THE TEXAS AGRILIFE RESEARCH & EXTENSION CENTER AT LUBBOCK

COTTON ENTOMOLOGY RESEARCH REPORT

2011

TECHNICAL REPORT 12-4

TEXAS AGRILIFE RESEARCH, CRAIG NESSLER, DIRECTOR
THE TEXAS A&M SYSTEM, COLLEGE STATION, TEXAS
2012

COTTON ENTOMOLOGY PROGRAM

RESEARCH ACTIVITY ANNUAL REPORT

2011

SUBMITTED TO:

PLAINS COTTON IMPROVEMENT COMMITTEE

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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 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 current boll weevil eradication program for the High Plains area has progressed much more rapidly than anticipated. Now, the successful boll weevil eradication program has eliminated the boll weevil from this region for over 7 years. The team effort of PCG, Texas AgriLife Research and Texas AgriLife Extension Service over many decades has resulted in a very comprehensive understanding of boll weevil ecology and behavior.

With a successful boll weevil eradication program and increased adoption of the Bollgard technology (now >90%), the cotton insect research and extension program focus has changed considerably during the last 8-9 years. Our current research and 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 hope to continue this partnership as we challenge ourselves to deliver the best cotton insect-pest management recommendations to our Texas High Plains producers. The departure of our Cotton Extension Specialist, David Kerns, may pose some interim challenges for the 2012 summer, but Dr. Parajulee's Program has exceptionally talented people on the team who, together with seasoned IPM Agents we have in the region, can address insect management issues for our producers without them noticing this transition.

Texas AgriLife Research & Extension Center at Lubbock

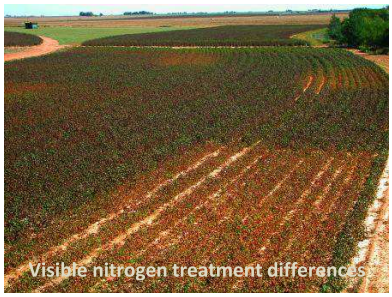
COTTON ENTOMOLOGY PROGRAM

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 high-input Texas High Plains cotton production system has been conducted for the last ten years. Differential nitrogen fertility has been shown to significantly affect cotton plant physiological parameters, thereby influencing arthropod population dynamics.



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

(IN COOPERATION WITH MONSANTO COMPANY)

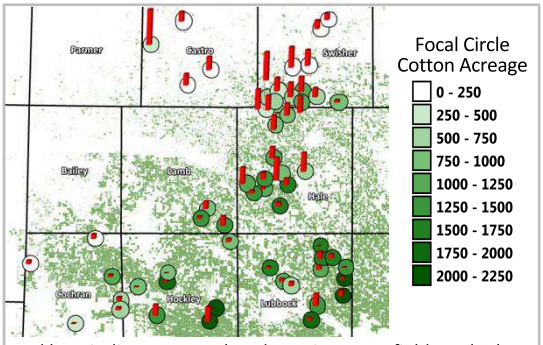
As part of an ongoing Monsanto program to develop commerically available *Lygus*-tolerant cotton germplasm, numerous cotton lines, genetically modified to confer *Lygus* tolerance via protein expression (similar to Bt), are being evaluated for effectiveness under whole-plant cage field conditions, as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

COTTON YIELD AND FIBER QUALITY COMPENSATORY RESPONSE TO *LYGUS*-INDUCED FRUIT LOSS

A major Program focus has been cotton yield and quality compensation following *Lygus*-induced fruit loss. Currently, two transgenic cotton varieties (DP 104 B2RF or "short-season" and DP 161 B2RF or "long-season") are being evaluated under a high-input drip irrigation system for their responses to simulated chronic infestations with *Lygus hesperus* insects. Weekly COTMAN™ plant mapping is performed to monitor fruit set and retention profiles. The late-season cotton variety exhibited greater compensatory potential, though it may be more vulnerable to commonly observed late-season *Lygus* infestations.

INFLUENCE OF NON-COTTON PLANT HOSTS ON *LYGUS* POPULATIONS IN COTTON: DEVELOPING A LANDSCAPE-LEVEL APPROACH TO PEST MANAGEMENT

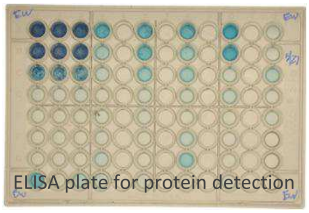
Sixty cotton fields were selected from across the Texas High Plains for weekly arthropod sampling and seasonal monitoring of the agricultural landscape composition within a 3-km radius of each field. Among other significant variables, corn and sunflower acreages and overall habitat heterogeneity correlated strongly with increased *Lygus* abundance in cotton focal fields, while surrounding cotton acreage correlated negatively. This study is expected to provide impetus for future investigations into landscape-level approaches to pest population management, and our efforts to develop such approaches are ongoing.



Red bars indicate *Lygus* abundance in cotton fields and 3-km circles are colorized to indicate encompassed cotton acreage.

LYGUS SOURCE-SINK DYNAMICS IN COTTON-ALFALFA AGROECOSYSTEMS AND POPULATION GENETIC DIVERSITY

Lygus movement in a cotton-alfalfa agroecosystem was monitored in the Texas High Plains using protein markers. An enzyme-linked immunosorbent assay (ELISA) was used to precisely detect spray-applied protein markers adhering to *Lygus* insects collected from cotton and alfalfa. *Lygus* movement in the experimental cotton-alfalfa system was proven to be bidirectional, and was significantly affected by cotton phenological stages and alfalfa crop condition (as affected by water availability, senescence, or mowing). The genomic DNA of eight *Lygus* populations from across the Texas High Plains was genotyped using microsatellite markers developed in our laboratory, revealing three genetically distinct regional populations. Our continuing investigations of pest population movement and genetic structure will help in developing regional-level pest management strategies for Texas High Plains producers.



