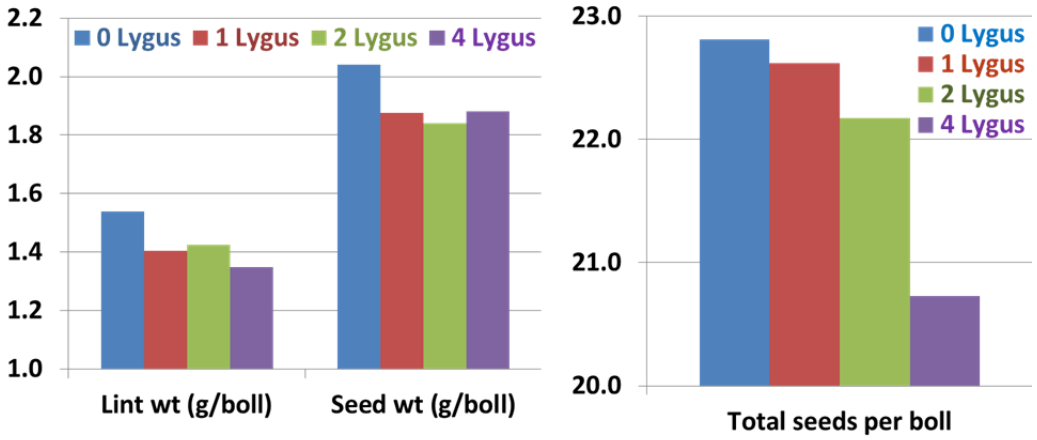

Figure 12. Influence of varying levels of *Lygus* infestations at 350 HU on lint and seed yields, Lubbock County, TX, 2013.

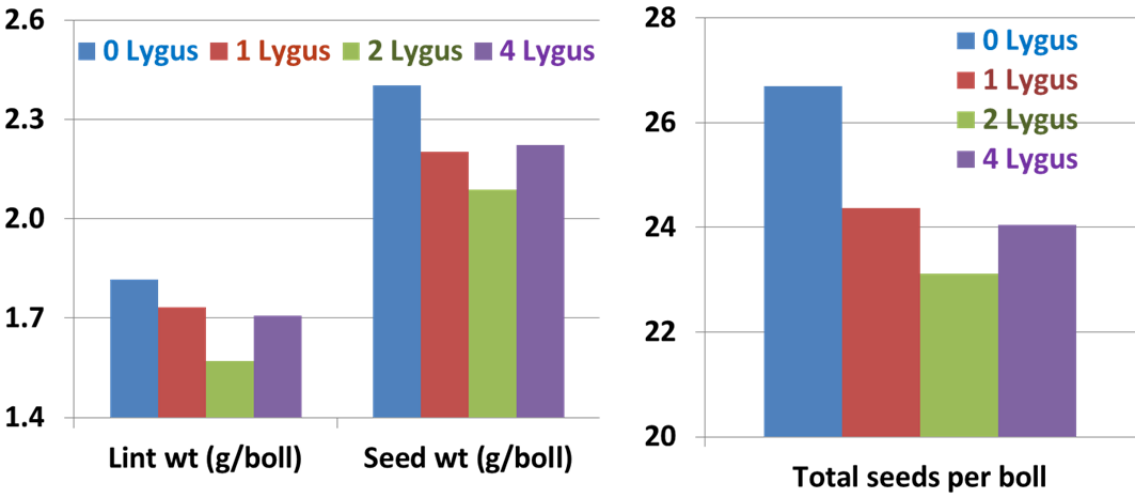

Figure 13. Influence of varying levels of *Lygus* infestations at 550 HU on lint and seed yields, Lubbock County, TX, 2013.

Percentage reduction in lint and seed yield due to *Lygus* augmentation compared to that in untreated control ranged from 8 to 9 for both 350 and 550 HU (Fig. 14). Interestingly, the number of total seeds per boll did not decrease in 350 HU, but the seed density decreased by over 10% at 550 HU. We are unable to offer a reasonable explanation as to why the seed density per boll did not decrease at 350 HU together with lint yield, but it decreased by 10% in 550 HU. The seed weight, however, did not vary across *Lygus* augmented treatments.

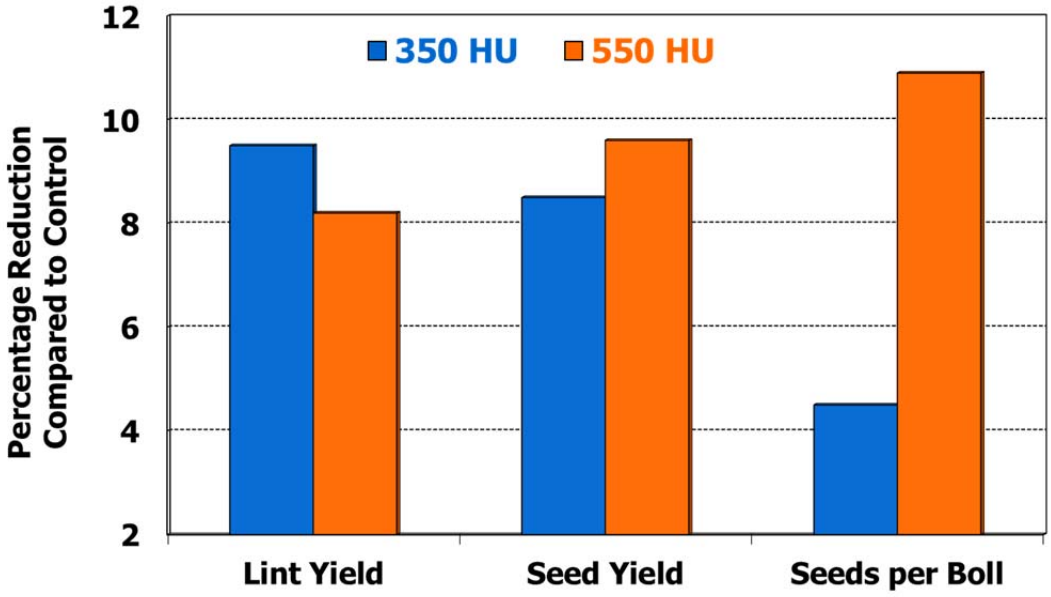

Figure 14. Percentage reduction in lint yield, seed yield, and seed density per boll in *Lygus*-infested bolls versus uninfested control bolls for 350 and 550 HU infestation of 1, 2, and 4 *Lygus* per boll augmentation. Data are averaged across infestation levels within a given HU, Lubbock County, TX, 2013.

Summary

There was a significant change in boll composition (boll profile) between the cotton plants at 350 and 550 HU from first flower. Despite a subtle variation between no-choice (cup-caged single boll feeding) versus choice (whole-plant cage with access to all boll types for feeding) situations, it appeared that bolls were relatively safe at 28-30 mm diameter size or 350 HU, which was approximately equivalent to two weeks old bolls. While year-to-year variation exists and the variation in boll susceptibility is expected across cropping system management (irrigation, planting date, fertility, etc.), maturing bolls should generally be safe from *Lygus* injury two weeks after white flower, especially for *Lygus* adults. We plan to investigate this relationship for *Lygus* nymphs in 2014. Cotton boll developmental rates may vary depending on the crop cultivar and crop management system, therefore the interactions between *Lygus* damage potential and other cotton cultivars and various crop management systems need to be investigated to determining the *Lygus* safe boll developmental stages. Future research of our program is expected to address these issues.

Acknowledgements

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TEXAS A&M AGRILIFE EXTENSION

Thrips Management in Texas High Plains Cotton

This is the Final Report (2013) for the Project 13-456TX entitled "*Thrips Management in Texas High Plains Cotton*." In this report I provide the information on the entire study that was conducted at the Texas A&M AgriLife Research farm located at Halfway, Texas. Although the study was replicated in three other locations, weather conditions (hail storm, wind and drought), and insignificant pest pressure did not allow us to continue the trial on those three locations. This year, cotton in the Texas High Plains has experienced harsh weather conditions ranging from drought, high velocity winds, to cold conditions immediately after planting. In addition to drought conditions, some of the region's cotton suffered damages due to hail storms and blowing sand.

Materials and methods

This study was conducted at the Texas A&M AgriLife Research farm located at Halfway, TX. Cotton seeds of variety FM1944 B2R were planted on 3 May, 2013. Each plot was 35 row-ft long and 4 rows wide (40-inch seedbed spacing). There were six different treatments: 1) Aeris® seed treatment, 2) Gaucho® + Poncho® seed treatment, 3) Avicta Complete® seed treatment, 4) Orthene® 97S @ 3 oz./A at threshold, 5) Vydate® @ 8.5 fl oz./A at threshold, and 6) untreated control. The initial thrips sampling at the cotyledon stage was conducted 25 days after planting on 28 May. Subsequently, three more weekly thrips counts were preformed to record thrips numbers (both adults and nymphs). From each plot (35 feet by 4 rows), 10 seedlings were visually inspected and numbers were recorded for both adult and immature thrips. In the respective experimental plots, one application of both Orthene® and Vydate® were made as the thrips population was above the recommended threshold level. Insecticides were applied using a hand-held 2-row boom with 40-inch nozzle spacing, flat fan TeeJet XR8003VS nozzles, and 30 psi (resulted in 10 gpa total spray volume). Prior to harvest, plant height and the number/location of 1st-position harvestable and non-harvestable bolls were recorded to evaluate the effect of treatments on plant growth, especially with regard to delayed maturity. Finally, plots were hand harvested from 10 row feet (approximately 20-22 plants) and processed for ginning to obtain the lint yield.

Results and discussion

Cool weather conditions immediately after planting delayed germination/seedling emergence by more than 2 weeks. The first week of thrips sampling indicated the number of thrips in the three seed treatments were significantly lower than the untreated control (Fig. 1A). Thrips numbers in the two foliar treatments are basically pre-treatment counts (first week of sampling; Fig. 1A).

The plots for these two foliar treatments received the first insecticide applications immediately after the pre-treatment count, thus allowing the effect of the foliar application to be observed at the time of the 2nd week sampling (Fig. 1B). Although the number of thrips, especially the adults, during the first week of sampling was lower in the plots with seed treatments than the control, the number of thrips exceeded the recommended economic threshold. This situation would necessitate additional, curative foliar applications on the seed treatment plots. However, limited reproduction, as evident by number of immature thrips, occurred on the seed treatment plots, especially on the Aeris® and

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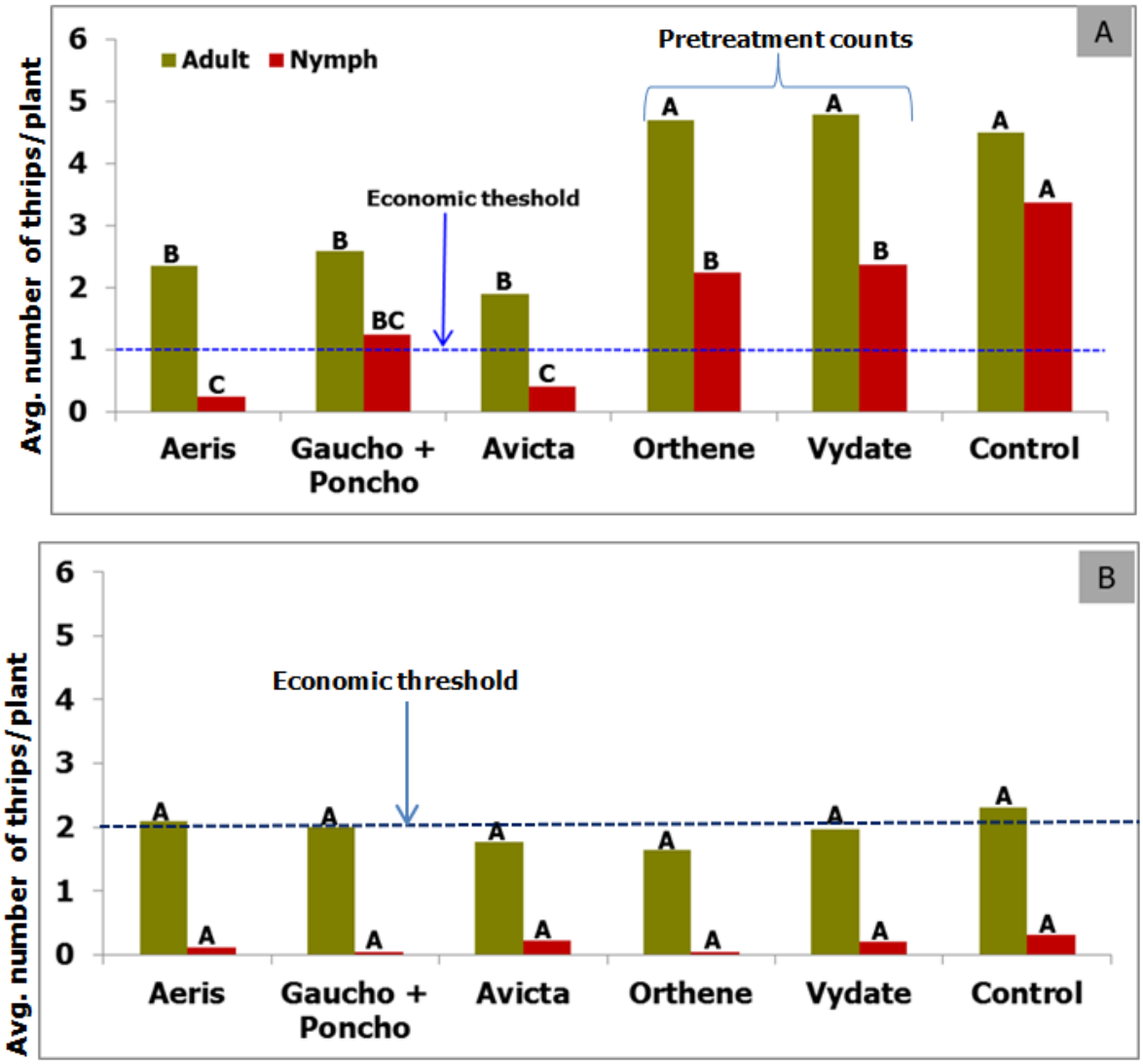
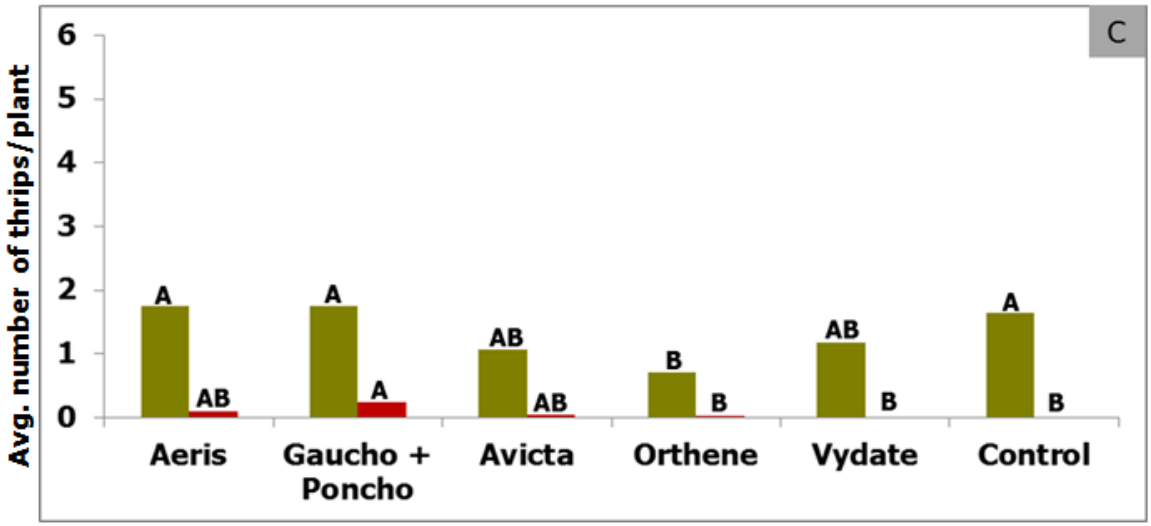
Avicta Complete® as compared to the control plots where more than three immature thrips per plant were recorded on the first week of sampling.



The second week of sampling revealed that the overall thrips numbers in all the treatments were lower than the previous week and the numbers were not statistically different. It appears that the first applications of the two foliar insecticides (Orthene® and Vydate®) were able to reduce the number of thrips considerably (Fig. 1B). We recorded negligible reproduction of thrips during this period, irrespective of the treatments. Usually it is expected that the number of thrips would increase in the untreated control on the subsequent sampling dates. However, we did not see that trend and we speculate that there was no re-infestation of thrips into the study field and likely the weather conditions, such as low temperature and gusty winds, might have prevented the development of thrips during that period of time.



The number of thrips (adults) observed in the seed treatment plots were approximately two per plant, which suggests that the efficacy of the chemicals on the seed treatments was low. The diminishing efficacy of seed treatments at this stage (33 days after planting) is relatively clear. Several studies conducted across the cotton belt have indicated that seed treatments are not highly effective beyond 3-4 weeks after planting. Therefore, if producers encounter situations where insecticide treated seeds are delayed in their germination and seedling emergence, the seed treatments are likely not able to fully protect the plants from thrips. The third week of sampling indicated that the Orthene® applied plots had fewer thrips than the Aeris® and Gaucho®+ Poncho® treatments (Fig. 1C).

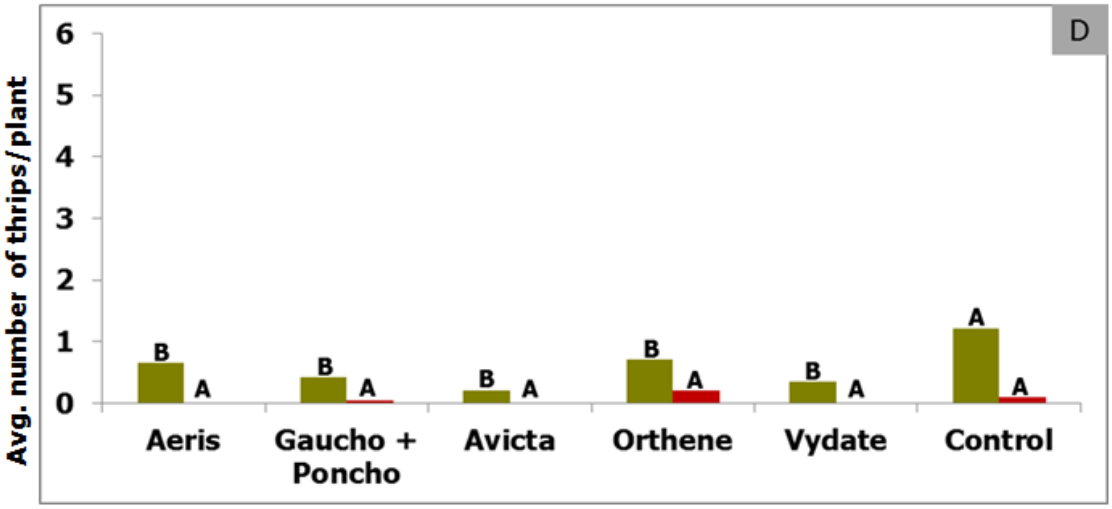


Figure 1. Number of adult and immature thrips per plant at four sampling dates/days after planting (DAP). A. 25 DAP, B. 35 DAP, C. 44 DAP, and D. 51 DAP.