Influence of Soil Nitrogen Level on Seasonal Activity of Cotton Arthropods and Lint Yield under Drip Irrigation

M.N. Parajulee, S.C. Carroll, R.B. Shrestha, A.M. Cranmer, 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: Experimental plots of DP 104 B2RF cotton were planted on June 14, 2011; delayed due to lack of seed-bed planting moisture. The experiment was 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 lbs/acre. Cotton was planted (approximately 56,000 seeds per acre) in 30-inch rows and was irrigated with a subsurface drip irrigation system. We took soil samples from the experimental plots on April 27 in order to determine the residual nitrogen. Crop growth and arthropod activity were monitored throughout the season. Fertility treatments were applied on August 3 with a soil applicator ground rig.

Results: Cotton arthropod activity remained non-existent throughout the 2011 growing season due to unusually high temperatures and drought throughout the crop growing season. Higher rates of applied N (>100 lbs/A) resulted in significantly higher leaf chlorophyll content compared to that in lower or zero N plots. A strong correlation was found between leaf chlorophyll content and lint yield.

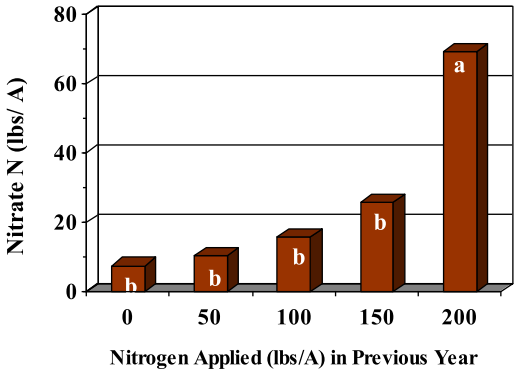


Fig. 1. Effect of previous years' nitrogen application rates on residual nitrogen in 2011.

Soil residual N was significantly lower in zero, 50, 100, and 150-lb N treatments compared with that in 200-lb N after the 2010 crop (Fig. 1). 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. Nevertheless, crop cut-out occurred abruptly at around the same time (60 DAP) in all treatments due to extreme temperature.

Although not significant, zero-N plots produced the lowest yield and yield increased curvilinearly, with highest average yield occurring in 150 lb N/acre treatment (Fig. 2). Because planting was severely delayed, overall yield was much lower than expected.

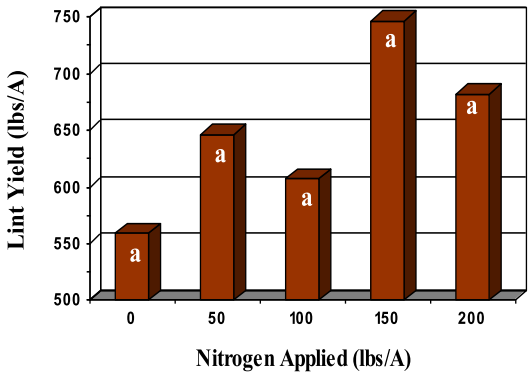


Fig. 2. Effect of nitrogen application rates on lint yield after 9 years of repetitive applications, 2011.

Cotton Cultivar Compensatory Response to *Lygus*-Induced Fruit Loss Under Low and High Irrigation

AUTHORS:

Megha Parajulee, Owen McSpadden, Ram Shrestha, Stanley Carroll, Wayne Keeling; Professor, Technician II, Research Associate, Research Scientist, Professor, Texas AgriLife Research

MATERIALS AND METHODS:

Plot Size:	4 rows by 50 feet, 3 replications
Planting Date:	May 3
Varieties:	DP 0935 B2RF, AMC 1532 B2RF
Fertilizer:	100-35-0
In-season irrigation:	Low = 6.6"; High = 13.2"
Insect treatment:	5 and 8 *Lygus* bugs (late instars) released per plant (5PP and 8PP) and Control (three treatments)
Insect release dates:	June 28, July 6, 12, and 19
Plant mapping dates:	June 28, July 5, 12, 19, and 27
Harvest Date:	October 17, 2011 (Hand-harvested)

Two cotton varieties (DP 0935 B2RF and AMC 1532 B2RF) were evaluated under low and high irrigation levels. *Lygus* bugs were released in each treatment combination (3 insect release treatments x 2 water levels x 2 cultivars x 3 replications = 36 plots) for four consecutive weeks to mimic a natural early season chronic infestation. The five and eight bugs per plant treatments were designed to exert significant insect pressure on fruiting cotton plants. Plant mapping was conducted immediately prior to each insect augmentation event and one additional plant mapping beyond the last bug release date to monitor the fruit set and retention profile as influenced by the bug augmentation treatment.

RESULTS AND DISCUSSION:

Lygus augmentation treatments resulted in significantly greater percentages of fruit shed than control plots in high-irrigation treatment, but low irrigation treatment plots were not significantly influenced by insect-augmentation treatments (Tables 1-2). For both cultivars, control plots underwent higher percentage of physiological fruit abscission in low-irrigation regime compared with that in high irrigation regime. Simultaneously, higher amount of irrigation water favored greater damage by *Lygus* (Table 2). Nevertheless, cultivars did not vary in their response to *Lygus* infestation and damage. Overall, lint yield was similar between the two cultivars (DP 0935 B2RF: 423 lb/A; AMC 1532 B2RF: 406 lb/A), whereas high-irrigation regime resulted in significantly greater yield than low-irrigation regime in both cultivars (Table 3). However, both cultivars were able to fully compensate the early fruit loss caused by *Lygus* injury (Table 3).

Table 1. Percentage square abscission in cotton induced by varying levels of three consecutive releases of *Lygus* nymphs in water x cultivar treatments, Lamesa, Texas, 2011.

Insect Density	Cultivar			
	AMC 1532 B2RF		DP 0935 B2RF	
	Low Water	High Water	Low Water	High Water
Control	13 a	8 b	16 a	5 b
Low	18 a	9 b	20 a	13 a
High	18 a	26 a	35 a	12 a

Table 2. Percentage square abscission in cotton induced by varying levels of three consecutive releases of *Lygus* nymphs compared between two cultivars, Lamesa, Texas, 2011.

Insect Density	Cultivar	
	AMC 1532 B2RF	DP 0935 B2RF
Control	11 b	11 b
Low	13 ab	16 ab
High	21 a	23 a

Percentage abscission varied with insect density treatment. Cultivars did not vary within insect density treatment.

Table 3. Lint yield (lb/A) in cotton after *Lygus*-induced pre-flower square loss in water x cultivar treatments, Lamesa, Texas, 2011.

Insect Density	Cultivar			
	AMC 1532 B2RF		DP 0935 B2RF	
	Low Water	High Water	Low Water	High Water
Control	287	543	250	707
Low	189	414	199	594
High	286	718	307	580
Average	254 b	558 a	219 b	627 a

Combined over water level, insect-induced fruit losses were all compensated in both cultivars. High-water regime resulted in significantly higher yield compared with that in Low-water regime in both cultivars.

QUANTIFICATION OF COTTON PLANT GROWTH RESPONSE TO COTTON FLEAHOPPER INFESTATIONS

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R. B. Shrestha
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Y. Yang
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Abstract

Cotton fleahopper (*Pseudotomoscelis seriatus* Reuter) is an important early-season cotton pest in Texas. Cotton fleahopper adults and nymphs feed upon cotton squares, inflicting heavy early-season square loss, and potentially altering the cotton plant growth pattern. The growth responses of cotton cultivars to various levels of cotton fleahopper injury are yet to be clearly characterized. In this study, the plant growth responses of two commercially available cotton cultivars (DP 161B2RF and FM 9063B2F) to cotton fleahopper injury were evaluated versus control plants. Control plants received no insect augmentation. Cotton fleahoppers were laboratory-reared on green beans to third- to fourth-instar nymphs and then carefully released in cotton plant terminals at the rate of 4-6 nymphs per plant to create the "high" cotton fleahopper infestation treatment. Cotton plant growth, development, and yield parameters were then monitored. Cotton plant height, root length, numbers of nodes, leaves, fruits and root-shoot biomasses were recorded from treated and control plants. DP 161B2RF plants were significantly taller than those of FM 9063B2F. Early-season fleahopper-induced fruit losses did not affect the cotton plant growth. In both study years, no significant plant height differences were observed between cotton fleahopper-infested and control plots. Unusually high temperatures and low precipitation in 2011 complicated comparisons between years. No significant plant biomass differences between control and fleahopper-infested plants were observed in 2010. However, in 2011, total plant biomass was significantly higher in fleahopper-infested plots than in control plots. In both cultivars, lint yield data suggest that cotton plants were able to compensate for 15-20% of cotton fleahopper-induced early-season fruit loss.

Introduction

USDA statistics show that Texas is the leading state in terms of annual cotton production. Texas produced 43.5% of total U.S. cotton in 2010. The Texas High Plains represents the largest virtually contiguous area of cotton cultivation in the world. Economically, cotton yields in the United States are impacted annually by a number of arthropod pest species (Williams 2011), making arthropod pest management an important factor in growing cotton. In fact, cotton arthropod pests adversely impacted U.S. cotton lint yields by 3.91% in 2010 (Williams 2011). Of approximately 25 major arthropod pest species encountered in Texas cotton fields, the cotton fleahopper (*Pseudatomoscelis seriatus*) is among those which are economically important (Parajulee *et al.* 2006, and 2008; Parker *et al.* 2000), and according to Williams (2011), ranked fourth, at 0.36%, in 2010 U.S. cotton losses.

Predominantly, cotton fleahoppers feed upon pinhead-sized or smaller squares, which results in abortion of these young fruits, thereby impacting yields. While cotton fleahopper feeding preferences serve as a baseline for their management in cotton fields, a detailed understanding of cotton plant responses to fleahopper damage remains unachieved. Cotton plant growth is sensitive to numerous environmental and management input factors. Cotton growth responses to various input factors are well-documented and growth models have been developed. However, the specific cotton plant responses to differential levels of injury inflicted by cotton fleahopper feeding remain unclear. In this study, a clearer understanding of these responses was the primary objective. A controlled experiment was conducted in a high-input subsurface drip-irrigated cotton field in the Texas High Plains to compare the responses of cotton, in terms of plant growth parameters and yields, to differential levels of early-season, induced cotton fleahopper injury. Two cotton cultivars were selected for testing.

Dr. L. T. Wilson's Agroecosystems Group in Texas (Texas AgriLife Research and Extension Center, Beaumont, Texas) has been developing a comprehensive Cotton Crop Production Decision System (CropDSS). They have

already developed a physiologically based cotton model, and irrigation and fertilizer management applications, or "advisors" have been incorporated in the model. A current goal of Dr. Wilson's team is to develop an integrated pest management advisor to augment the current system, and the secondary objective of this study was to provide comprehensive cotton plant response data in furtherance of that goal.