The fourth week of sampling indicated that, except for the control, all treatments had significantly lower number of thrips (<1 thrips/plant; Fig. 1D). By this time, the plants were at the 4-true leaf stage, thus plants were beyond cotton plants' thrips susceptibility window.

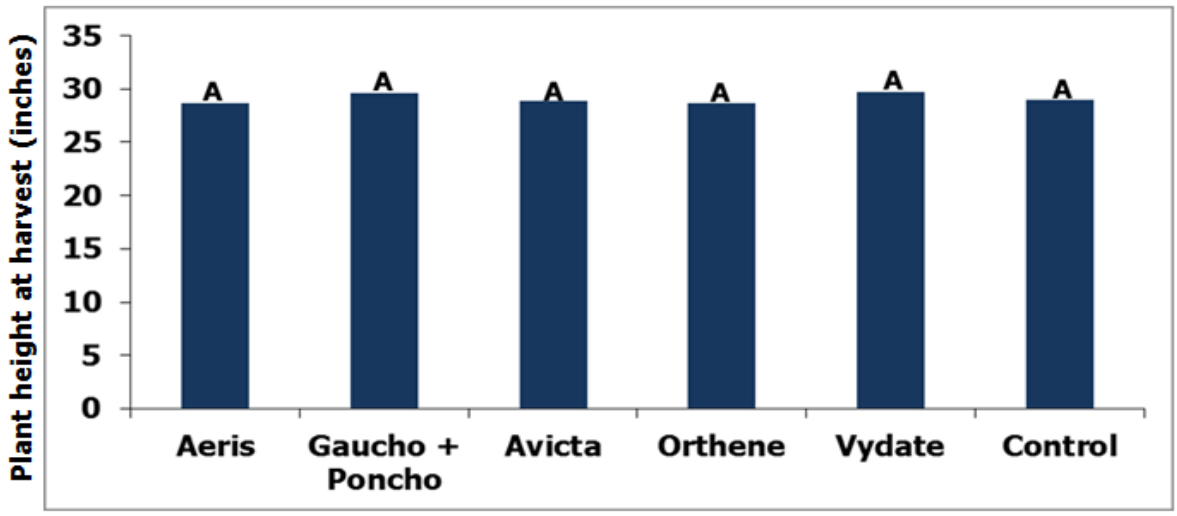


Figure 2. Average plant height (inches) during the pre-harvest crop stage.

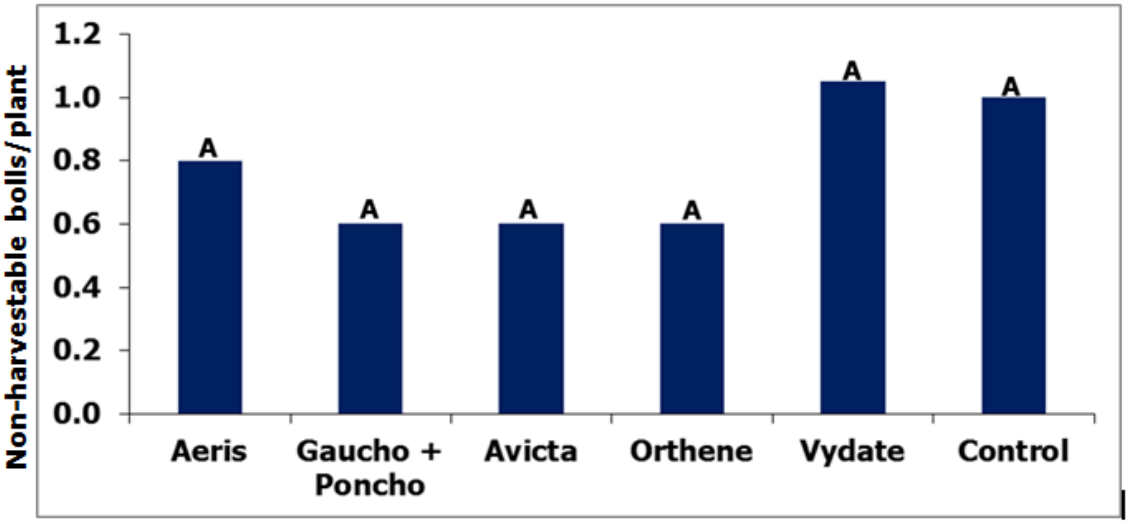


Figure 3. Average number of non-harvestable bolls per plant in different treatments.

Pre-harvest plant mapping indicated that there were no significant differences in plant growth among the different treatment plots, as evidenced by the pre-harvest plant heights (Fig. 2). The number of non-harvestable bolls also did not vary significantly among the treatments, which suggests that there were no differences in crop maturity (Fig. 3).

Although we observed high thrips numbers early in the season, likely these thrips did not colonize fully in order to cause extensive long-term injury. We observed numerical differences between the treatments and control plots in lint yield, but none of the differences were statistically significant (Fig. 4).

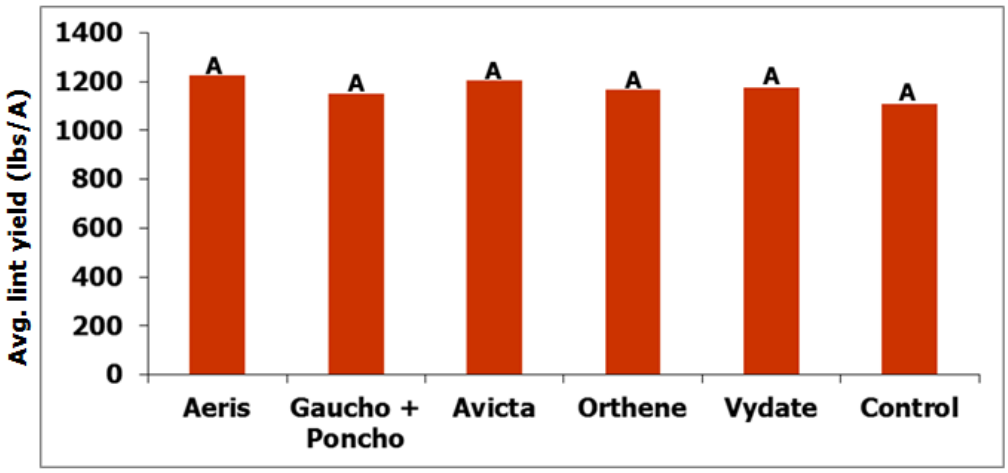


Fig. 4. Average lint yield in different treatments.

Summary

Based on the results from this study, seed treatments appear to be effective in reducing the number of thrips, especially Aeris® and Avicta Complete®, both performed equally in minimizing immature populations. However, realized protection from seed treatments may be less than expected in the event that seeds do not germinate in a timely manner. Foliar application of Orthene®, when thrips populations were above the action threshold, resulted in good thrips control, which means producers can use Orthene® as remedial applications if seed treatments do not provide adequate control. Outcome of thrips injury in terms of delayed maturity and yield reduction can vary from year to year. This is especially true for thrips since plants have enough time to compensate the injury received early in the season provided that the cotton receives good weather conditions, adequate moisture, and protection from late season pests. However, in the Texas High Plains region, growing conditions are typically characterized by periods of low rainfall and could also be limited by cool temperature during the fall, both of which call for attention in early season thrips management to get the plants off to a good start. This study will be repeated next year in multiple locations to hopefully observe the variation in crop response to thrips injury. Additionally, we will be recording the thrips species composition in our studies to understand if there are any relationships in efficacy of these seed treatments with specific thrips species.

Future directions

Results from this study indicate that all the three insecticide seed treatments are equally effective on the thrips population in the study location. Comparing our results with the results from other similar studies conducted in the mid-south (Louisiana, Mississippi, Arkansas, and Tennessee) regions, it appears that the suspected "resistance to thiamethoxam" found in mid-south is not present in High Plains. The thrips species involve in "resistance to thiaomethoxam" is tobacco thrips, *Frankliniella fusca*. In contrary, the predominant thrips species in Texas High Plains is believed to be western flower thrips, *Frankliniella occidentalis*. Our future approach would be to document thrips species composition and conduct the same seed treatment trial in different regions of Texas, where there is difference in thrips species composition. That will provide us the information whether the tobacco thrips in Texas is "resistant to thiamethoxam" or not. We would also try to include other variables such as soil type, irrigation level and planting date to evaluate the insecticide seed treatment efficacy on thrips.

Long-term Survey of Bollworm Moth Flight Activity and Pyrethroid Resistance Monitoring in the Texas High Plains

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INTRODUCTION

The Texas High Plains is recognized as the most intensive cotton growing area in the world. In this region, the bollworm is classified as an important economic pest of cotton. Seed from genetically modified cotton is available with Bollgard II (*Bt*) technology which provides excellent crop protection from lepidopteran pests. Continued bollworm population monitoring is important because of the significant cotton acreage that is not planted with this technology, particularly on reduced-input dryland which accounts for approximately 50% of the cotton acreage. In addition, the percentage of lower-input dryland cropping is increasing as irrigation capacity is steadily decreasing across the region.

Trapping Studies. In 2002, an ongoing trapping study was initiated to investigate the weekly flight activity patterns of the cotton bollworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), and beet armyworm, *Spodoptera exigua* (Hübner) in the southern Texas High Plains region. Since the pyrethroid resistance study discussed below was conducted only for cotton bollworms, the trapping/flight data discussed in this report will also be limited solely to bollworms. Insect traps (Hartstack pheromone trap; Fig. 1) were used to measure the seasonal abundance and flight profiles of cotton bollworm adult males as they responded to baited pheromone traps.

Bollworm Pyrethroid Resistance Monitoring. Beginning in 2007, the Lubbock Texas AgriLife Cotton Entomology Program began cooperating in a multi-state cotton bollworm pyrethroid resistance monitoring study. Please refer to Musser et al. (2013) for a comprehensive review of the entire Beltwide Resistance Monitoring Program (2007-2012 time period). In this report, only the portion from the Texas Southern High Plains region will be highlighted.

Figure 2. Stanley Carroll servicing a Hartstack pheromone trap containing male cotton bollworm moths (left panel). After counting the moths for the flight profile portion of the study, the freshly captured moths were placed individually into glass vials (right panel) of two types, clean 20-ml vials (untreated controls) or vials treated with a concentration of 5-µg/vial of cypermethrin (diagnostic dose).

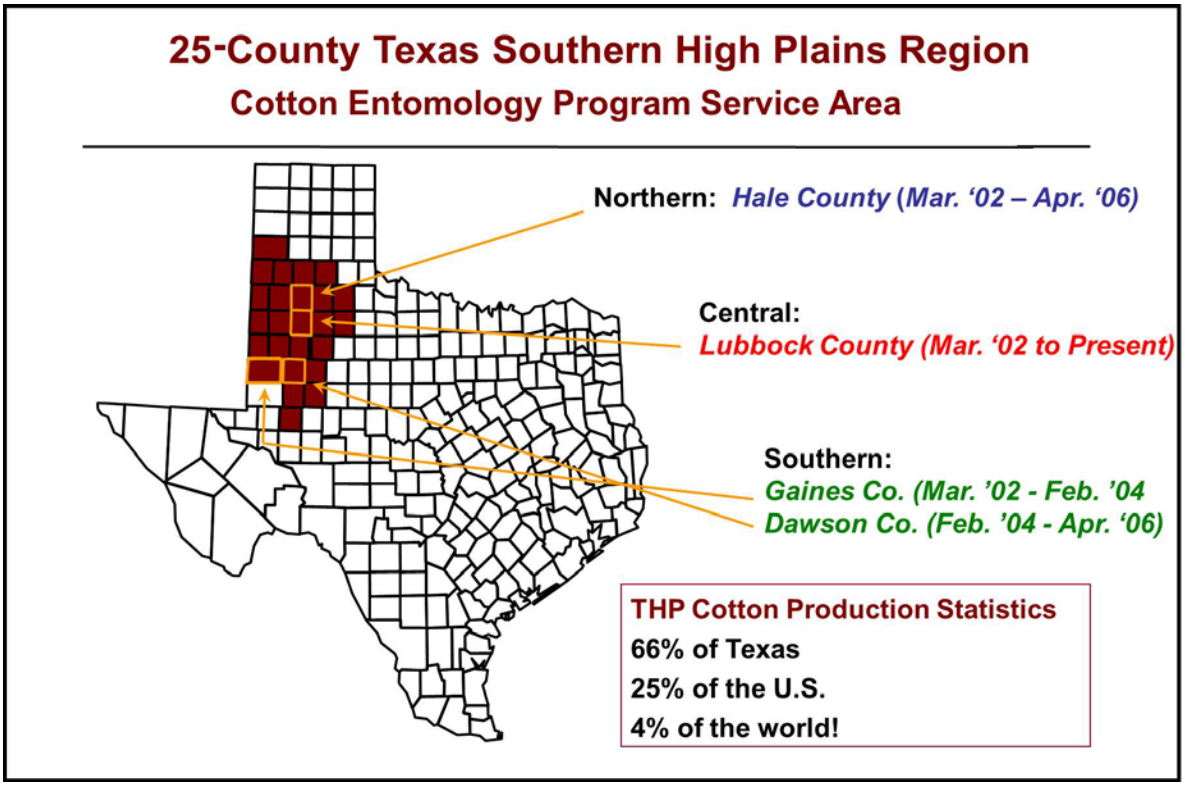

Figure 2. Selected counties and trapping durations for a pheromone trapping study conducted in the Southern Texas High Plains to investigate the seasonal moth flight patterns of cotton bollworm, 2002-2013.

MATERIALS and METHODS

Cotton Bollworm Trapping.

Study Duration: March 2002 to Present

Study Sites: Hale, Lubbock and Gaines/Dawson counties, Texas (Fig. 2)

Sampling Protocol:

- Three traps (Hartstack et al. 1979) baited with bollworm pheromone lures were placed in each of the selected counties representing the northern, central, and southern areas of the Texas High Plains (Fig. 2). Traps located in Gaines Co. were moved to neighboring Dawson Co. after the first year of the study to facilitate more frequent monitoring. Trapping sites within a county were geographically separated by a minimum distance of 5 miles.
- Traps were monitored throughout the year at intervals of approximately one week during active flight periods (spring, summer, fall) and bi-monthly during periods of low flight activity (winter).
- Cotton bollworm specific pheromone lures were replaced at two-week intervals on the traps.

Cotton Bollworm Pyrethroid Resistance Monitoring.

Study Duration: 2007 to Present

Study Sites: Lubbock County, Texas

Sampling Protocol:

- Freshly captured healthy male moths were taken from pheromone traps located at three Lubbock County sites (same sites as described above) and after return to the lab, placed into either clean 20-ml scintillation vials (untreated controls) or identical vials treated at Dr. Fred Musser's laboratory (Mississippi State University) with a concentration of 5-µg/vial of cypermethrin (diagnostic dose) (Fig.1).
- Moth survival/mortality was monitored 24-hr later for the moths held in both untreated control vials and cypermethrin treated vials. Moths capable of controlled flight were counted as "alive", while dead and/or those unable to fly were classified as "dead".
- Moth survival observed from treated vials was corrected for control mortality as reported by Abbott (1925).

RESULTS

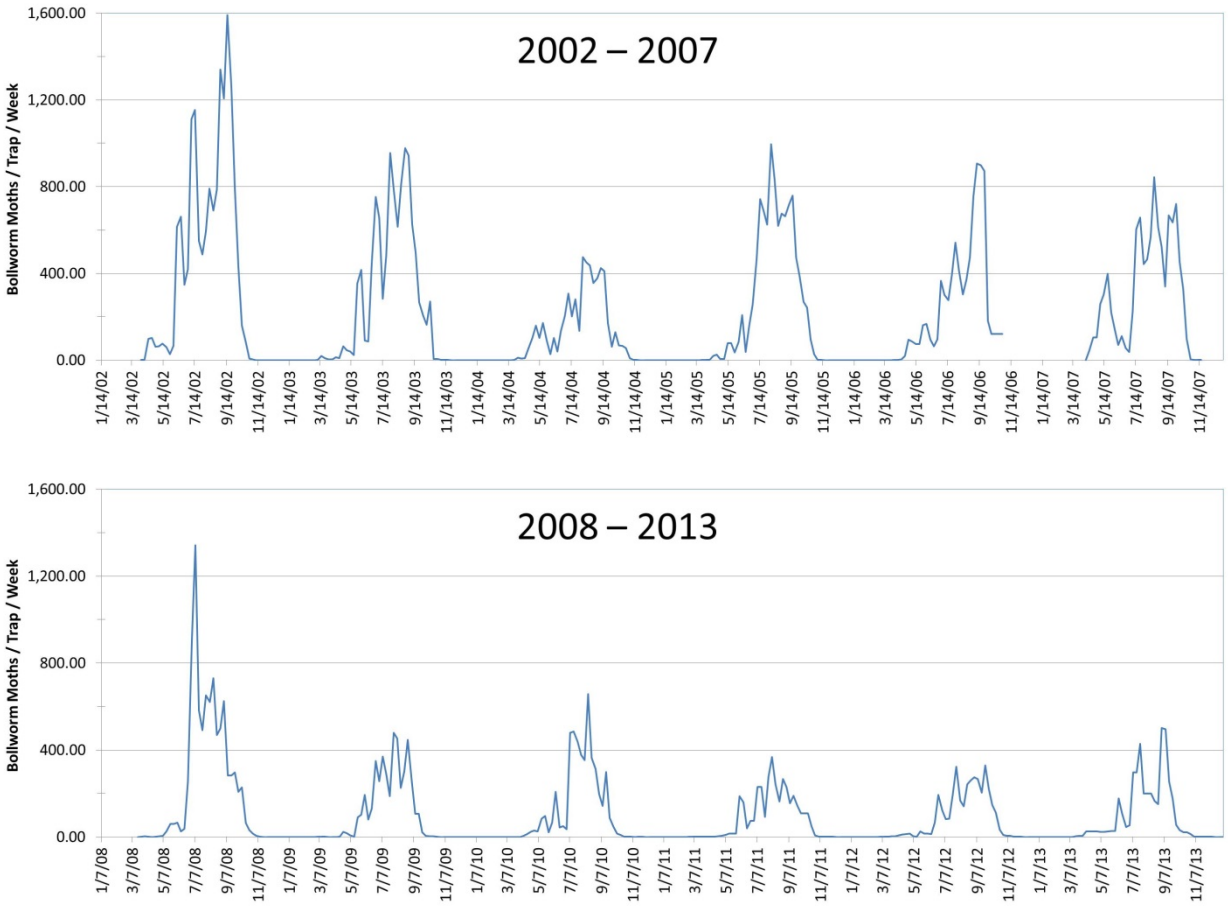

Figure 3. Annual seasonal flight profiles based upon average weekly cotton bollworm moths captured in pheromone traps positioned in rural cotton producing areas of Lubbock County, TX. 2002-2013.

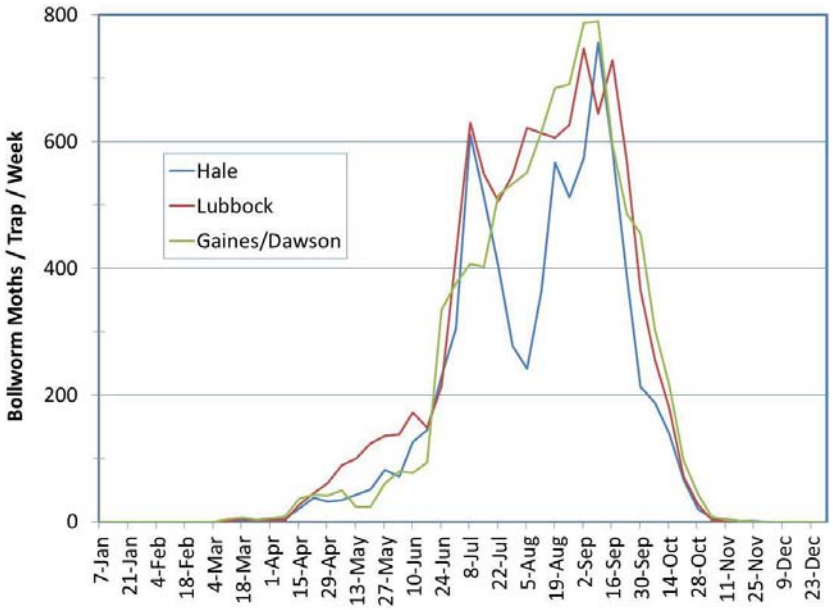

Figure 4. Cotton bollworm historical flight profiles (each colored-coded line represents the mean weekly trap captures averaged across four years). Multiple county flight profiles are shown so that comparisons can be made for areas roughly representing the northern (Hale), central (Lubbock) and southern (Gaines/Dawson) regions of the Texas Southern High Plains. 2002-2005.

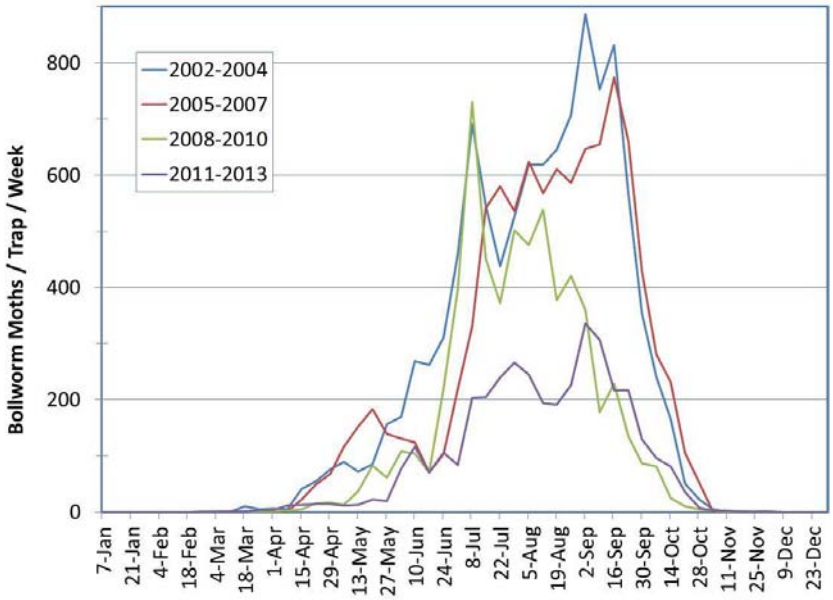

Figure 5. Average number of bollworm moths/trap/week, Lubbock County. The 12 years of male moth flight profiles (see Fig. 3) are grouped into four 3-year profiles representing boll weevil eradication/early Bollgard® adoption period (2002-2004), increased Bollgard® adoption (2005-2007), Bollgard® adoption peak (2008-2010), and the three most recent years (2011-2013).

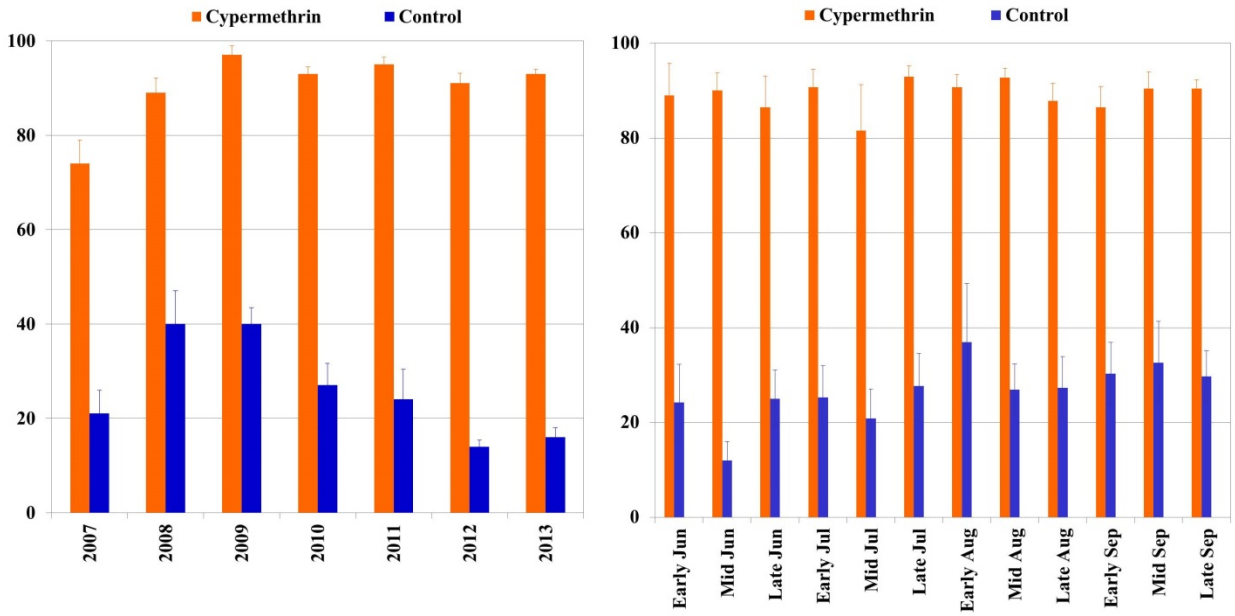

Figure 6. Cotton bollworm moth susceptibility (measure in terms of % mortality on y-axis) to cypermethrin in a vial bioassay, 2007-2013. The data in the left panel show the year-to-year variation in cypermethrin susceptibility of individual bollworm moths into vials treated with 5 µg cypermethrin (treated) and moth mortality in clean vials without cypermethrin (control). The data presented in the right panel show within-season variation in cypermethrin susceptibility of bollworm moths averaged across seven years of the study, Lubbock, TX.

DISCUSSION

Cotton Bollworm Flight Profiles.

Twelve annual bollworm moth flight profiles for bollworms in Lubbock County are shown in Figure 3. Overall, the abundance of bollworms decreased over the study years. With the exception of 2004, bollworm trap captures during the first seven years of the study were noticeably higher than in the last five years. Overall population levels detected in Lubbock County were relatively similar from 2003 to 2007, except for 2004 which exhibited a much reduced population similar to what was observed in later years (2009-2013).

Figure 4 illustrates the calculated historical bollworm flight profiles (based upon pheromone trap captures) for the three counties. Bollworm flight activity in the region was low or non-existent during the period from mid-November to mid-March. An extended period of high bollworm moth activity occurred during the mid-June to mid-October time period which overlays the entire period that cotton is vulnerable to fruit damage. Within this extended period of activity, the highest numbers of moths responded to traps from early August to mid-September.

During the first four years (2002-2005), seasonal flight profiles were monitored in three areas representing northern (Hale County), central (Lubbock County), and southern (Gaines/Dawson counties) regions of the Texas High Plains. Although individual yearly flight profiles can vary greatly, Figure 4 clearly indicates that when averaged across several years, the flight profiles from the different north-south regions of the THP do not differ greatly in timing and/or magnitude of peak bollworm flight activities.

Figure 5 illustrates four 3-year bollworm flight profiles roughly representing the years immediately following boll weevil eradication and the beginning of Bollgard® adoption (2002-2004), the increased Bollgard® adoption years (2005-2007), Bollgard® adoption peak years (2008-2010), and the three most recent years (2011-2013). The most recent three years can be characterized by the presence of continued drought, some crop failure, low crop yields, and decreased irrigation capacity across the region. The flight profiles of the first six years (2002-2004 & 2005-2007) started earlier (early April), lasted longer, and had later and larger peaks of activity than the 2008-2010 flight profile. The average moth flight profile from the last three years (2011-2013) clearly indicates the bollworm flight activity started much later and had relatively low numbers of bollworms responding to traps.

Bollworm Pyrethroid Resistance Monitoring.

Bollworm moths in the Texas High Plains, specifically the Lubbock County populations, were highly susceptible to 5 µg cypermethrin in the vial bioassay, with 90-97% mortality in 6 of the 7 years of the study; the 2007 study showed an average of 74% seasonal mortality (Fig. 6).

Although vial bioassays were performed on fresh moths collected within a 24-hour trapping period, control vials had 20-40% mortality. Corrected mortality (Abbott 1925) due to cypermethrin (5 µg) ranged from 80 to 93%.

Averaged over 7 years, within-season mortality of cypermethrin-treated moths did not vary significantly. Mortality values fluctuated around 90% throughout the season, except for mid-July populations that showed about an 80% mortality (Fig. 6).

ACKNOWLEDGMENTS

Funding for this study was provided by Texas A&M AgriLife Research Cotton Entomology Project 8810, Cotton Incorporated Core Program, and Plains Cotton Growers, Inc., Lubbock, TX.

The bollworm cypermethrin resistance monitoring portion of this poster is a subset of a multi-state, multi-year Beltwide program. All cypermethrin-treated and control vials used in this bioassay were furnished by Dr. Fred Musser of Mississippi State University.

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Compensation of *Lygus hesperus* Induced Preflower Fruit Loss in Cotton

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ABSTRACT A 3-yr field study quantified the compensatory ability of cotton (*Gossypium hirsutum* L.) to preflower fruit damage by *Lygus hesperus* Knight in the Texas High Plains under limited irrigation. Experiments were designed to achieve varying levels of preflower fruit loss by augmenting *Lygus* bug populations using nymphal bugs reared in a laboratory colony. Treatments included 1) three bugs per plant (3PP), 2) one bug per plant (1PP), 3) naturally occurring background bug density or untreated control (NC), and 4) 0 bugs achieved through insecticide spray applications (SC). *Lygus* release treatments (3PP and 1PP) were initiated at early fruiting (squaring) and repeated weekly for a total of three consecutive weeks. Two levels of *Lygus* bug infestations, one insect per plant (1PP) and three insects per plant (3PP), inflicted fruit loss percentages of 24–38 during the maximum fruit set period. Observations on the number of fruit lost at the crop preharvest stage indicate that plants receiving the 3PP and 1PP treatments exhibited higher ability to restrain physiological fruit loss when compared with the two control treatments (NC and SC). Cotton plants could not fully compensate the yield loss because of fruit damage caused by *Lygus* bugs at the observed level of damage. The total lint yields in the 1PP and 3PP treatments were 114 and 118 kg/ha lower, respectively, compared with that in treatment SC. The reduction in yield was primarily because of the loss of first fruiting position bolls. However, lint yields from bolls other than first position of the cotton plant were similar across treatments. Fiber quality data indicated an increase in fiber length from insect release treatment plants compared with the two control treatments.

KEY WORDS cotton, plant compensation, *Lygus hesperus*, insect damage, lint yield

Plant compensation to herbivore damage has been documented in several plant–insect studies (Sadras 1995, Rosenheim et al. 1997, Gavloski and Lamb 2000, Pilson and Decker 2002, Blatt et al. 2008, Wise et al. 2008, Lu and Ding 2012). Understanding the compensatory ability of any crop has special significance as it can quantify the economic importance of the associated herbivorous insect pest (Brook et al. 1992, Bednarz and Roberts 2001, Rosenheim et al. 2006). Crop response as a result of herbivore damage has a direct bearing in determining the pest status and economic threshold level of any pest, and the formulation of integrated pest management (IPM) strategies (Fitt 1994, Wilson et al. 2003).

In response to herbivore damage, plants could use several mechanisms to compensate for fruit loss. A cotton plant (*Gossypium hirsutum* L.) may respond to insect damage by responding actively or passively, also by responding instantaneously or in a time dependent manner (Sadras 1995). The result of this compensatory response could be increase in fruit set, increase in number of fruiting sites, setting of heavier fruits, and increased rate of late flowering (Sadras 1995). One can consider these plant parameters to measure plant compensation after damage by an insect pest.

Literature has shown that in compensation studies, damage to the plant is either inflicted by release of an insect pest of interest (Mulrooney et al. 1992, Holman and Oosterhuis 1999), by simulated mechanical damage (Sadras 1996, Lei 2002, Herbert et al. 2006), or by using growth regulating hormones (Pettigrew et al. 1992). In cotton, research on compensation ability after damage by thrips (Terry 1992, Sadras and Wilson 1998), larvae of *Heliothis* complex (Mann et al. 1997, Holman and Oosterhuis 1999), aphids (Rosenheim et al. 1997), and to some extent the plant bug (*Lygus* sp.) (Leigh et al. 1988, Teague et al. 2001, Teague et al. 2002) has been conducted in the past. Past research shows variable results depending on the method used, so, it is still not clear if simulated mechanical damage is equivalent to damage by actual insect pests. Involvement of chemical factors such as enzymes, toxins, or other hormonal substances is absent in the case of simulated damage (Stewart and Sterling 1988, Burden et al. 1989, Holman and Oosterhuis 1999, Wilson et al. 2003).

It is evident from previous findings that fiber yield compensation in cotton is influenced by regional environmental conditions (Trumble et al. 1993, Rosen-

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heim et al. 1997, Bednarz and Roberts 2001). Studies conducted on cotton compensation to fruit damage in different regions of the U.S. Cotton Belt reported that the cotton crop has the ability to sustain a low level of insect damage (15–20% fruit loss) without significant economic yield loss. The regions were as follows: Arkansas (Holman and Oosterhuis 1999, Teague et al. 2001), North Carolina (Jones et al. 1996), South Carolina (Mann et al. 1997), Virginia (Herbert et al. 2006), New Mexico (Pierce 2006), Louisiana (Holman 1996), and California (Montez and Goodell 1994). A similar study was conducted by Baugh et al. (2003) in the Texas High Plains, which produces ≈70% of the cotton grown in Texas. After manual removal of first position fruit during the preflowering growth stage, results indicated that irrigated cotton could compensate for 20–40% fruit loss without reduction in yield or delay in crop maturity. Among different plant parameters in measuring compensatory ability of cotton after fruit loss, fiber yield and fiber quality are most commonly considered (Rosenheim et al. 1997).

The aforementioned examples clearly indicate that the cotton plant has the ability to compensate for insect pest related fruit damage, but the extent of yield compensation is dependent on the crop growth stage during damage, available water and fertility resources, growing conditions, and the nature of damage inflicted. Therefore, in measuring the compensation potential of the cotton crop to insect damage and resulting yield loss, all these factors should be taken into consideration. In this study, focus was on the compensation of cotton to fruit damage at the preflowering stage following in situ *Lygus hesperus* infestations under typical Texas High Plains growing conditions that are often characterized by short growing seasons, hot summers, and limited precipitation.

Materials and Methods

The experiment was conducted at the Texas A&M AgriLife Research and Extension Center farm, Lubbock, TX during 2005, 2006, and 2007. In 2005 and 2006, seeds of Paymaster cotton, 'PM 2326 RR,' were planted on 20 May and 15 May, respectively. In 2007, seeds of Stoneville cotton, 'ST 4554 B2RF,' were planted on 15 May. Because a few incidences of bollworm damage in the experimental fields during the 2006 growing season were encountered, the next year (2007), it was decided to use ST 4554 B2RF, a Bt-transgenic cultivar, to proactively eliminate possible damage of cotton fruits from the bollworm–budworm complex. In-furrow aldicarb at 850 gm ai/ha was applied at planting and acephate at 204 gm ai/ha was sprayed onto the seedlings during the third week after planting to protect the seedlings from thrips damage. Approximately 130,000–162,000 seeds/ha were planted in a furrow-irrigated field fertilized with nitrogen at 90 kg/ha. In each year, the irrigation amount and frequency consisted of four irrigation events with ≈7.6 cm of water applied per irrigation and deployed at 8–10 d preplant, peak squaring, peak flowering, and at crop cutout when plants reached four to five nodes above white flower.

Table 1. Plant mapping, insect release, and insecticide spray activities during a 3-yr cotton compensation study, 2005–2007, Lubbock, TX

Year	Activities	Dates
2005	Plant mapping	6 July, 13 July, 20 July, 27 July, 6 Aug.
	Insect release	7 July, 14 July, 21 July
	Insecticide spraying	28 June, 5 July, 22 July, 29 July
2006	Plant mapping	28 June, 5 July, 13 July, 22 July
	Insect release	29 June, 6 July, 13 July
	Insecticide spraying	23 June, 3 July, 7 July, 23 July
2007	Plant mapping	10 July, 17 July, 24 July, 2 Aug.
	Insect release	11 July, 18 July, 24 July
	Insecticide spraying	6 July, 11 July, 18 July, 24 July, 15 Aug.

The Insects. The necessity of having large numbers of *Lygus* bugs of similar life stage at one time warranted establishment of a reliable insect rearing facility. A nucleus culture of *L. hesperus* was obtained from the Western Cotton Research Laboratory, USDA-ARS, Phoenix, AZ during early 2005, and was augmented with locally collected *L. hesperus* to maintain the colony's vigor and to increase the colony size. Initially, all life stages were fed with a premixed artificial diet procured from Bio-Serv (Frenchtown, NJ) to establish the insect colony. After that, the diet was prepared in-house using the *L. hesperus* diet recipe described by Debolt (1982) during the remainder of the study period. All life stages were reared inside growth chambers (model 818; Precision, Winchester, VA) programmed for a constant temperature of 26 ± 2°C, 40–50% RH, and a photoperiod of 14:10 (L:D) h. Under these conditions, *L. hesperus* completed a life cycle in 28–30 d.

Treatments and Insect Release. There were four treatments: 1) Plots receiving three *Lygus* bugs per plant (3PP) per release. 2) Plots receiving one *Lygus* bug per plant (1PP) per release. 3) Natural control (NC), plots were not sprayed and no bugs were released. 4) Sprayed control (SC), plots were sprayed at weekly intervals for 4 wk (starting from the day of the first insect release treatment) with acetamiprid at 30 gm ai/ha to exclude other sucking insect pests. Three releases of *L. hesperus* nymphs (second-third instar) were made in consecutive weeks during the preflowering (first 4 wk of squaring) stage of the crop. Plots were 15.24 m in length by eight 1.01-m spaced rows. Insect release and data collections were performed in two adjacent 3.04-m-row sections within each plot. The details of the release periods and associated observations are shown in Table 1.