Materials and Methods

A 2-yr study was conducted in a "high-input" subsurface drip-irrigated cotton field at the Texas AgriLife Research farm near Lubbock, Texas (2010-2011). Two cultivars, DP 161B2RF and FM 9063B2F, were evaluated. Experimental plots measured 12 rows (40-inch spacing) by 100 ft, and were separated by 5-ft fallow alleys. Centrally located 10' sections of cotton were flagged in each plot for insect treatment deployment. Extraneous plants in each plot served as treatment buffers. In both years, experiments were laid out in a randomized complete block design with two cotton cultivars and two insect augmentation levels (control versus 4-6 cotton fleahopper nymphs per plant) with three replications. Woolly croton, a cotton fleahopper weed host, was harvested from locations in and near College Station, Texas, and then transported to Lubbock and placed in cold storage until fleahoppers were needed for the fleahopper augmented treatments. Conditions conducive to cotton fleahopper emergence were simulated in a laboratory environment in order to induce hatching of overwintered eggs embedded in the croton stems, and emerged cotton fleahoppers were subsequently reared using fresh green beans as a feeding substrate. At approximately ten days post-emergence, fleahopper nymphs were provided fresh cotton squares as a training substrate prior to field release. Considerable effort was expended to ensure synchronization of rearing efforts with cotton crop development for optimal release timing. Nymphal cotton fleahopper releases were initiated upon first observation of pinhead-sized squares in all plots, at which point cotton had progressed to the 4-5 true leaf stage. Weekly releases were conducted 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 rates of 4-6 nymphs per plant.

Weekly monitoring of cotton fruiting patterns via COTMAN^{TM} was initiated immediately prior to initial insect augmentation, and continued until physiological cotton cut-out [nodes above white flower (NAWF) = 5]. Plant biomass measurements were assessed at two-week intervals for a total of five sampling dates, and included detailed classification of plant parts. At each sampling date, five plants per plot were extracted from plots reserved specifically for destructive plant sampling. As may have been necessary for practical reasons, plants were stored in a large walk-in cooler (~41°F) until processing. As quickly as was feasible following plant extraction, plants were dissected and their variously classified parts separated for measurement. Plant height, root length, and numbers of nodes, main stem leaves, reproductive branches and leaves, vegetative branches and leaves, and total fruits (squares, flowers, and bolls) were recorded. Separated plant parts were then exposed to low-intensity heat for duration sufficient to achieve thorough desiccation, after which "dry" biomasses of the variously classified plant parts were recorded. Yield monitoring was conducted by hand-harvesting in flagged portions of treatment plots.

Results and Discussion

Cotton Fruit Loss:

The fruit loss monitoring data, as acquired via the SQUAREMAN component of COTMAN^{TM}, showed that augmented cotton fleahopper nymphal infestations significantly increased the percentage of first-position cotton square loss in both years. Average percent square loss was significantly higher in DP 161B2RF than in FM 9063B2F in 2011 (Fig. 1), but not in 2010. Although cotton fleahopper nymphs were released weekly for three weeks, evidence of square loss was not apparent until the third week, suggesting a possible 1-2 week delay in cotton plant response to fleahopper-induced injury. This delay is further evidenced by cotton plant responses detectable as many as fourteen days beyond the final insect augmentation treatment.