Three consecutive insect releases were made when the crop reached the desired plant-growth stage. Before insect release, 3.04-m sections were flagged in the two middle rows of each plot. Each section contained ≈30–35 plants (60–70 plants per plot). Insects were released during morning hours (6:00–9:00 a.m.) to avoid extreme afternoon temperatures that could hamper initial insect establishment. Small, plastic snap boxes were used to carry the insects to the field and insect(s) were placed on plant's upper terminal by

using a camel hair artist's brush. Care was taken to keep the insect(s) in place on the plant surface until they could establish footing.

Experimental Design, Observations, and Data Analysis. The four different treatments (SC, NC, 1PP, and 3PP) were assigned randomly to plots in a completely randomized design with four replications. Each year the experiment was conducted in a different field of the research farm. Plant mapping is a reliable method to record plant response to damage by insect pests or management practices (Constable 1991). Detailed plant mapping was conducted during the active growing season (in-season plant mapping) and before harvest (preharvest plant mapping) to measure the number of fruits that were retained, lost, open, and nonharvestable (bolls that are hard-locked or not fully opened) on every fruiting terminal. Plant height and number of nodes were recorded during both observation periods. The two-row sections of each plot later were hand harvested and ginned to obtain yield parameters. Physical properties of lint samples were analyzed through the high volume instrumentation system at the Fiber and Biopolymer Research Institute, Lubbock, TX. Data were analyzed using a two-way factorial (split-plot) analysis where year was considered as main plot and treatment as subplot. The year × treatment and year × replication interactions were considered random effects, whereas treatments were fixed effects using PROC MIXED (SAS Institute 2003). Denominator degrees of freedom for each test of treatments were determined by the Satterthwaite option. Treatment means were separated by Fisher's least significant difference at the $\alpha = 0.05$ level.

Results

Plant response toward the release of the *Lygus* bugs during preflowering stage of cotton was measured at two distinct crop stages: one after each of the three insect releases, referred to as "in-season plant mapping," and another immediately before harvesting, termed "preharvest plant mapping." At each sampling event, overall plant response based on plant height, number of nodes, number of intact fruits, and number of fruits shed was recorded. Results on total lint yield and fiber quality parameters in the different treatments also are reported.

In-Season Plant Mapping. It was observed that average plant height in the insect released treatments (1PP and 3PP) was significantly different ($F = 4.55$; df = 3, 42; $P < 0.007$) from both of the two control treatments (Fig. 1A). Plants in insect release treatments were at ≈7–8 cm shorter than control plants during the growth stage. However, the average number of nodes per plant was not significantly different ($P = 0.77$) among the treatments (Fig. 1B). The number of squares (flower buds) intact on a cotton plant determines the potential yield. The average number of intact squares on each plant after all three insect releases indicated that *Lygus* bugs significantly reduced ($F = 20.58$; df = 3, 42.1; $P < 0.001$) square retention (Fig. 1C). There was a difference of about four squares between SC and 3PP treatments (Fig. 1C). Considering total number of fruiting positions (all potential fruiting sites) per plant across all the treatments, it is evident from the results that there was no significant difference ($P = 0.51$) in average number of fruiting positions regardless of treatment received (Fig. 1D). The plants in all four treatments behaved equally in terms of fruit bearing potential, which was in the range of 12–13 fruiting positions per plant (Fig. 1D). Percent fruit loss provides an estimate of potential yield loss and a yardstick for management decisions. It was observed that the *Lygus* bug at the present release rate had a significant effect ($F = 73.56$; df = 3, 42.1; $P < 0.001$) on fruit loss during the early growing stage of a cotton plant (Fig. 1E). Three releases of three *Lygus* bugs per plant (3PP) caused 38.66% fruit loss against a physiological fruit loss (SC) of 7.27% (Fig. 1E). Thus, net fruit loss because of the *Lygus* bug was 31 and 17% in treatment 3PP and 1PP, respectively.

Preharvest Plant Mapping. Average plant height in the four treatments ranged from 57 to 60 cm (Fig. 2A) with no significant differences ($P = 0.11$) among the treatments. Average number of nodes per plant at preharvest was significantly different ($F = 12.61$; df = 3, 33; $P < 0.001$) among the treatments (Fig. 2B). Plants in the insect release treatments (1PP and 3PP) had significantly more nodes than that in the SC treatment. A difference of at least one node per plant was observed when comparison was made between the 3PP (15.81 nodes per plant) and SC (14.23 nodes per plant) treatments. Observation on average number of open bolls per plant indicated that plants in the SC treatment (8.12 open bolls per plant) had significantly more ($F = 5.13$; df = 3, 33.1; $P = 0.005$) open bolls per plant than the plants in the rest of the treatments (Fig. 2C). Average number of lost fruits, which could have potentially contributed to yield, was significantly higher on plants receiving insect release treatments than the control treatments (Fig. 2C). Number of nonharvestable bolls indicates that the bolls did not have enough time to mature and open. Average number of nonharvestable bolls per plant was higher ($F = 4.80$; df = 3, 42.1; $P = 0.005$) in the NC, 1PP, and 3PP treatments than in the sprayed control (SC) (Fig. 2C).

Lint Yield. Total lint yield is a quantitative measure of compensation by the cotton crop following damage by insect pests. There was a significant effect of insect augmentation treatment on total lint yield ($F = 5.8$; df = 3, 33; $P = 0.003$). When total lint yield was considered, SC treatment resulted in a significantly higher yield (1,256.5 kg/ha) compared with 1PP and 3PP treatments, which differed by 114.0 and 118.2 kg/ha, respectively (Fig. 3A). There was no significant total lint yield difference between the SC and NC treatments. The total lint yield was divided further into the contributions from first position fruit versus the remaining boll positions of a plant. First position bolls produced significantly higher ($F = 5.80$; df = 3, 33; $P = 0.002$) lint weights in control treatments (i.e., SC and NC) than two of the insect release treatments (Fig. 3B). The yield differences in NC, 1PP, and 3PP

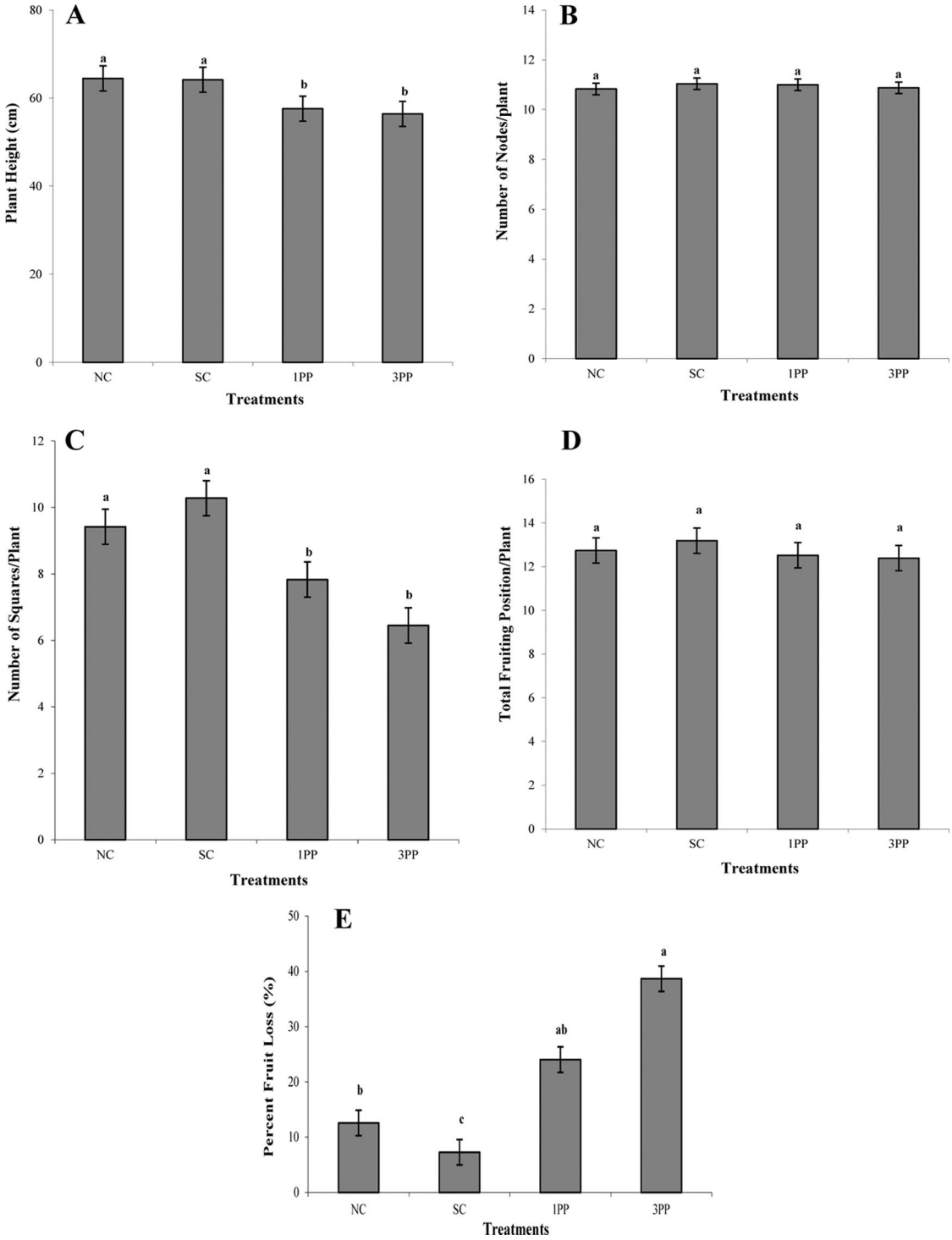

Fig. 1. In-season plant mapping observations as influenced by *L. hesperus* augmentation treatments (SC, spray control; NC, natural unsprayed control; 1PP, one bug per plant; 3PP, three bugs per plant): plant height (A), number of nodes per plant (B), number of squares per plant (C), total fruiting positions per plant (D), and percent fruit loss (E). The data value bar represents the treatment mean and the error bar represents (±) standard error of the mean. Treatment means listed with the same letter are not significantly different ($P = 0.05$).

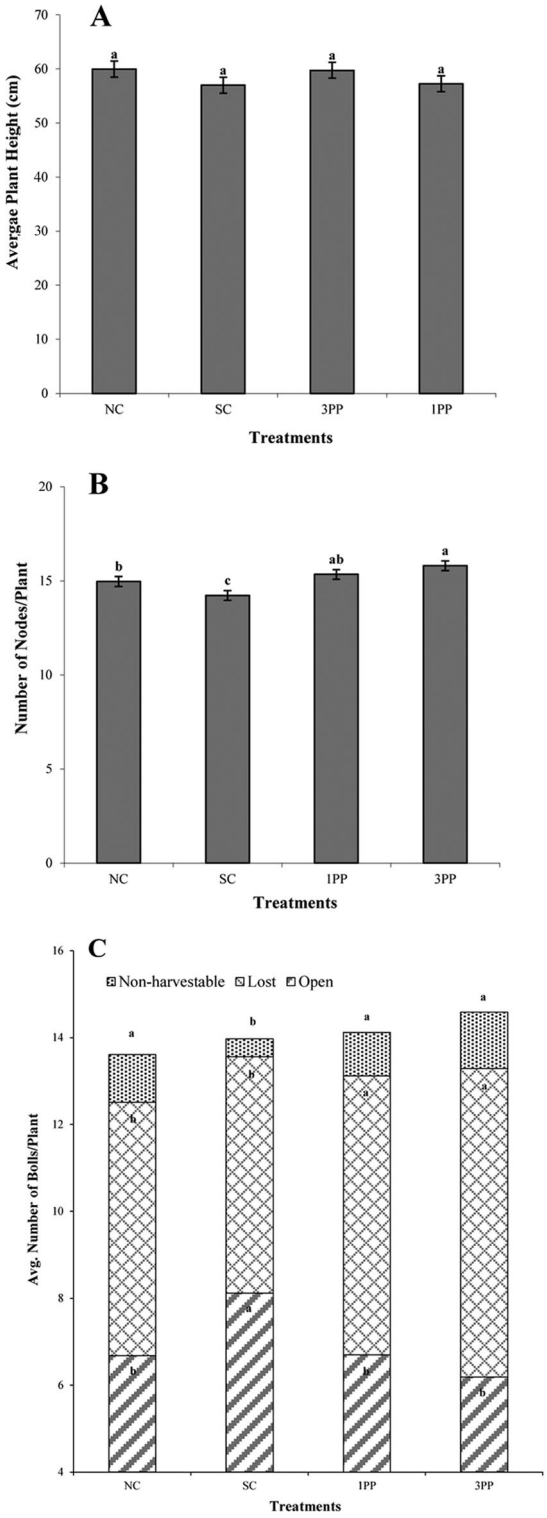

Fig. 2. Preharvest plant mapping observations as influenced by *L. hesperus* augmentation treatments (SC, spray control; NC, natural unsprayed control; 1PP = one bug per plant; 3PP, three bugs per plant): plant height (A), number of nodes per plant (B), and average number of open, lost, and nonharvestable bolls per plant (C). The value bar represents treatment mean and the error bar represents (±) standard error of the mean. Treatment means listed with the same letter are not significantly different ($P = 0.05$).

treatments from SC treatment were 43.0, 138.3, and 127.9 kg/ha, respectively. Lint yield from bolls other than first position of the cotton plant in all the treatments did not show significant difference ($P = 0.55$). This yield indicates that bolls from other than first position fruit in each treatment produced similar amounts of lint (Fig. 3C), which ranged from 212.9 (NC) to 255.9 (1PP) kg/ha.

Lint Quality. Several quality parameters were measured on lint from all four treatments. Statistical results on five parameters (micronaire, length, uniformity, strength, and elongation) are shown in Table 2. Among the five parameters, only fiber length was found to differ significantly among the treatments (Table 2). Statistically, the fiber length of the two treatments receiving insect releases was higher ($F = 5.43$; df = 3, 33; $P = 0.003$) than the two control treatments. Although not significant at $\alpha = 0.05$, fiber strength also appeared to be marginally influenced ($P = 0.06$) by insect-induced fruit loss that resulted in compensatory lint. Because fiber length and fiber strength are weakly but positively correlated (Kloth 1998), these two parameters were influenced similarly by the insect augmentation treatments.

Discussion

This study provides a foundation for understanding the compensation ability of cotton to damage by the *Lygus* bug during the crop's preflowering stage. The two regimes of insect pressure, one insect per plant (1PP) and three insects per plant (3PP), mimic two different population densities (low and high densities, after field survivorship is considered) of this pest in cotton fields, and have been adequate to allow successful quantification of the extent of damage corresponding to two different population densities.

Among the plant parameters, plant height and number of nodes indirectly explain plant response to herbivore damage. During in-season plant mapping, there was a reduction of plant height by 6–8 cm in the insect release treatments compared with natural control (Fig. 1A). However, no significant change in the number of nodes was observed among the treatments (Fig. 1B). These in-season observations on plant height and number of nodes indicate that although there was a short period of slow growth in plants receiving insect release treatments, the plants did not exhibit this difference in terms of number of mainstem nodes. However, preharvest plant mapping data show that the plant height was not significantly different (Fig. 2A), but there was an increase of at least one node in the plants receiving insect release treatments (Fig. 2B). These results indicate that the cotton plants might have responded to fruit loss because of *Lygus* damage by increasing nodes toward the end of the growing

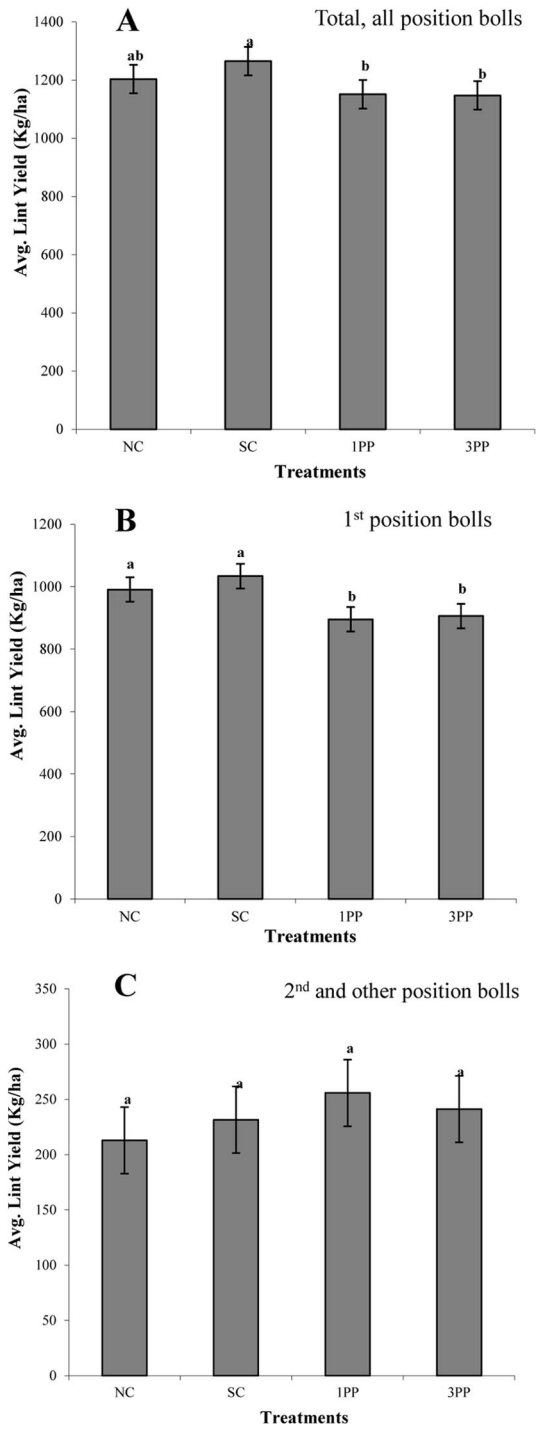

Fig. 3. Cotton lint yield (kg/ha) as influenced by various *L. hesperus* augmentation treatments (SC, spray control; NC, natural unsprayed control; 1PP, one bug per plant; 3PP, three bugs per plant): total lint yield (A), lint yield from first-terminal position (B), lint yield from other than first-terminal positions (C). The data value bar represents the treatment mean and the error bar represents (±) standard error of the mean. Treatment means listed with the same letter are not significantly different ($P = 0.05$).

period. In the U.S. midsouth, plant height and number of nodes normally increase with increased plant bug damage, but the harsh growing conditions (hot and dry with low precipitation) of the Texas High Plains may be attributed to a slower in-season growth of the insect-infested plants in our study (Hanny et al. 1977).

Insect damage can be quantified by comprehensive observations on the fruiting profile of a plant. Observations on total number of fruiting positions during the growing season (in-season plant mapping) show that plants in all four treatments had equal ability to bear fruits; that is, plants did not grow differently in terms of its fruit bearing capacity (Fig. 1D). Because the plants in all the treatments had an opportunity to bear an almost equal number of fruits per plant, this served as a baseline to measure the effect of insect releases in terms of lost or intact fruit number. During in-season plant mapping, it was observed that an average of 4.6 and 2.8 fruits per plant were lost in 3PP and 1PP treatments, respectively. This fruit loss could have been saved if plots were treated with insecticide. This is an important piece of information for pest management strategies, particularly in developing an IPM module.