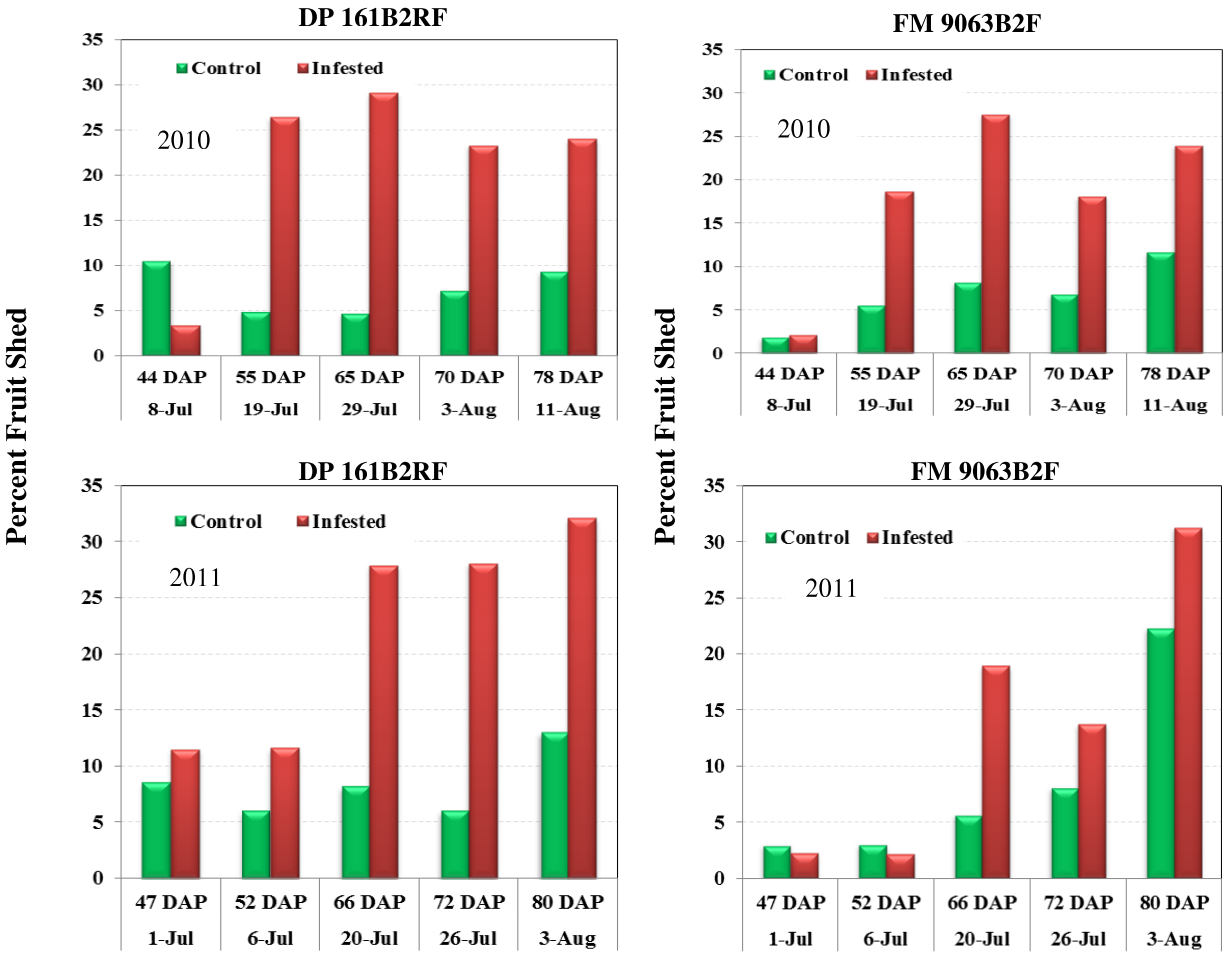

Figure 1. Percent first-position fruit shed induced by cotton fleahopper injury.

Plant Height:

Overall, plants were significantly taller in 2010 than in 2011, likely owing to the more favorable 2010 growing season. In both years, DP 161B2RF plants were significantly taller than those of FM 9063B2F (Fig. 2). In neither study year were significant plant height differences observed between cotton fleahopper-infested and control plots. In 2011, cotton fleahopper-infested plants were slightly, numerically taller than their control counterparts (Fig. 2). Cotton fleahopper-induced fruit shed, as indicated by 5-30% first-position square loss, was insufficient to significantly alter cotton plant heights in these two cotton cultivars.

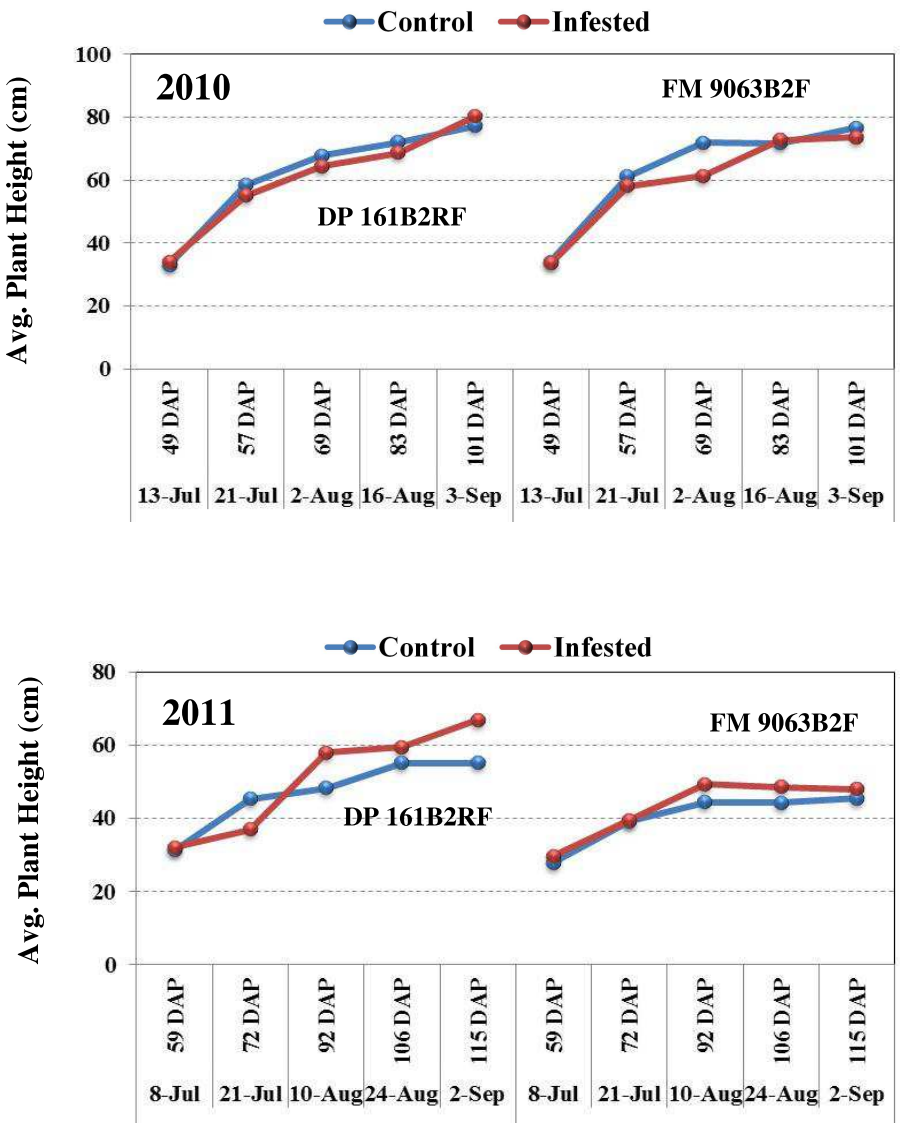

Figure 2. Effect of fleahopper injury on plant height.

Plant Biomass:

Cotton plant growth was rapid and total plant biomass significantly increased weekly until 80 DAP. No significant plant biomass differences between control and fleahopper-infested plants were observed in 2010 (Fig. 3). However, in 2011, total plant biomass was significantly higher in fleahopper-infested plots than in control plots (Fig. 3), primarily due to significant increases in root, branch, and fruit biomasses. As was previously mentioned, FM 9063B2F plants were significantly shorter than DP 161B2RF plants in both years. However, FM 9063B2F plants exhibited significantly greater leaf, root, branch, fruit, and total biomasses than their DP 161B2RF counterparts in 2010. More seasonable growing conditions in 2010 may have facilitated expression and observation of this varietal difference.