One indicator often used in deciding the economic threshold level for cotton insect pests is the percent fruit retention during the crop growing season. For example, under Texas High Plains conditions, it is commonly recommended to apply an insecticide treatment if cotton square retention drops below 75% after the third week of squaring (Layton 2000). Similar levels of square retention have been adopted by other states such as Arizona, California, and North Carolina in their cotton pest management strategies (Layton 2000). In this experiment, we established two distinct fruit retention (conversely subtracting percent fruit loss from 100; Fig. 1E) levels (76% for 1PP and 61% for 3PP). We believe that this is one of the few compensation studies where actual insect populations were used successfully to achieve the desired insect-induced damage levels and the first one conducted under Texas High Plains conditions. Similar results also were observed by Teague et al. (2004) when *Lygus lineolaris* (Palisot de Beauvois) was used to establish fruit damage in preflowering cotton to study compensation of cotton grown in Arkansas.

Once the damage level was established in the growing season, we measured the persistence of the effect of insect injury as well as plant response at the preharvest stage. Preharvest plant mapping provides information on the fruiting profile of a cotton plant, which represents the total lint yield. The preharvest plant mapping showed that there were similar numbers of open bolls per plant in all treatments, except in the SC treatment, which recorded approximately two more bolls per plant. It appears that the systematic insecticide spraying during the time of squaring saved some of those first position fruits, which in other treatments had been damaged by released *Lygus* bugs. The insect release treatments showed significantly higher fruit loss than the controls, but the magnitude of difference in the degree of fruit loss between control and

Table 2. Effect of *L. hesperus* augmentation treatments on fiber quality parameters obtained from HVI analysis

Parameters	SC	NC	1PP	3PP	F	P
Micronaire (unit)	4.71a	4.73a	4.67a	4.62a	0.97	0.419
Fiber length (cm)	2.74b	2.74b	2.79a	2.82a	5.43	0.004
Uniformity (%)	82.23a	82.32a	82.16a	82.54a	0.74	0.533
Strength (g/tex)	27.70a	27.70a	27.80a	28.30a	2.64	0.066
Elongation (degree)	7.96a	8.13a	7.91a	7.78a	1.70	0.186

ANOVA; df = 3, 33.

insect release treatments at two different observation periods (in-season and preharvest) was not similar. A significantly greater fruit loss during the growing season in 3PP and 1PP treatment was recorded as compared with its sprayed (SC) counterpart (4.8 and 3.2 versus 1.0 fruit lost per plant) (Fig. 1E). However, in a similar comparison between the insect release treatments (1PP and 3PP) and SC treatment at preharvest stage, the difference became smaller (7.1 and 6.4 versus 5.4 fruit lost per plant) than what was observed previously during the early plant growth stage (in-season plant mapping, Fig. 2C). This result also signifies an important physiological aspect of the cotton crop, the natural or physiological shedding of cotton fruit during its growth period. Once we compared two stages of crop growth, i.e., in-season–preflowering and preharvest–postreproductive, it was observed that in control treatments the initial percent fruit loss was in the range of 7.4–12.7% (Fig. 1E), which increased by 30% (in-season fruit loss minus preharvest measure of seasonal fruit loss) at the end of the growing season (Fig. 2C). As all the experimental plots were protected with insecticides after the insect release treatments, in theory, there should not be any kind of insect pressure between then and harvest. Hence, the 30% of fruit loss after the first plant mapping to preharvest plant mapping in control treatments was not due primarily to the insect damage, but as a result of natural fruit shedding. This result is in agreement with previous findings, where 50–60% natural fruit loss in cotton was reported (Hearn and Room 1979).

Another notable part of this experiment was the quantification of how the cotton plant redistributed its available energy to compensate for early loss of fruit because of insect infestation. As mentioned in previous discussion, even the control treatments (SC and NC) showed a fruit shed of 30% at the preharvest crop stage. If we impart the same magnitude of natural fruit shed in insect release treatments (1PP and 3PP), there would be 55–69% fruit loss per plant in 1PP and 3PP treatments, respectively. But in reality, we observed only 45 and 48% total fruit loss per plant at the preharvest stage, suggesting that plants in those treatments (1PP and 3PP) did not shed as many fruit as the control treatments. Therefore, it is likely a compensation mechanism might be operating in cotton plants where earlier insect-induced fruit loss had driven the plants to not shed relatively more fruit in their later growth stage. It is also possible that insect-induced fruit loss left plants less vulnerable to environmental stresses, and extreme western Texas summer conditions (temperature and low moisture) caused less physiological fruit shedding than that observed on plants holding a higher fruit load during the mid- to late summer. A similar observation was made by Lei (2002) using simulated damage of cotton fruit by *Helicoverpa* spp. larvae. Although there might be other physiological mechanisms operating in cotton plants in this compensation process, the aforementioned mechanism has been the one most commonly documented by other researchers (Brook et al. 1992, Mulrooney et al. 1992, Pierce 2006). It is worth mentioning that as opposed to 30% of fruit shed in control treatments, plants in insect release treatments exhibited only 10–20% during their entire growing period (here fruit shed refers to loss of fruit because of physiological reasons and estimated based on in-season plant mapping). However, the question arises in the process of compensation for natural fruit shed by the cotton plant. Is early fruit loss equivalent to fruit shed at a later stage of plant growth? It is often observed that the natural fruit shed in a cotton plant mostly consists of fruits from second- to fourth-terminal positions, which were shed as either squares or young bolls (Constable 1991). Therefore, the first-position fruits, which eventually contribute more to the final yield, remain intact in control treatments unless there are biotic or abiotic stresses. However, plants in insect release treatments, although they compensate for early fruit loss by reducing later season fruit shed, also compensate for insect-induced fruit loss by adding both vertical and horizontal fruiting nodes.

Overall, there were significant yield differences between insect release and control treatments, which indicates plants in the insect release treatments could not fully compensate for lint yield loss because of insect injury. Although statistically not significant, bolls other than first position in 1PP and 3PP treatments contributed more to the total lint yield as compared with the controls (Fig. 3C). It appears that plants did compensate for the yield loss caused by the first-position fruit loss by retaining more fruits in second and third fruiting positions in insect-infested plants. Our results corroborate the study by Heitholt (1997), which demonstrated that although manual removal of first-position fruits stimulated retention of second-position fruit, that full recovery may not be realized.

We also have observed that total lint yield did not significantly vary between 1PP and 3PP treatments (Fig. 3C), where one would expect significantly higher yield reduction at higher insect density (3PP).

It is not clear why this is the case, but the authors observed activities of predators such as ants and spiders, which could have lowered the effective number of *Lygus* nymphs remaining on the plants after release, compromising the feeding efficiency of the released insects, or both. Usually, when there were more *Lygus* nymphs (as in case of 3PP treatment) released onto a single plant, it might be easier for predators to find at least one or two of those nymphs. This increase may be why we did not observe a significantly higher (in relation to the bug release ratio of 1:3) damage level in our 3PP treatment. A similar record of ant predation on different mirids, including cotton fleahopper [*Pseudatomoscelis seriatus* (Reuter)] has been documented in a previous study (Breene et al. 1990).

One of the main issues regarding plant compensation, particularly in cotton, is the delay in crop maturity. It is especially important where the growing period is constrained by weather conditions and late season insect pest attacks (Gore et al. 2000). In Texas High Plains conditions, cotton crops encounter decreasing heat units beyond the crop maturity stage, plus other weather limitations including high wind, early frost, or both. A delay in crop maturity may lead to problems because of late season pests and also yield loss from adverse weather conditions (Stewart and Sterling 1988). Therefore, if a considerable delay in crop maturity is expected because of insect-induced fruit loss and resulting young compensatory fruits, seasonal length must also be considered while accounting for plant compensatory potential. In our 3 yr of conducting this experiment, we did not observe any significant delays in crop maturity, so we were unable to assess the influence of season length on plant compensatory potential. Another caveat in our research was the use of a different cotton cultivar in study year 2007. We acknowledge that cultivar characteristics could play a significant role in the compensatory ability of cotton. However, for any given treatment, year-to-year variation was not statistically significant, which suggests that the use of different cultivars most likely would have had insignificant impact in the overall result of this study.

Although fruit-feeding insects such as the *Lygus* bug cause direct yield loss in cotton, they could also negatively impact the lint quality parameters—a significant factor in cotton price determination in the world market. In this study, we analyzed all major fiber quality parameters but did not observe any significant differences across treatments, except in fiber length where a significant increase (0.05–0.08 cm) was observed in insect release treatments compared with that in control plots. However, this small difference in fiber length does not significantly impact the overall value of the lint (Bradow and Davidonis 2000). The effect of insect-induced fruit loss on fiber quality generally would manifest from the delayed maturity and shorter seasonal length.

In conclusion, considering the intertwining effect of both biotic (insect pest, predator, plant cultivar) and abiotic (water, heat units, fertility) factors on the ability of cotton to compensate for fruit loss, there is a need to focus on the influence of a specific factor, while holding others constant. This should allow progress toward fully understanding the plant's compensatory potential and its ramifications in pest management recommendations. Cotton is cultivated in a wide geographic area with large variations in rainfall, soil type, and cultivation practices. Variations in production practices (dry land, limited irrigation, full irrigation, narrow-row, minimum tillage, low and high input) and climatic and edaphic parameters would significantly influence the degree of plant susceptibility to insects, as well as plant's ability to tolerate/compensate for insect-induced injury and fruit loss. Through our continuing research, we plan to further clarify the relationships between *Lygus*-inflicted injury to cotton and cotton's compensatory potential under various production scenarios.

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Population Genetic Structure of *Pseudatomoscelis seriatus* (Hemiptera: Miridae) in the Cotton-Growing Regions of the United States

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ABSTRACT The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae) is an economically important insect pest of cotton in the United States. However, reports of cotton fleahopper infestation and its management in cotton fields are restricted primarily to Texas, Oklahoma, and Arkansas. The objective of this study was to understand the genetic diversity of cotton fleahopper populations infesting cotton in the cotton-growing areas of the United States. Amplified fragment length polymorphism markers were used to detect genetic diversity and to characterize geographic genotypes across the distribution of the cotton fleahopper in the United States. We used 172 individuals and 559 amplified fragment length polymorphism loci in this study and found significant, but low, level of genetic differentiation among geographic populations ($F_{ST} = 0.02$; $P < 0.0001$). Molecular fingerprints of cotton fleahopper populations were partitioned into three broad regional genetic populations with a western, central, and eastern distribution. The western (Arizona) and eastern (Florida, Georgia, South Carolina, and North Carolina) populations are genetically distinct, whereas the central (Texas, Oklahoma, Arkansas, Mississippi, Louisiana, and Alabama) population represents an admixed population, which include both western and eastern populations. These results suggest considerable gene flow among the populations within regions but restricted gene flow among populations from eastern and western region.

KEY WORDS cotton pest, AFLP, population structure, gene flow, dispersal

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), is a native insect to the southern United States and northern Mexico (Knutson et al. 2002). In the United States, the cotton fleahopper has a wide distribution, from west (California) to east (North Carolina) and north (Nebraska) to south (Texas) (www.discoverlife.org). The cotton fleahopper is a polyphagous insect and uses >160 different plant species belonging to 35 different families (Esquivel and Esquivel 2009). However, it has been studied mostly in light of its pestiferous association with cotton. Cotton fleahoppers prefer and perform better on native weed hosts than in cotton (Gaylor and Sterling 1976, Holtzer and Sterling 1980, Beerwinkle and Marshall 1999, Barman et al. 2012). This host preference and performance pattern might be indicative of the relatively longer association of this insect with its native wild host plant species than with cotton in the United States.

Cotton as a cultivated crop was introduced to the United States during the 1600s, and subsequent cultivation of cotton expanded to most of the southern states (Lewis and Richmond 1966). The cotton fleahopper was first reported as a pest of cotton during early 1920s (Reinhard 1926). Currently, cotton is commercially grown in 17 states in the United States, out of which 10 have reported cotton fleahopper infestations in cotton, mostly in the southwest and mid-south region (Williams 2011). The extent of crop loss because of cotton fleahopper infestation in these states differs considerably. Cotton in Texas, Oklahoma, and Arkansas are affected the most by cotton fleahopper (Williams 2011), whereas in the remaining states it does not seem to be a problem. Currently, we do not have clear understanding why there would be such drastic variation in the level of cotton fleahopper infestation to cotton in different areas.

Insect pest species with wide geographic distributions and host ranges have been shown to be composed of genetically distinct subpopulations owing to the populations' geographic and host plant affiliation (Brunner et al. 2004, Meng et al. 2008, Seyahooei et al. 2011). Thus, cotton fleahoppers in different cotton-growing states could be genetically distinct, considering the differences in eco-geographic factors and variable presence of this insect in the cotton fields across the southern United States. In nature, insect populations may become genetically distinct because

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Table 1. *P. seriatus* populations collected from different cotton-growing states in the United States

State	State code/population code[a]	Latitude (north) dd min	Longitude (west) dd min	Elevation (m above sea level)
Arizona	AZ	33 01.986	111 34.999	421
Texas	TX	30 32.100	96 26.640	72
		34 09.300	101 57.000	1,066
		31 25.320	100 08.400	358
Oklahoma	OK	34 37.415	99 53.659	341
Tamaulipas, Mexico	MX[b]	26 00.057	97 44.245	17
Arkansas	AR	36 03.035	90 22.654	77
		36 20.537	90 13.088	87
		35 23.609	92 23.405	208
Mississippi	MS	33 42.902	90 59.458	39
		33 24.454	90 54.673	71
Louisiana	LA	32 21.057	91 30.662	33
		32 54.643	91 44.833	32
Alabama	AL	30 31.632	88 14.558	68
		30 38.820	87 45.668	56
Florida	FL	30 57.643	85 08.034	59
Georgia	GA	31 08.327	84 48.688	49
South Carolina	SC	33 41.586	80 41.618	86
		33 31.659	80 44.995	63
North Carolina	NC	36 12.165	76 26.573	4
		35 49.683	77 36.313	36
		35 42.379	77 49.728	40

[a] Insect samples collected from several sites in a state are considered as one population representing that state.
[b] MX- is not a representation of any U.S. state. Insects were collected from Tamaulipas, Mexico.

of reproductive isolation or lack of gene flow between different populations (Roderick 1996, Mallet 2001, Laffin et al. 2004). Further, reproductive isolation is influenced by several factors such as individuals' dispersal ability, ecological isolation, geographic barriers, and local adaptation after natural selection (Feder et al. 1988, Via 1999, Mopper et al. 2000, Sosa–Gomez et al. 2005, St Pierre et al. 2005, Voudouris et al. 2012). Unraveling the genetic differentiation among populations, in other words, finding the genetic population structure of a pest species may allow us to understand variation in traits relevant to their control.

In the past few decades, there has been growing interest in studying population genetic structure of different insect taxa to incorporate this information in the realm of practical applications such as conservation and pest management (Porretta et al. 2007, Franklin and Myers 2008, Lavandero et al. 2009, Kobayashi et al. 2011). In case of agricultural pest management, population genetic studies have provided information about host races, population migration, pheromone races, efficient biological control, and source of invading pest populations (Pashley 1986, Thomas et al. 2003, Kim and Sappington 2006, Lozier et al. 2009, Hartfield et al. 2010, Medina et al. 2010, Zepeda–Paulo et al. 2010). Population genetic studies have also documented how genetic differentiation may exist between insecticide-resistant populations and susceptible populations or pest populations encountering variable level of management pressure such as crop rotation (Franck et al. 2007, Miller et al. 2007, Chen et al. 2012). It is possible that the populations of a widely distributed species may have undergone variable rates of natural selections at different geographic locations. As a result, locally adapted populations may exist, which are currently subdivided because of reproductive isolation. Thus, population genetic studies could generate valuable information on the ecology and evolution of pest populations, which can be incorporated into the effective pest management strategies.

In this study, we used amplified fragment length polymorphism (AFLP) markers to assess the structure of genetic variation in geographically distant cotton fleahopper populations associated with cotton throughout the cotton-growing belt of the southern United States.

Materials and Methods

Sample Collection and DNA Extraction. Insects were collected from 11 cotton-growing states in the United States and one location in Mexico (Tamaulipas). Insects were collected during May to July of 2010 from cotton fields in areas where cotton is extensively cultivated within each state (Table 1; Fig. 1). *P. seriatus* adults were collected during the reproductive stage of cotton using a standard sweep net and a motorized blower also known as a "keep-it-simple" (KIS) sampler (Beerwinkle et al. 1997). In total, 172 adult cotton fleahoppers were used for AFLP analyses. Insects were preserved in 95% ethanol at 4°C until used for DNA extractions. Genomic DNA was extracted using Qiagen DNeasy kit (Qiagen, Valencia, CA) following the manufacturer's recommended protocol for animal tissue. DNA concentration and purity were measured for each specimen using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., DE).

AFLP Procedure. AFLP markers were generated using the protocol proposed by Vos et al. (1995), with slight modifications. Restriction digestion and ligation steps were performed by adding 5.5 μl (average 50 ng/μl) of genomic DNA to 5.5 μl of a master mix containing 1.1 μl of 10× T4 DNA ligase buffer, 1.1 μl of 0.5 M NaCl, 0.55 μl of diluted bovine serum albumin

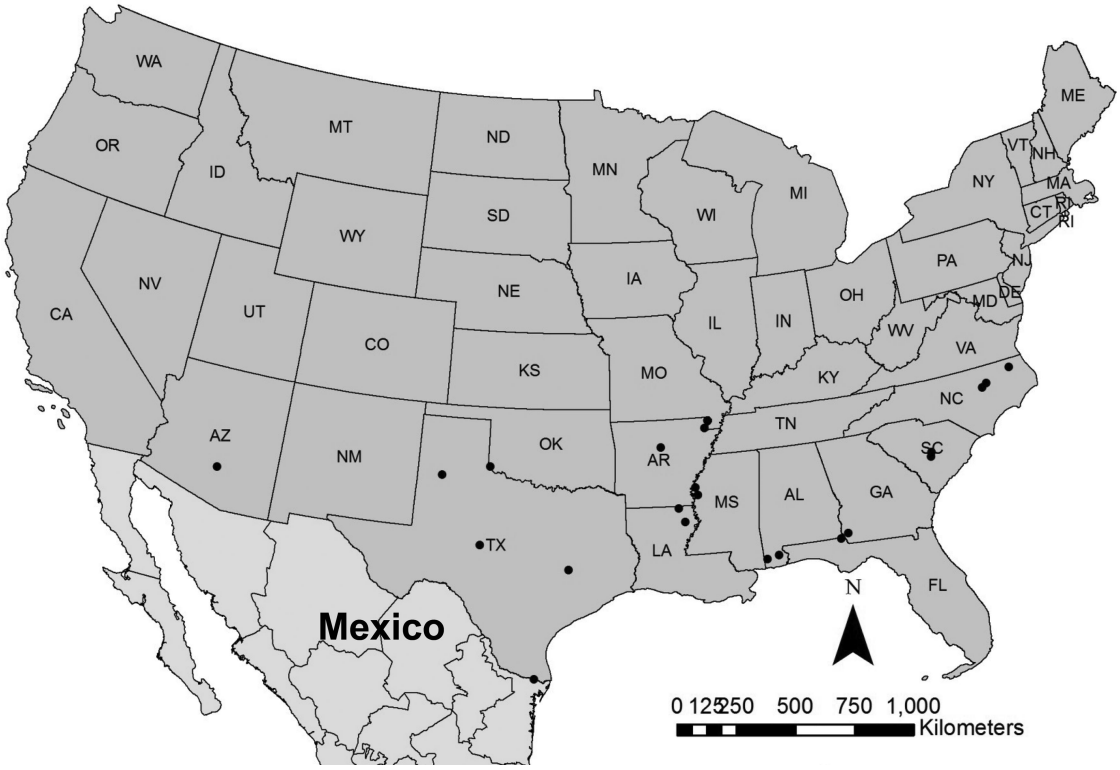

Fig. 1. Cotton fleahopper collection sites in different cotton-growing states of the United States. Each collection site is represented by a black dot in the map.

(1 mg/ml), 0.05 μl of MseI (New England BioLabs Inc., Ipswich, MA), 0.05 μl of EcoRI (New England BioLabs Inc.), 0.03 μl of T4 DNA ligase (New England BioLabs Inc.), 1 μl of MseI and 1 μl of EcoRI adaptors (Applied Biosystems, Foster City, CA), and 0.61 μl of ultra-pure water (18.2 mega-ohm/cm). The entire reaction incubated at 37°C for 2 h for adequate digestion. Subsequently, each reaction was diluted to 1:18 (11 + 189 μl) ratio with buffer TE_{thin} (15 mM Tris of pH 8.0, 0.1 mM EDTA). Preselective polymerase chain reaction (PCR) amplification was performed in a 20-μl reaction containing 4 μl of the diluted restricted or ligated DNA and 16 μl of a mixture containing 1 μl of EcoRI and MseI AFLP preselective primers mix (Applied Biosystems) with 15 μl of AFLP core mix (Applied Biosystems). The PCR protocol for the preselective amplification consisted of 95°C for 1 min followed by 20 repetitive cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 90 s with a final hold at 75°C for 5 min followed by a storing temperature of 4°C. The amplified product was diluted 20-fold by adding 190 μl of buffer TE_{thin} to each reaction. For selective PCR amplification of restriction fragments, 4 μl of the diluted preselective PCR product was added with 15 μl platinum super mix (Applied Biosystems), 1 μl of primers EcoRI-AAC or EcoRI-ACG, and 1 μl of MseI-CAC or MseI-CTC (Applied Biosystems). The PCR parameters were an initial warm-up at 95°C for 30 s, 12 cycles of 95°C for 10 s, 65°C for 40 s with a lowering of 0.7°C per cycle, 72°C for 5 min, followed by 35 cycles of 95°C for 11 s, 56°C for 30 s, 72°C for 2 min, and finally a hold of 75°C for 5 min before storing the samples at 4°C.

Samples were analyzed using capillary electrophoresis. Each reaction was prepared by adding 0.5 μl of 400 HD-ROX-size standard (Applied Biosystems), 9 μl of HiDi formamide, and 1 μl of selective PCR amplification product. Samples were analyzed in an ABI 3130 genetic analyzer (Applied Biosystems). Electropherograms were evaluated in GeneMapper 4.0 (Applied Biosystems) using a 1 bp bin width. Fragments between 50 and 400 bp with a threshold peak of 100 or more relative florescent units (RFU) were considered for final analysis.

AFLP Data Analysis. Data obtained from two primer pairs were combined and analyzed as a single matrix. The percent polymorphic loci (%P) and expected heterozygosity (He) were estimated using GenAlEx 6.3 (Peakall and Smouse 2006). Principal coordinate analysis (PCA) was performed on genetic distances among geographic populations based on Nei's genetic distance (Nei 1972). Bayesian clustering of individual was performed in STRUCTURE 2.3.1 (Pritchard et al. 2000, Falush et al. 2007). The STRUCTURE run followed an admixture model, with 10 replicates for each K, assuming $K = 1–12$ and 50,000 burn-in followed by 50,000 MCMC replications. The best estimate of K was determined by the method

Table 2. Molecular diversity for geographic populations of cotton fleahopper

Populations	*N*	%P	He	SE of He
AZ	16	59.2	0.104	0.0060
TX	21	71.0	0.110	0.0057
OK	12	59.9	0.108	0.0058
MX	14	57.4	0.102	0.0058
AR	18	67.9	0.109	0.0057
MS	16	64.9	0.108	0.0058
LA	18	66.2	0.109	0.0057
AL	13	55.8	0.105	0.0059
FL	6	36.7	0.096	0.0068
GA	20	66.9	0.107	0.0057
SC	17	60.8	0.108	0.0059
NC	17	60.3	0.109	0.0059

%P, percent polymorphic loci; He, expected heterozygosity; SE, standard error of heterozygosity.

described by Evanno et al. (2005), which takes into account the rate of change in the probability of data between successive K [Ln Pr(X K)] values and graphically finds the uppermost hierarchical level of population structure for the tested scenario. Evanno et al. (2005) method for determining the likely value of K was performed using the STRUCTURE HARVESTER web service (Earl and vonHoldt 2012). Analysis of molecular variance attributed to different hierarchical groups was calculated using ARLEQUIN version 3.1 (Excoffier et al. 2005). We measured isolation-by-distance (IBD) by regressing genetic distance, $F_{ST}/(1-F_{ST})$, over geographic distance (kilometer) among populations as described by Rousset (1997) using GenAlEx 6.3. The significance of the correlation coefficient (r^2) was calculated with a Mantel test based on 9,999 random permutations.

Results

The number of individuals (172) and AFLP bands (559) used in this study were sufficient (SESim = 0.002) to reveal the presence of population structure (Medina et al. 2006). 71.1% of the AFLP bands were polymorphic. Molecular diversity, as indicated by percent polymorphism, was highest for the Texas (TX) population and lowest for the Florida (FL) population (Table 2). Expected heterozygosity was similar for all populations. Principal coordinate analysis (PCA) of the 12 geographic populations sampled, revealed that populations were grouped into at least four clusters (Fig. 2). Out of these four clusters, the cotton fleahopper populations obtained from FL and Arizona (AZ) formed two separate clusters, whereas Georgia (GA), South Carolina (SC), and North Carolina (NC) populations clustered together. Individuals from the remaining states (i.e., TX, OK, AR, MS, LA, and AL), including individuals from Tamaulipas, Mexico, grouped together. Tests for significance of genetic differentiation indicated that overall, there was significant, yet genetic differentiation among geographic populations ($F_{ST} = 0.02$; $P < 0.0001$).

The STRUCTURE analysis indicated that there could be two or five possible genetic populations among all the 12 populations analyzed. Analysis of likely K value using Evanno et al. (2005) indicated that there were more than one clear peak for ΔK, which was at $K = 2$ and 5 (Supp. Fig. 1 [online only]). Upon examining the graphical outputs from STRUCTURE and assignment probabilities of individuals into different inferred genetically distinct populations (Supp. Fig. 2 [online only]), we interpret that at $K = 5$, there are three major genotypes that represent biologically meaningful population structure for our dataset (Fig. 3). At $K = 5$, the structure of genetic variation in the cotton fleahopper populations appears to be partitioned into three regional genetic populations: western (AZ); central (TX, OK, Mexico, AK, MS, LA, and AL); and eastern (FL, GA, SC, and NC).

Analysis of molecular variance (AMOVA) of the12 geographic populations indicated that most of the genetic variation existed within populations (97.6%), and the remaining proportion (2.4%) of genetic vari-

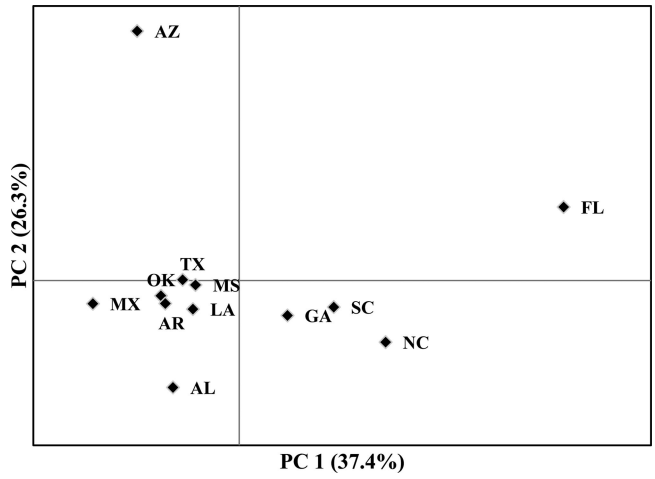

Fig. 2. PCA on genetic distances of cotton fleahoppers from 12 geographic locations.

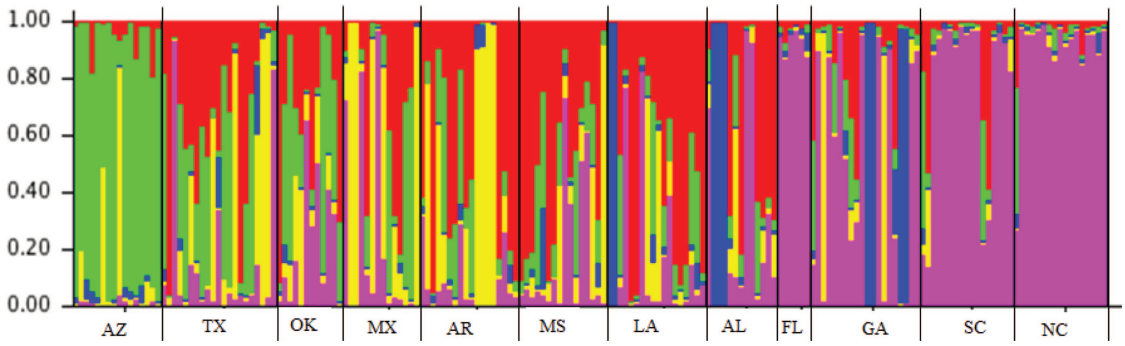

Fig. 3. Results of STRUCTURE analysis for cotton fleahoppers from 12 locations across its geographic distribution at $K = 5$. Individuals are organized by the state where they were collected (see Table 1 for state codes). A vertical black line separates each of the states considered. (Online figure in color.)

ation was explained by geographic location (Table 3A). PCA also grouped the 12 geographic populations we studied into three regional groups (i.e., western, central, and eastern). AMOVA further confirmed that the regional grouping was able to explain significant genetic variation (1.9%; $F_{CT} = 0.02$; $P = 0.0008$). At the next hierarchical level (within region), the geographic population(s) explained significant ($F_{SC} = 0.01$; $P < 0.0001$), but (1.2%) genetic, variation (Table 3B). The correlation analysis (Mantel test) between genetic and geographic distance among different geographic populations was significant ($r^2 = 0.26$; $P = 0.02$) (Fig. 4).

Discussion

The AFLP analysis of cotton fleahopper populations collected from 12 geographically distant cotton-growing locations showed that cotton fleahoppers within each population have significant genetic variation (Table 3). High genetic variation in case of cotton fleahopper is expected considering the native range of this insect in the southern United States. Several studies have documented that genetic variation at insects' native ranges tend to be greater than at introduced ranges (Grapputo et al. 2005, Husseneder et al. 2012). For example, relatively high level of genetic diversity (both at nuclear and mitochondrial level) was recorded in Colorado potato beetle (*Leptinotarsa decemlineata* Say) populations collected from central United States, which is the native range of this insect, whereas populations collected from different locations in Europe, an introduced range for this insect pest, exhibited significantly reduced genetic diversity. We found that the 12 geographic populations we studied are genetically structured into three broad regional populations, viz., western (AZ), central (TX, MX, OK, AR, MS, LA, and AL), and eastern (FL, GA, SC, and NC) populations. Several other insect species, distributed across the United States, have also shown a similar pattern of regional genetic structure. For example, the potato tuberworm (*Phthorimaea operculella* (Zeller)) (Medina et al. 2010), the pecan nut casebearer (*Acrobasis nuxvorella* (Neunzig)) (Hartfield et al. 2012), the Hessian fly (*Mayetiola destructor* Say) (Morton et al. 2011), the sorghum plant bug (*Stenotus rubrovittatus* (Matsumura, 1913)) (Kobayashi et al. 2011), the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Grapputo et al. 2005), and the cotton boll weevil (*Anthonomus grandis* Boheman) (Kim and Sappington 2006), all showed regional population structure similar to the one found in the cotton fleahopper. Our findings suggest that cotton fleahoppers within a particular region (i.e., eastern, central, or western) exhibit relatively high levels of gene flow when compared with populations among regions, where gene flow seems to be somewhat restricted. However, populations within regions vary in their degree of genetic differentiation. That is, populations within the central region show some degree of genetic differentiation, whereas populations within the eastern and western region are more homogeneous (Fig. 3; Table 3). These differences suggest there is variation in the degree of gene flow experienced by populations within the central region when compared with population within the eastern and western regions. Restriction in gene flow among individuals from regional cotton fleahopper populations might be because of geographic and ecological barriers.

Geographic barriers such as mountain ranges, rivers, and stretches of unsuitable habitat can potentially keep insect populations physically isolated, and thereby promote genetic differentiation (Fairley

Table 3. AMOVA of 12 geographic populations without (A) and with (B) regional grouping

	Sources of variation	df	% variation	*F*-statistics	*P* value
A	Among geographic populations	11	2.4	0.023	<0.0001
	Among individuals within population	176	97.6		
B	Among geographic regions	2	1.9	0.019	0.0008
	Among populations within region	9	1.2	0.012	<0.0001
	Among individuals within population	176	96.9		

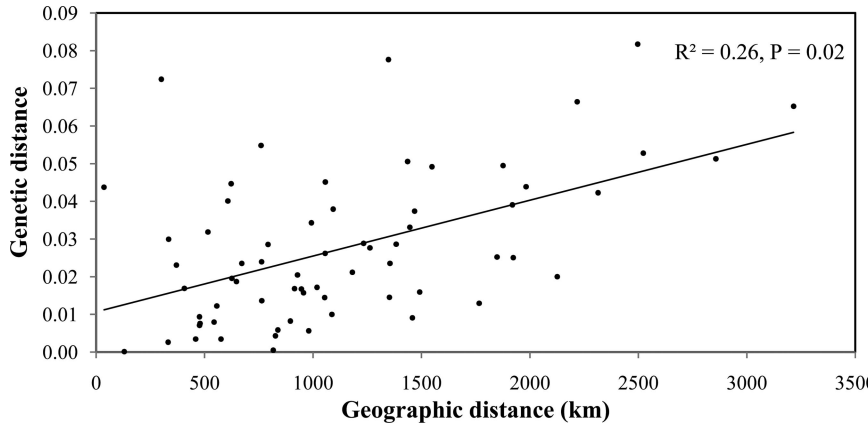

Fig. 4. IBD among fleahopper individuals from 12 locations (11 U.S. states and 1 Mexican state) based on AFLP data. The graph shows the result of Mantel test for correlation between genetic and geographic distances among different populations.

et al. 2000, Forister et al. 2004, Seyahooei et al. 2011). For example, the Rocky and Appalachian Mountains in the United States are suggested as geographic barriers in keeping western and eastern potato tuberworm (*Ph. operculella*) populations isolated and possibly playing a significant role in its current genetic structure (Medina et al. 2010). Cotton fleahopper populations in the western cotton-growing region (e.g., AZ) are likely to be geographically separated from central and eastern cotton-growing regions because of several mountain ranges bordering TX and New Mexico (e.g., Guadalupe mountains). Furthermore, cotton-growing areas in AZ are separated from central and eastern areas by a long stretch of land lacking any known cotton fleahopper host plants. In this scenario, it is not surprising to find that the AZ fleahopper population is genetically distinct from the rest of the populations we sampled (Figs. 2 and 3).

The population sizes of cotton fleahoppers infesting cotton in different states vary considerably (Williams 2011). Small population sizes of fleahoppers in the eastern cotton-growing region (e.g., GA, SC, and NC) may lead to faster genetic drift in this population facilitating genetic differentiation. One explanation for small population size in the eastern region may be the absence, or lesser occurrence, of natural habitat for primary wild host plants of fleahopper, such as horsemint (*Monarda punctata* L.) and woolly croton (*Croton capitatus* (Hogwort)). Except for the south–central TX and the MS Delta (areas of intensive cotton cultivation), no other cotton-growing areas have been thoroughly surveyed for alternate wild host plants of the cotton fleahopper (Snodgrass et al. 1984, Esquivel and Esquivel 2009). Our sampling efforts and communications with collaborators in eastern cotton-growing states (e.g., GA, SC, and NC) suggest that these primary wild host plants are relatively uncommon in the eastern United States, especially the horsemint. Unlike the central United States, where it is common to find these host plant species adjacent to cotton fields, horsemint and woolly croton are not observed adjacent to the cotton fields in the eastern part of the country. The absence or relatively low abundance of these primary wild hosts in the eastern cotton-growing states may in part explain the smaller cotton fleahopper population in this region compared with the insect's population size in some of the central states such as Texas and Oklahoma.

The IBD (Wright 1943) shows that there is a positive and significant correlation between genetic and geographic distance in cotton fleahopper populations. Currently, there is no empirical data available on the dispersal potential of cotton fleahoppers, but field observations (not documented) indicate that this insect appears to be a weak flyer, and do not seem to engage in high altitude flights (Almand et al. 1976). Being a weak flyer, a characteristic of the members in the family Miridae (King 1973, Stewart and Gaylor 1994, Lu et al. 2009), cotton fleahoppers might not be able to disperse long distance. This might explain the observed pattern of IBD and regional clustering of cotton fleahopper populations. A meta-analysis by Peterson and Denno (1998) indicated that phytophagous insects with high mobility show weak IBD, whereas insects with moderate mobility show pronounced or significant IBD. Unlike insects with similar geographic distributions showing panmictic populations, such as *Ostrinia nubilalis* (Hübner) and *Helicoverpa zea* (Boddie, 1850) (Krumm et al. 2008, Groot et al. 2011), the relatively limited dispersal ability of cotton fleahoppers may also explain the genetic differentiation we have observed. Future studies should look into the dispersal behavior and potential of cotton fleahopper within cotton fields and at the landscape level using available mark–recapture techniques as have been implemented in several other insect systems (Hagler and Jackson 2001, Russell et al. 2005, Sivakoff et al. 2012). Such information along with the results from this study should allow us to combine data on physical movement and gene flow of cotton fleahopper populations to better understand the structuring of genetic variation and its implications in the management of this pest.