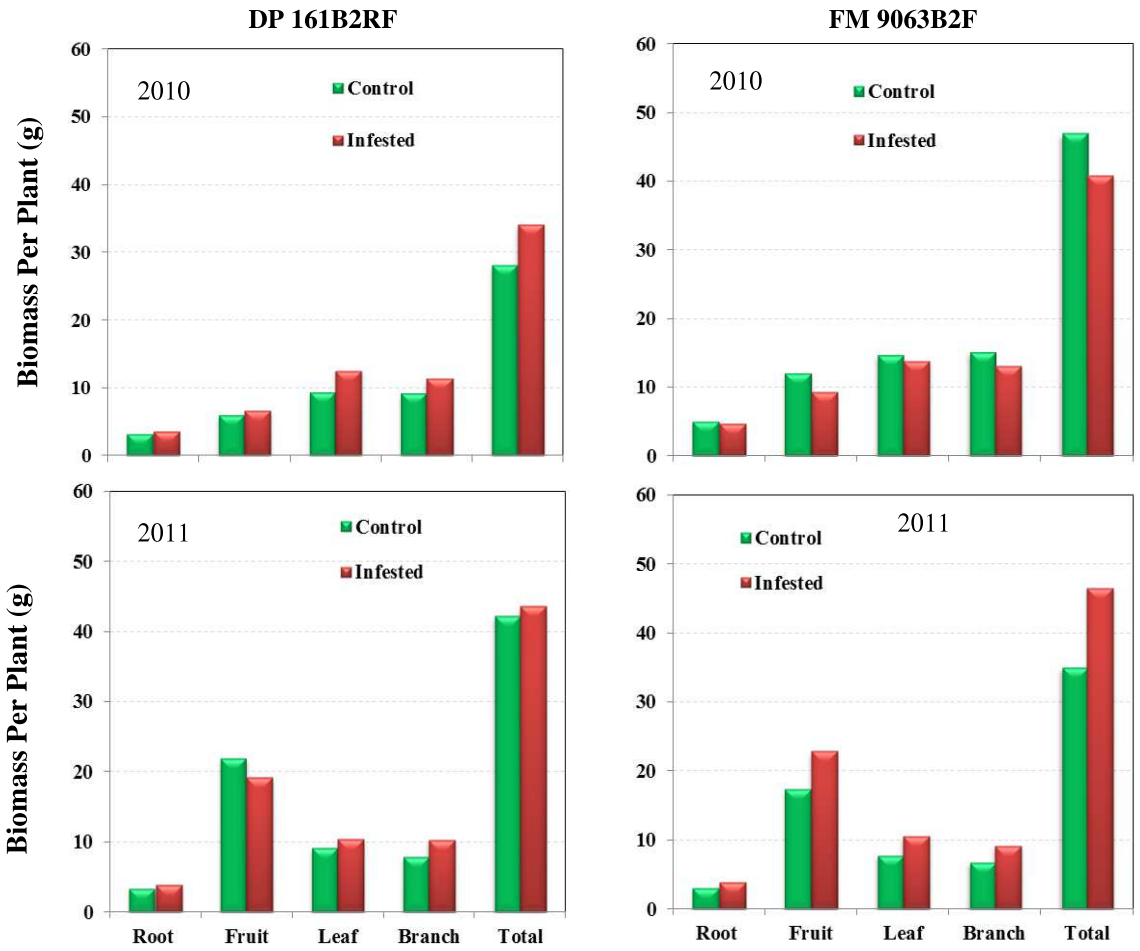

Figure 3. Effects of cotton fleahopper injury on the biomass of segregated cotton plant parts.

Lint Yield:

For both cultivars, plants were significantly shorter and lint yields were numerically lower in 2011 compared to that in 2010. There were no statistical differences in lint yields between these two years (Fig. 4). Neither cultivar showed statistical differences in lint yield between cotton fleahopper-infested and control plots. DP 161B2RF controls consistently exhibited higher numerical lint yields than their fleahopper-infested counterparts. Fleahopper-infested FM 9063B2F plants produced numerically higher second-position-or-greater lint yields than control plants, suggesting the possibility of a fruiting pattern response to infestation. These observations suggest the possibility of a differential varietal response to cotton fleahopper infestation; however, for clarification, further study with higher cotton fleahopper densities is recommended. Our data revealed that cotton may easily compensate for 5-30% fleahopper-induced, first-position fruit loss. The compensatory capacity of FM 9063B2F numerically exceeded that of DP 161B2RF in both years. Regardless of year, cultivar, or treatment, first-position contributions to total lint yield exceeded combined contributions from other fruiting positions.

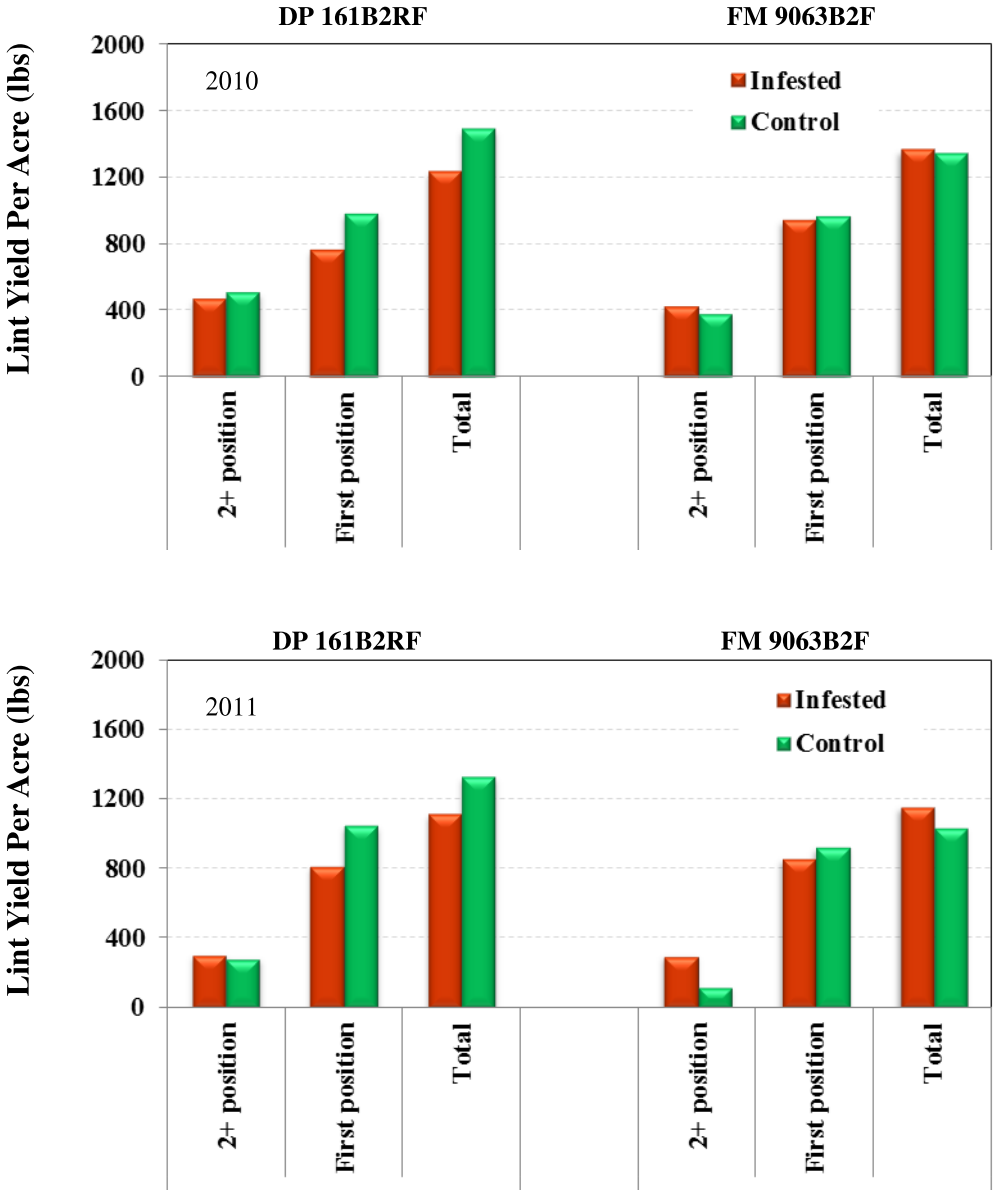

Figure 4. Effects of cotton fleahopper infestations on cotton lint yield.

Summary

It is clear that the stark contrasts between the two studies' growing seasons complicated analyses and interpretation of results. Although in 2011, cotton fleahopper-infested plants were numerically taller than their control counterparts, two-year combined data analysis failed to reveal significant differences in plant height between fleahopper-infested and control plots. In general, early-season fleahopper-induced fruit loss did not result in statistical differences in plant biomass. Yield data suggest that cotton plants are able to compensate for 5-30% of early-season fruit loss induced by cotton fleahoppers in both cultivars. Future investigation may provide clearer answers to questions posed in this study.

Acknowledgements

The authors wish to thank Kamala Adhikari, Anup Bastola, Michele Fekete, Andrew Stevens, and the late Anabel Reid, whose technical assistance was invaluable. Partial funding was provided by a grant from Texas AgriLife Research Cropping Systems Initiative.

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POTENTIAL OF ORGANIC PESTICIDES FOR WESTERN FLOWER THRIPS MANAGEMENT IN SEEDLING COTTON: EFFECT ON PLANT PARAMETERS

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Abstract

Western flower thrips [*Frankliniella occidentalis* (Pergande)] is an important pest of seedling cotton. Seed treatments, seed-bed applications of aldicarb or foliar applications of various insecticides are common practices for managing this early-season cotton pest. However, available options for thrips management in organic cotton production systems are very limited. The efficacy of three organic products: Entrust® Naturalyte® Insect Control (spinosad microbial), PyGanic® Crop Protection EC 5.0 II (pyrethrum), and Surround® WP Crop Protectant (kaolin clay) were evaluated and compared to an untreated control. Treatments were initialized during the week of cotton seed germination and applied weekly for three weeks thereafter. In conjunction with treatment applications, cotton was sampled weekly via visual and absolute (plant washing) methods. In addition to thrips densities, cotton seedling total (above and below soil surface) biomass, leaf area, and leaf chlorophyll indices were recorded. Despite relatively low seasonal thrips densities, Entrust® was observed to be most effective in controlling thrips, followed by Surround®, PyGanic®, and the control. Cotton seedling total biomass was highest in Surround®-treated plots, followed by PyGanic®, Entrust®, and control.

Introduction

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 during 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). Williams (2011) reported that for 2010, Texas had an estimated total of 5,343,620 acres of cotton infested with thrips, which resulted in 8,937 cotton bale loss due to thrips damage.