Information gained from population genetic studies have the potential to improve insect pest monitoring and management practices (Denholm and Rowland 1992, Bourguet et al. 2000, Endersby et al. 2006, Malausa et al. 2007, Lozier et al. 2008, Medina 2012). For example, knowing the way in which genetic variation is organized may improve the design of strategies to delay resistance to transgenic crops expressing insecticidal toxins (Tabashnik 1994, Carriere et al. 2010). Few studies have been able to link population genetic structure to specific traits relevant to pest control. It is clear that individuals belonging to the same pest species are far from being genetically and phenotypically homogeneous. Future studies should investigate how the organization of genetic variation may translate into vulnerability of pests to different control practices.

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Intraspecies mixture exerted contrasting effects on nontarget arthropods of *Bacillus thuringiensis* cotton in northern China

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Abstract

1 Row-intercropping is a type of multiple cropping with two or more crops grown simultaneously in alternate rows in the same area. It is a traditional agronomic practice and is still prevalent in modern Chinese agricultural ecosystems. Many studies have proposed that intercropping at the crop species level can significantly contribute to pest management when properly managed. However, the performance of intercropping at the plant genotype level is still largely unknown.

2 A multiyear field experiment was conducted to examine the effects of intraspecies *Bacillus thuringiensis (Bt)*/non-*Bt* crop mixture on nontarget arthropods. Densities of dominant pests and predators were assessed via direct visual observations.

3 Cotton aphid population levels in monoculture *Bt* cotton fields were greater than that observed in non-*Bt* cotton, whereas the row-mixture planting of *Bt* and non-*Bt* suppressed the abundance of cotton aphids compared with that in monoculture of either genotype. Investigations also demonstrated that the intraspecies row-mixture increased whitefly abundance compared with monoculture of either genotype. However, the mixture exerted neutral effects on population sizes of mirid bugs and predators.

4 These results suggest that crop cultivation management is insufficient to control secondary pests of *Bt* cotton, and thus multiple pest suppression strategies are warranted.

Keywords *Bt* cotton, cotton aphid, intercropping, mirid bug, predator, whitefly.

Introduction

Cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), once the major cotton pest in northern China, has been effectively controlled by the adoption of transgenic cotton expressing a δ-endotoxin from *Bacillus thuringiensis* (*Bt*). However, cultivation of *Bt* cotton led to substantial variations in crop composition and pest management practices, which in turn changed the arthropod community structures within cotton ecosystems, resulting in a greater herbivore population size in *Bt* cotton compared with that in non-*Bt* cotton (Wilson *et al.*, 1992; Cui & Xia, 1998, 2000; Greene *et al.*, 1999; Herron *et al.*, 2000; Wu *et al.*, 2002; Deng *et al.*, 2003; Lu *et al.*, 2010). For example, Cui and Xia (1998, 2000) found that populations of *Aphis gossypii* Glover (Hemiptera: Aphididae), *Tetranychus cinnbarinus* Boisduval (Prostigmata: Tetranychidae), *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) and *Empoasca biguttula* Ishida (Hemiptera: Cicadellidae) were elevated in *Bt* cotton fields compared with that in non-*Bt* cotton. Herron *et al.* (2000) and Deng *et al.* (2003) found that cotton aphid populations in *Bt* cotton were significantly larger compared with non-*Bt* cotton. Wu *et al.* (2002) and Lu *et al.* (2010) reported that the widescale use of *Bt* cotton has led to a frequent outbreak of mirid bugs in northern China. However, population densities of major predator species in *Bt* cotton fields were significantly greater than those in conventional cotton receiving pesticide applications (Wu & Guo, 2005; Sisterson *et al.*, 2007;

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Lu *et al.*, 2012). Finally, evidence suggests that *H. armigera* populations in northern China have developed field-evolved resistance to *Cry1Ac*-expressing *Bt* cotton (Liu *et al.*, 2010). Therefore, the longevity of *Bt* cotton is dependent on its control effect on the resistance development of target pests and outbreaks of nontarget pests.

Various refuge strategies have been field-tested for delaying the resistance development of target pests to *Bt* crops (Gould, 1998; Tabashnik *et al.*, 2005) with promising effects (Tabashnik *et al.*, 2008, 2009; Wu *et al.*, 2008). At the same time, much effort has been directed toward managing the secondary pest complex in *Bt* cotton, and increasing the biological control effect of natural enemies is an effective strategy for overall *Bt* cotton management. Numerous studies suggest that the enhancement of predator abundance and diversity through increasing plant diversity exerts positive effects on pest control in many cropping systems (Andow, 1991; Parajulee *et al.*, 1997; Parajulee & Slosser, 1999; Men *et al.*, 2004; Gardiner *et al.*, 2009).

In most agro-ecosystems, strip intercropping, namely the planting of two or more crops simultaneously in different strips in a manner to permit independent cultivation, as well as allowing the crops to interact agronomically (Vandermeer, 1992), is the principal strategy in plant diversity enhancement. Such strip intercropping could be achieved via interspecies or intraspecies row-mixtures. An interspecies row-mixture refers to the planting of two or more species of crops simultaneously in the same field, whereas an intraspecies mixture is the planting of two or more genotypes of the same crop species simultaneously in the same field. A few studies have documented the effects of intraspecies mixtures on the predator complex and any resulting pest control in cotton fields (Sisterson *et al.*, 2004; Yang *et al.*, 2012). For cotton fields, the intraspecies row-mixture of *Bt* and non-*Bt* cotton is equivalent to setting a structured refuge.

The present study aimed to explore the feasibility of utilizing a structured refuge to suppress nontarget pests of *Bt* cotton in small-holder agro-ecosystems of northern China. We hypothesized that a row-mixture planting of *Bt* and non-*Bt* cotton would exert a positive effect on pest control, and that this effect would be irrespective of cotton genotype. We also hypothesized that the effect of an intraspecies mixture on pest and predator abundance would be consistent across growing seasons.

Materials and methods

Field experimental design

Field experiments were conducted at the Langfang Experiment Station (39.538°N, 116.708°E) of the Chinese Academy of Agricultural Sciences (CAAS), located in the Jiuzhou County of Hebei Province. Before 2008, field corn was grown in the selected fields. Based on the current *Bt* cotton adoption rate of the Hebei Province and the refuge size for target pests recommended by Vacher *et al.* (2003), we set up three experimental treatments: (i) monoculture of a *Bt* cultivar; (ii) monoculture of a non-*Bt* cultivar; and (iii) intercropping of 75% *Bt* and 25% non-*Bt*. Intercropping plots were planted in a repeated pattern: one row of non-*Bt* and then three rows of *Bt*. The pattern continued until all rows within a plot were occupied. A randomized complete block design was used with four replications. Each plot within a block encompassed approximately 0.33 ha (length 20 m, width 16.5 m), which is a typical cotton field size in the Hebei Province. Seeding was performed at a rate expected to produce 40 000 plants per planted ha. A 3-m fallow space was left between plots and among blocks to decrease insect dispersion among treatments (Wu & Guo, 2003; Li *et al.*, 2010). Cotton was maintained with agronomic practices standard to northern China, although no fungicides or insecticides were applied to the experimental plots. Plot layout and management practices were identical across all three study years.

Cotton genotypes

The cotton genotypes used in the present study included a genetically modified *Bt* cotton (cv 'GK-12', expressing a δ-endotoxin from *Bt*) and a non-*Bt* cotton (cv 'Simian-3', the parental line of 'GK-12'). The seeds of the two genotypes were provided by colleagues from the Biotechnology Research Center of CAAS. Cotton genotypes exhibited marked differences in leaf trichome density (Xue *et al.*, 2008), *Bt* toxin content (Zhang *et al.*, 2006) and associated resistance to lepidopteran species.

Arthropod sampling

Arthropods sampled included three pest species groups [cotton aphid *A. gossypii*; mirid bug complex *Lygocoris lucorum* Meyer-Dur, *Adelphocoris suturalis* Jackson and *Adelphocoris fasciaticollis* Reuter; and whitefly *Bemisia tabaci* (Gennadius) biotype B] and four predator groups [ladybirds beetles *Coccinella septempunctata* L. and *Propylaea japonica* Thunberg; lacewing *Chrysoperla sinica* (Tjeder); spiders complex and *Orius similis* Zheng]. In each growing season, arthropod sampling was conducted every 10 days from early June until mid-September, corresponding to 4 weeks after cotton seedling emergence to plant defoliation for harvest preparation. Arthropod groups were sampled by visually inspecting 20 cotton plants at five randomly chosen sampling sites distributed across the two diagonal lines of the plot (100 plants per plot) *in situ*. Because of practical concerns as a result high densities, cotton aphid and whitefly populations were quantified by visually inspecting three leaves each from the upper, middle and lower main stem portions of the plant, respectively. In total, nine leaves per selected plant were investigated. For other arthropods, entire plants were visually inspected in the morning (8.00–10.00 h) or afternoon (16.00–18.00 h), with particular attention being paid to flowers and squares, which are likely hiding places for feeding insects.

Statistical analysis

Data obtained from the 100 total plants from the five sampling sites within each experimental plot were pooled to correct for data dependency, and so each plot was used as a replication

unit. Arthropod density responses to treatments were analyzed by two steps. First, the overall effects of these factors on pests and predator abundances during the 3-year study were analyzed with a linear mixed effect model using planting pattern and cotton genotype as a fixed factor, and year as a random factor (SAS Institute, 2003). Then, the effects of planting pattern (monoculture or row-mixture), cotton genotype (*Bt* or non-*Bt*), sampling date, and their interactions, on the abundance of natural enemies and herbivores in each growing season were further analyzed separately with a PROC MIXED procedure in repeated measures analysis of variance (SAS Institute, 2003). Differences in arthropod abundances on specific sampling dates were separated using Fisher's least significant difference. When necessary, the data were $\sqrt{(n+0.5)}$ transformed or $\log(n+1)$ transformed to satisfy assumptions of normality and homogeneity of variance before analysis of variance.

Results

Row-mixture intercropping arrested the abundance of cotton aphids

Cotton aphid population size varied significantly across years and sampling dates. The abundance of cotton aphid on *Bt* cotton was higher that that of non-*Bt*, and row-mixture intercropping markedly decreased the abundance of cotton aphid throughout all 3 years of the study (Figs 1 and 2). In addition, the interaction between planting pattern and cotton genotype was statistically significant (Table 1).

In each growing season, cotton aphid population levels varied significantly across sampling dates (Figs 1 and 2 and Table 2). Row-mixture intercropping significantly depressed the abundance of cotton aphid compared with the *Bt* or non-*Bt* monoculture (Fig. 1). At the same time, the densities of cotton aphid varied greatly within cotton genotypes in monoculture fields and across growing seasons (Fig. 2 and Table 2). The effect of cotton genotype on cotton aphid densities changed with sampling date, as did the effect of planting pattern. The impact of *Bt* cotton on population size of cotton aphid varied greatly among years. In 2008, the abundance of cotton aphid in *Bt* cotton was markedly higher than that in *non-Bt* cotton (Fig. 2A–D), whereas, in 2009 and 2010, no significant differences in cotton aphid were found between *Bt* and non-*Bt*. In addition, the interaction between cotton genotype and planting pattern was not significant for cotton aphid, except for the 2008 growing season (Table 2).

Row-mixture intercropping exerted a neutral effect on the abundance of mirid bugs

Abundances of mirid bugs showed significant variations across years and sampling dates, although planting pattern and genotype had no marked impact on mirid bugs activities. Yet, the interactions between year and planting pattern were statistically significant (Table 1).

Discernible fluctuations of mirid bugs abundance were found across the sampling dates for all years (Fig. 3 and Table 2), although comparable numbers of mirid bugs were found between *Bt* and non-*Bt* cotton fields at the same sampling date. Row-mixture intercropping showed no pronounced effect on the abundance of mirid bugs compared with the monocultures of

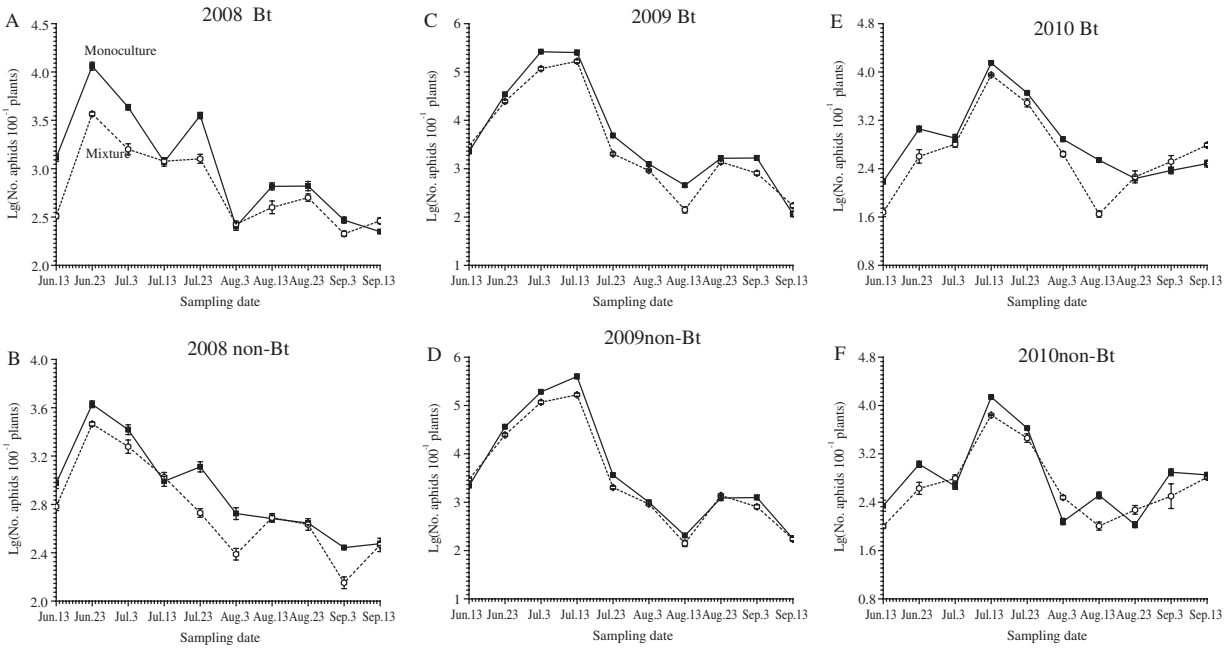

Figure 1 Dynamics of the cotton aphid on the same cotton genotype under different planting patterns [monoculture *Bacillus thuringiensis (Bt)* cultivar, monoculture non-*Bt* cultivar, and mixed-rows of same *Bt* and non-*Bt* cultivars] from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the line graphs represent population sizes (mean ± SE) of the monoculture fields, whereas the dotted-lines represent those of the mixture of *Bt* and non-*Bt* cotton at a row ratio of 75% to 25%, respectively.

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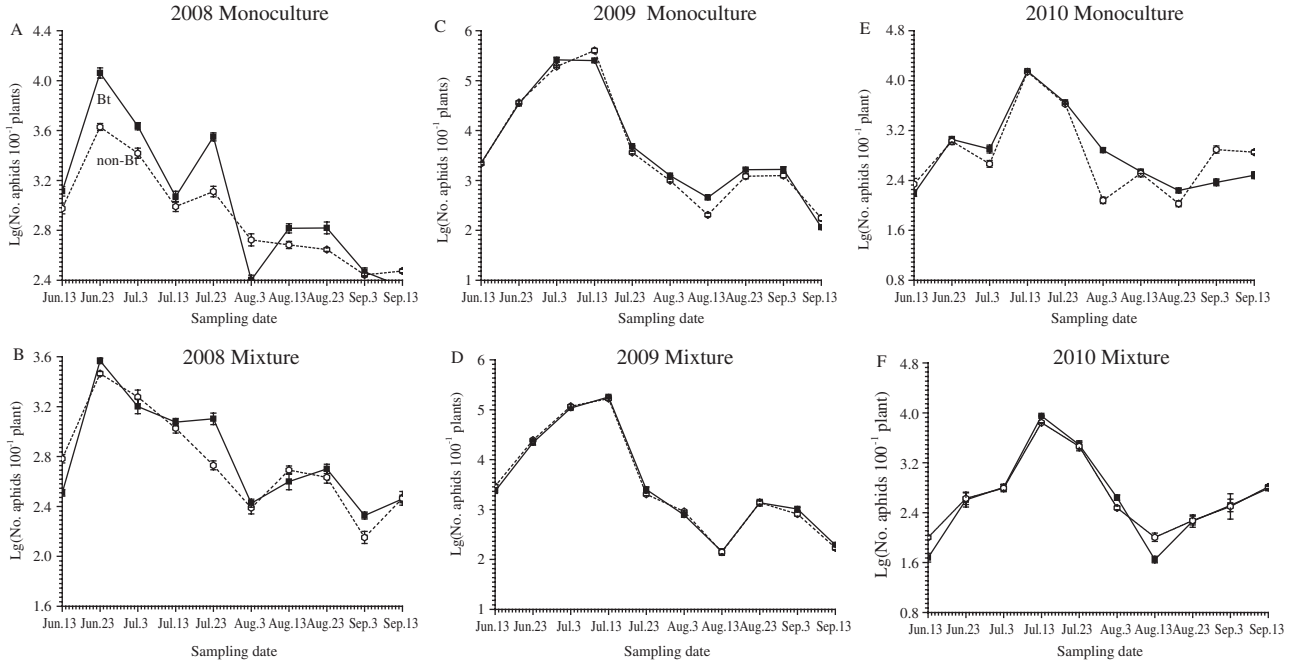

Figure 2 Dynamics of cotton aphid on *Bacillus thuringiensis (Bt)* cotton and non-*Bt* cotton under different planting patterns (monoculture *versus* mixture) from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the graphs represent population sizes (mean ± SE) of monoculture fields, whereas the dotted-lines represent those of the mixture of *Bt* and non-*Bt* cotton at a row ratio of 75% to 25%, respectively.

Table 1 *F*- and *P*-values from the linear mixed model estimated effect of cotton genotype, planting pattern, year and their interactions on population size of herbivores in northern China cotton fields in 2008, in 2009 and 2010

		Cotton aphid		Mirid bugs		Whitefly	
Factor	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Year	2,40	2300.90	< 0.0001	289.63	< 0.0001	51.28	< 0.0001
Genotype	1,40	10.66	0.002	0.08	0.781	0.49	0.488
Pattern	1,40	268.34	< 0.0001	3.85	0.057	17.64	0.001
Date	9, 459	82.77	< 0.0001	104.77	< 0.0001	618.25	< 0.0001
Year × Genotype	2,40	10.91	0.002	0.30	0.589	1.84	0.183
Year × Pattern	2,40	1.34	0.273	3.29	0.049	0.35	0.707
Genotype × Pattern	1,40	5.44	0.008	2.33	0.112	2.16	0.129
Genotype × Pattern × Year	2,40	0.18	0.835	1.57	0.221	1.01	0.372

either the *Bt* or non-*Bt* genotypes in the 2009 and 2010 growing seasons (Fig. 3 and Table 2). However, in 2008, the population size of mirid bugs was higher in intercropping fields compared with the corresponding cotton genotype in monoculture fields (Fig. 3C, D). The interaction between genotype and planting pattern was significant for the growing season of 2008 (Table 2). In conclusion, no clear trends were found because the effect of cotton genotype and planting pattern on the population size of mirid bugs changed with sampling date.

Row-mixture intercropping increased abundances of whiteflies

The results of a linear mixed model indicated that there were significant variations in the abundances of whiteflies across years and sampling dates. In addition, the row-mix planting pattern consistently showed increased whitefly densities. However, differences as a result of cotton genotype were not detectable. Furthermore, neither the interactions between each of two factors (year, planting pattern and genotype), nor the interactions of all the factors were statistically significant (Table 1).

Whitefly densities varied significantly across sampling dates. There were significant differences in abundance of whitefly between the two planting patterns (monoculture versus mixed-row plantings) in most of the investigating periods (Fig. 4 and Table 2). The row-mixture plantings increased the abundance of whitefly compared with the same genotype monocultures, whereas the effect of cotton genotype on whitefly abundance was negligible in most cases, whether under monoculture or mixture. Moreover, the interaction between planting pattern and

Table 2 *F*-values of the repeated measures analysis of variance testing the effects of planting pattern, cotton genotype and sampling date on population sizes of cotton aphid, mirid bugs and whitefly in northern China cotton fields in 2008, 2009 and 2010

Year	Factor	d.f.	Cotton aphid	Mirid bugs	Whitefly
2008	G	1,12	39.46***	4.7	5.93*
	P	1,12	238.67***	5.21*	16.45**
	D	9,108	501.33***	149.26***	2875.59***
	G × D	9,108	18.41***	4.14***	7.14***
	P × D	9,108	19.01***	14.34***	2.88**
	G × P	1,12	11.34**	7.65*	0.39
	G × P × D	9,108	9.98***	8.62***	2.36*
2009	G	1,12	3.59	1.11	0.85
	P	1,12	88.03***	3.55	1.52
	D	9,108	3829.37***	128.47***	389.24***
	G × D	9,108	6.09***	2.78**	0.32
	P × D	9,108	19.44***	4.90***	7.12***
	G × P	1,12	2.75	0.02	0
	G × P × D	9,108	6.26***	4.24***	0.92
2010	G	1,12	0.04	0.98	0.88
	P	1,12	42.55***	1.46	18.86***
	D	9,108	404.33***	218.71***	668.19***
	G × D	9,108	13.10***	3.01**	0.5
	P × D	9,108	20.35***	14.81***	11.72***
	G × P	1,12	1.79	0.75	2.59
	G × P × D	9,108	7.74***	2.59**	1.34

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

G, cotton genotype; P, planting pattern; D, sampling date; G × D, interaction between cotton genotype and sampling date; P × D, interaction between planting pattern and date; G × P, interaction between cotton genotype and planting pattern. G × P × D, interaction between cotton genotype, planting pattern and sampling date.

sampling date was significant for all growing seasons. However, the interactions between planting pattern and sampling date, and between cotton genotype, planting pattern and sampling date, were only significant for the 2008 growing season.

Row-mixture intercropping failed to enhance the abundance of predators

Overall, the predator abundance significantly varied between treatments among years and across sampling dates (Table 3). Cotton genotype and planting pattern contributed little to the variances in predator abundance, and this effect was consistent among growing seasons. However, the interactions between year and planting pattern were significant for most of the taxa group (Table 3).

The densities of all predator taxa fluctuated greatly across sampling dates (Table 4) but did so equally between monoculture and row-mixture intercropping fields, whether for *Bt* or non-*Bt* cotton fields in most cases, except for the growing season of 2008 (Table 4). The abundance of predators, such as adult ladybirds, *O. similis* Zheng and spiders, was higher in the non-*Bt* cotton field compared with that in the *Bt* field, whether for monoculture or mixture fields in 2008. The effect of planting pattern on the population size of adult ladybirds changed with sampling date for the 2008 and for 2010 growing seasons. At the same time, the effect of cotton genotype and planting pattern on spiders abundance changed with sampling date, and no clear trends were found for all the years tested.

Discussion

Impact of row-mixture as a Bt resistance management approach on cotton aphid

Cotton aphid abundance was higher on *Bt* cotton than on non-*Bt* cotton in 2008, whereas, in 2009 and 2010, the population size of cotton aphid in *Bt* fields was similar to that of the non-*Bt* fields. Many other studies have also reported that the abundance of cotton aphid in *Bt* cotton is higher compared with that in conventional non-*Bt* cotton (Wilson *et al.*, 1992; Cui & Xia, 1998; Greene *et al.*, 1999; Deng *et al.*, 2003). The discrepancy observed among the seasons in the present study may be a result of varying environmental conditions and arthropod complexes across study years.

Furthermore, intraspecies intercropping has suppressed the abundance of cotton aphid. This result supports our hypothesis that intraspecies mixtures would improve pest suppression. This finding is congruent with previous studies indicating that intercropping exerts strong positive effects on pest control (Litsinger & Moody, 1976; Risch, 1981; Andow, 1991; Altieri & Nicholls, 2004; Bomford, 2004; Shrewsbury & Raupp, 2006; Björkman *et al.*, 2010). However, the effects of mixed-row intercropping showed a significant variation among years and within genotypes. Xue *et al.* (2008) stated that the outbreak of cotton aphid was more frequently observed in transgenic *Bt* cotton because the lower leaf trichome density of transgenic *Bt* cotton facilitated aphid feeding compared with conventional non-*Bt* cultivars. Accordingly, we would have expected an

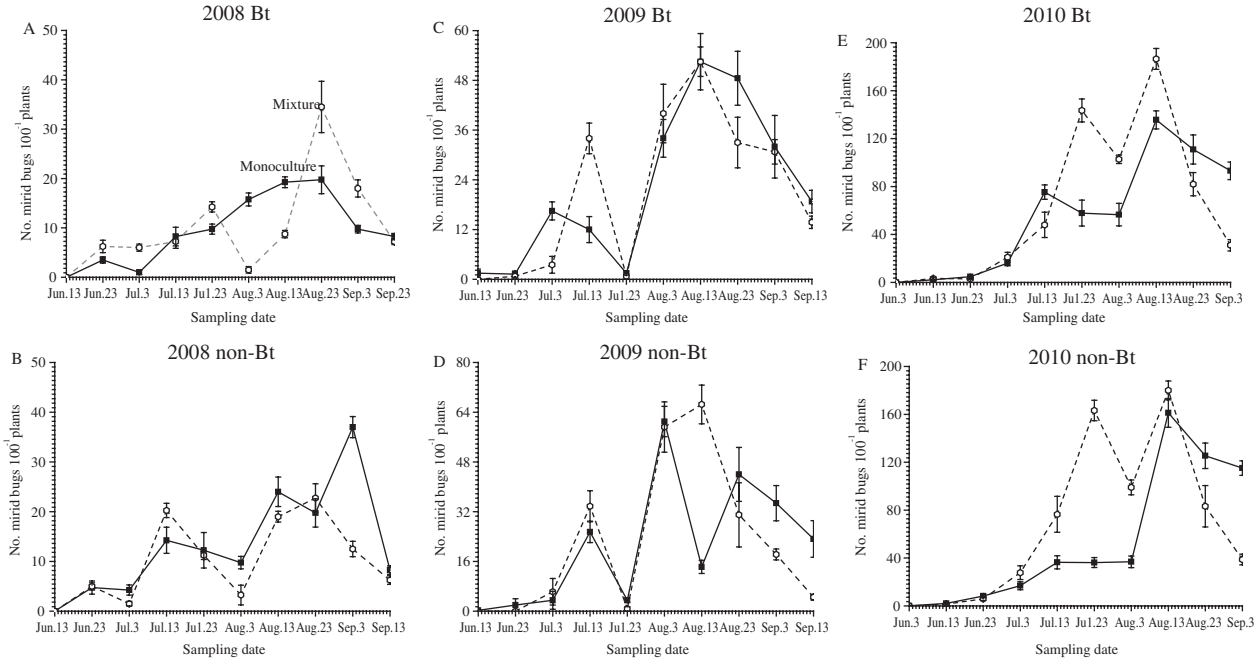

Figure 3 Dynamics of mirid bugs on *Bacillus thuringiensis (Bt)* cotton and non-*Bt* cotton with different planting patterns (monoculture *Bt* cultivar, monoculture non-*Bt* cultivar, and mixed-rows of same *Bt* and non-*Bt* cultivars) from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the line graphs represent population sizes (mean ± SE) of the monoculture fields, whereas the dotted-lines represent those of the mixture fields of *Bt* and non-*Bt* cotton at a row ratio of 75% to 25%, respectively.

intermediate cotton aphid population in mixture plots, with the highest densities in *Bt* plots and the lowest densities in non-*Bt* plots. However, the suppression effect of mixture on cotton aphid was similar between the two genotypes. This indicates that there may be other factors contributing to the observed phenomenon.

Influence of row-mix intercropping on whiteflies and mirid bugs

By contrast to our hypothesis, intercropping increased the occurrences of whitefly in the present study. At the same time, intercropping failed to alter the abundances of mirid bugs. The specific response of pests to intercropping may result from dispersion capability differences. Furthermore, the effect of intercropping on pests is partly determined by plant resistance, whereas plant resistance changes with the developmental age of plant (Barton & Koricheva, 2010). In addition, plants can modulate their defensive strategy based on neighbour identity (Broz *et al.*, 2010). The discrepancy of mixed-row plantings on mirid bugs among seasons may be the result of variation in climate and interactions among arthropods.

Neutral effects of row-mix intercropping on predator abundance

Planting pattern did not significantly influence the predator abundance in most of cases. Therefore, our expectation of increased predator activities in intercropped fields was rejected. Takizawa and Snyder (2011) suggested that higher predator biodiversity fostered the survivorship of juveniles, which in turn increased reproductive rates and contributed more offspring to succeeding generations, along with an increased foraging efficiency. In the present study, the abundances of predators, such as ladybirds and spiders, in intraspecies mixture cotton fields were higher than the corresponding genotype of monoculture cotton fields in 2008. However, this phenomenon was not observed in 2009 and 2010. In general, lower prey abundances are expected to aggravate intraguild predation and competition and thus lead to reduced activity and lower reproduction rates. Considering all of the factors noted previously, it is not unexpected that the intraspecies plantings in the present study did not enhance the occurrence of predators when prey is not sufficient.

Implications for future pest management

Although the widespread planting of *Bt* cotton has led to area-wide population suppression of key target pest species, such as *H. armigera* (Wu *et al.*, 2008), *Bt* cotton adoption has also led to the outbreak of mirid bugs (Wu *et al.*, 2002; Lu *et al.*, 2010) . Therefore, management of nontarget pests is a new requirement for the sustainable application of *Bt*-transgenic cotton. From the perspective of delaying resistance development in a target pest, Wu *et al.* (2008) argued that no structured refuge is advisable as a result of the presence of natural refuges provided by the wide diversity of crops in northern China. However, other studies report that the widescale planting of *Bt* cotton has led to an increased resistance frequency in target pests in some regions

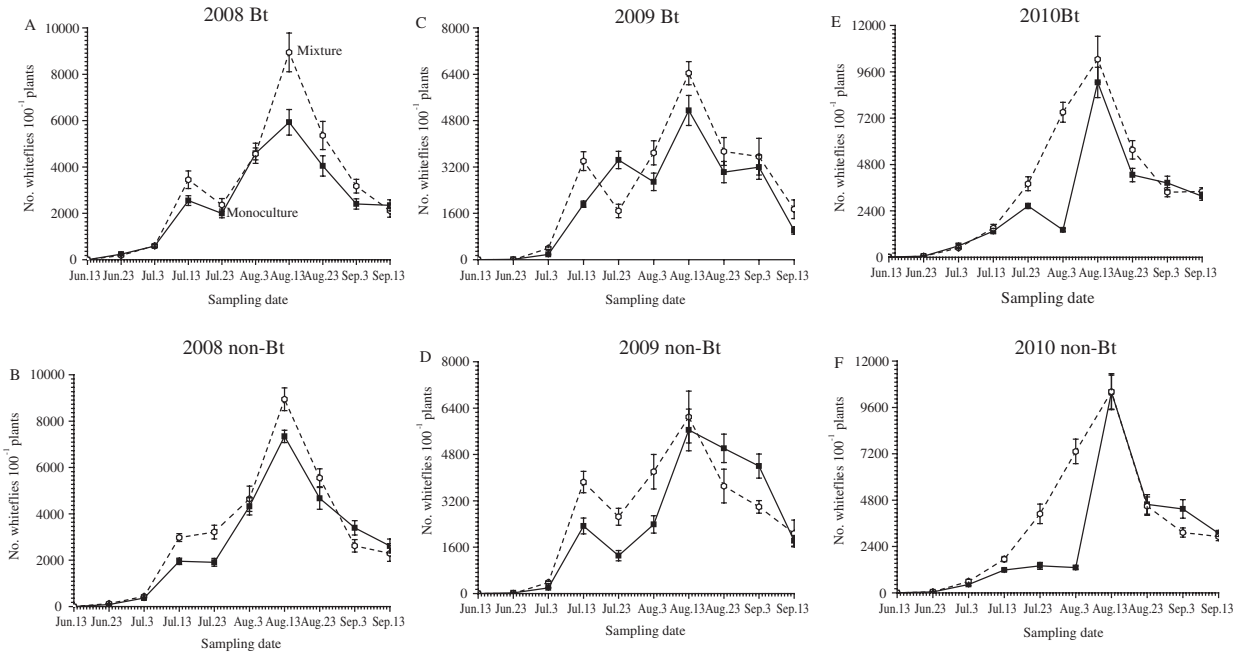

Figure 4 Dynamics of whitefly on *Bacillus thuringiensis (Bt)* cotton and non-*Bt* cotton with different planting patterns (monoculture *Bt* cultivar, monoculture non-*Bt* cultivar, and mixed-rows of same *Bt* and non-*Bt* cultivars) from mid-June to mid-September in (A, B) 2008, (C, D) 2009 and (E, F) 2010. Solid-lines on the line graphs represent population sizes (mean ± SE) of the monoculture fields, whereas the dotted-lines represent those of the mixture fields of *Bt* and non-*Bt* cotton at at a row ratio of 75% to 25%, respectively.

Table 3 *F*-values from the linear mixed model estimated effect of cotton genotype, planting pattern, year and their interactions on population size of predators in northern China cotton fields in 2008, 2009 and 2010

Factor	d.f.	Adult ladybirds	Larval ladybirds	Adult lacewing	Larval lacewing	*Orius similis*	Spiders
Y	2,40	410.97***	5.35**	60.04***	56.6***	264.99***	686.19***
G	1,40	0.85	1.50	0.87	6.91**	0.07	2.00
P	1,40	0.07	0.31	3.4	0.72	0.87	0.71
D	9,459	6.96***	9.32***	11.03***	5.01***	49.88***	101.28***
Y × G	2,40	0.00	0.01	0.15	11.93***	2.62	1.16
Y × P	2,40	7.89***	3.49*	0.59	4.78**	9.27***	43.04
G × P	1,40	0.69	0.02	0.09	8.04***	0.83	3.48*
G × P × Y	2,40	1.39	0.25	0.54	3.54*	3.08	7.17**

*P < 0.05; **P < 0.01; ***P < 0.001.

G, genotype; P, planting pattern; D, sampling date; G × D, interaction between cotton genotype and sampling date; P × D, interaction between planting pattern and sampling date; G × P, interaction between cotton genotype and planting pattern. G × P × D, interaction between cotton genotype, planting pattern and sampling date.

(Liu *et al.*, 2010; Wan *et al.*, 2012). To suppress secondary pest and to delay the resistance development of target pests, Wang *et al.* (2006) proposed that non-*Bt* crops should be planted concurrently with *Bt* crops.

The present study simulated the effects of structured refuge on secondary insects and their predators through intraspecies intercropping in northern China. The mixture in the present study's field scale (small scale) significantly suppressed the abundance of cotton aphid during the seedling and squaring (budding) stages and triggered higher whitefly densities, although it did not modify the population size of mirid bugs and the predator complex. These study results partly support the conclusion that the intraspecies mixture has a potential positive effect on pest control, although the effects are inconsistent with pest species and plant developmental stages. Therefore, future studies need to examine approaches that synchronize pest management regimes, pest species and plant developmental stages with respect to developing effective pest control programmes. In addition, a larger scale study may better determine the observed phenomenon to mimic the actual production scale. This is because the size and scope of intercropping can change the composition and diversity of landscape vegetation parameters. Because landscape structure dramatically influences the abundance, diversity and function

Table 4 *F*-values of the repeated measures analysis of variance testing the effects of cotton genotype, planting pattern, sampling date and their interactions on population sizes of predator in northern China cotton fields during in 2008, 2009 and 2010

Year	Factor	d.f.	Adult ladybirds	Larval ladybirds	Adult lacewing	Larval lacewing	*Orius similis*	Spiders
2008	G	1,12	8.35*	4.09	0.67	2.97	13.01**	5.69*
	P	1,12	11.45**	9.83**	3.2	2.07	1.8	60.69***
	D	9,108	14.42***	49.11***	14.51***	10.78***	25.25***	364.99***
	G × D	9,108	2.25*	10.38***	3.86***	2.09	5.74***	3.89***
	P × D	9,108	1.94	13.99***	0.43	3.41***	1.71	7.38***
	G × P	1,12	1.09	1.25	1.17	0.03	0.04	26.81***
	G × P × D	9,108	2.98**	0.9	1.65	2.48*	1.82	3.45***
2009	G	1,12	0.04	0.73	0.45	7.79*	1.94	4.46
	P	1,12	0.07	3.4	1.02	2.2	0.05	8.25*
	D	9,108	15.09**	18.58***	5.37***	12.53***	130.64***	408.59***
	G × D	9,108	1.23	1.03	1.01	1.77	8.68***	8.89***
	P × D	9,108	1.78	1.09	0.31	3.39***	23.18***	13.55***
	G × P	1,12	0.9	0.14	5.35*	4.80*	5.22	0.38
	G × P × D	9,108	0.71	0.6	1.3	1.91	3.50***	6.91***
2010	G	1,12	0.03	0.62	0.76	0.63	0.63	0.02
	P	1,12	2.63	2.78	3.93	1.19	2.51	0.37
	D	9,108	41.17***	16.4***	12.33***	8.49***	104.73***	0.85
	G × D	9,108	1.01	0.48	0.64	0.5	1.25	1.95*
	P × D	9,108	8.3***	1.17	1.77	1.99*	8.83***	1.67
	G × P	1,12	0.23	0.09	0	2.25	0.65	0.22
	G × P × D	9,108	0.95	0.21	0.64	0.39	1.41	2.87**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

G, genotype; P, planting pattern; D, date; G × D, interaction between cotton genotype and sampling date; P × D, interaction between planting pattern and sampling date; G × P, interaction between cotton genotype and planting pattern. G × P × D, interaction between cotton genotype, planting pattern and sampling date.

of natural enemies within croplands, optimizing the landscape structure through a reasonable arrangement of crop species or variety is crucial for developing ecologically intensive pest management approaches. Therefore, broadening the species pool of beneficial insects supported by a complex landscape and optimizing their activity should help to realize the benefits of habitat management.

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