Demand of organic cotton is increasing globally. The Organic Trade Association's 2010 Survey report indicated organic fiber sales in the United States grew by 10.4% in 2009 over the previous year, reaching a sales total of $521 million. In response to rising consumer interest in organic cotton, organic production systems, though a specialized niche representing a fraction of the US cotton market, are garnering increasing cotton producer attention. Organic production poses new and unique problems for cotton producers in terms of arthropod pest and weed control issues as compared to conventional production systems where a variety of agricultural chemicals are available and allowed. This is due to pesticide use limitations imposed by organic, or, as is often debated, “sustainable” guidelines. Insect pest management practices under organic cotton production remain essentially unexplored, and although numerous products approved and labeled for acceptable use in organic cotton production systems are available, a paucity of information directly comparing their efficacies against selected target key insect pests, such as thrips, represents an opportunity for beneficial, methodical scientific investigation. In addition, with the recent EPA/industry agreement to cancel all registered uses and remove aldicarb-containing products from the market, which in the past conferred superb thrips control in conventionally grown cotton for a number of years, there exists a need for effective alternatives, including those which may have parallel uses in organic production systems. It was recently announced that aldicarb will return to the market (new company, product trade name, labels and packaging) for an indefinite period of time with a restricted use classification. This development will likely be beneficial to conventional cotton production systems, but uses of these types of pesticides is not allowed under organic cotton production systems, thus other alternatives remain needed at the present time.

In this study, a selection of available organic products were evaluated to determine their efficacy in managing thrips in early-season cotton, with the intention of possibly supplying growers in both conventional and organic production systems with information which may facilitate thrips management decision-making in early-season irrigated, conventional and/or organically produced cotton. From among numerous products available for insect control (and successfully applied) in organic crops with potential efficacy against thrips in organically grown cotton, Entrust® Naturalyte® Insect Control (spinosad microbial), PyGanic® Crop Protection EC 5.0 II (pyrethrum), and Surround® WP Crop Protectant (kaolin clay) were selected for evaluation in this study.

Over-the-top organic or inorganic pesticide spray applications may affect cotton seedling growth and development in several ways. First, the pest population may be regulated, which may, in turn, alter the level of crop injury, the effects of which may be observed in terms of plant health, growth, and development. Second, the active ingredient of the product, or some other property of the product itself may directly alter plant photosynthesis, respiration, or transpiration, as examples. Third, these phenomena may manifest cumulatively or simultaneously. In addition to evaluating the thrips control efficacies of the products mentioned, a secondary objective of this study involved assessing, under an irrigated organic cotton production system, the effects of various levels of early-season thrips infestation, as actuated by the subject products, on cotton plant vegetative and reproductive development and yield.

In evaluating the two study objectives, it was hypothesized, perhaps broadly, that the selected organic products would suppress thrips densities in irrigated organic cotton differentially, and assuming as much, that the resulting differential thrips densities would facilitate evaluation of subsequent seasonal cotton growth, development, and yield parameters.

Materials and Methods

An irrigated organic cotton field was planted with cultivar FM 958 on 10 May 2011 near Muleshoe, Texas. The crop was cultivated using standard northern Texas High Plains organic cotton production practices. The experiment was deployed in a randomized complete block design with four treatments [three organic products - Entrust® Naturalyte® Insect Control (spinosad microbial), PyGanic® Crop Protection EC 5.0 II (pyrethrum), and Surround® WP Crop Protectant (kaolin clay) plus an untreated control] in order to achieve differential thrips densities in treated plots. Treatments were initiated during the week of cotton seed germination and applied weekly for three weeks thereafter. Simultaneously, adult and juvenile thrips were quantified via weekly visual sampling of ten plants per plot, via insect dislodgement into white polystyrene cups, facilitating observation by human samplers. In concurrence with and for verification of visual sampling data, adult and juvenile thrips densities were also quantified via "absolute" sampling, which involved whole-seedling immersion of ten plants per plot and subsequent, lengthy processes of sieve "washing" and vacuum filtration, followed by visual quantification under 10X or higher magnification. In addition, leaf chlorophyll, leaf area, plant height, and root length were measured on a weekly basis for ten weeks. Plant biomass (root, leaf, branch, and reproductive plant parts) was measured weekly for ten weeks. Cotton crop fruiting profiles were assessed via weekly COTMAN™ plant mapping. Lint yields were measured in each plot.

Results and Discussion

Record-breaking high temperatures and drought conditions during the 2011 growing season may have partially been the reason for the negligible (considerably below economic threshold levels) early-season western flower thrips densities in all of the experimental plots/treatments. Dry winters and springs result in poor germination and growth of small grain crops (wheat, rye, etc.) and roadside weeds, both of which provide non-cotton hosts for thrips densities to build up for later movement to small pre-squaring cotton plants. Under ideal conditions, growth and development parameters would be carefully evaluated in reference to normal or "expected" background thrips densities, yet the 2011 growing season provided the unique opportunity to evaluate the effects of differential thrips densities, as actuated by the selected organic product treatments, on plant root and shoot length, leaf area, and biomass of variously categorized plant parts.

Effect on Thrips Density

In untreated control plots, thrips densities were expected to increase seasonally due to the potential for thrips reproduction and, further, due to persistent immigration from nearby source habitats. However, thrips densities, as assessed weekly, decreased in all plots as the cotton plants grew older, indicating consistently decreasing cotton suitability as a thrips host across plots, perhaps due to unseasonably hot and dry conditions, which may have

reduced overall plant quality of the host. Consistent observations of decreasing thrips densities across plots suggests that any treatment effects on the thrips population were overcome by environmental or crop factors. However, despite relatively low overall thrips densities, the average number of thrips (adult+larvae and adults only) was significantly higher in untreated control plots than in Entrust®- and Surround®-treated plots (Fig. 1).

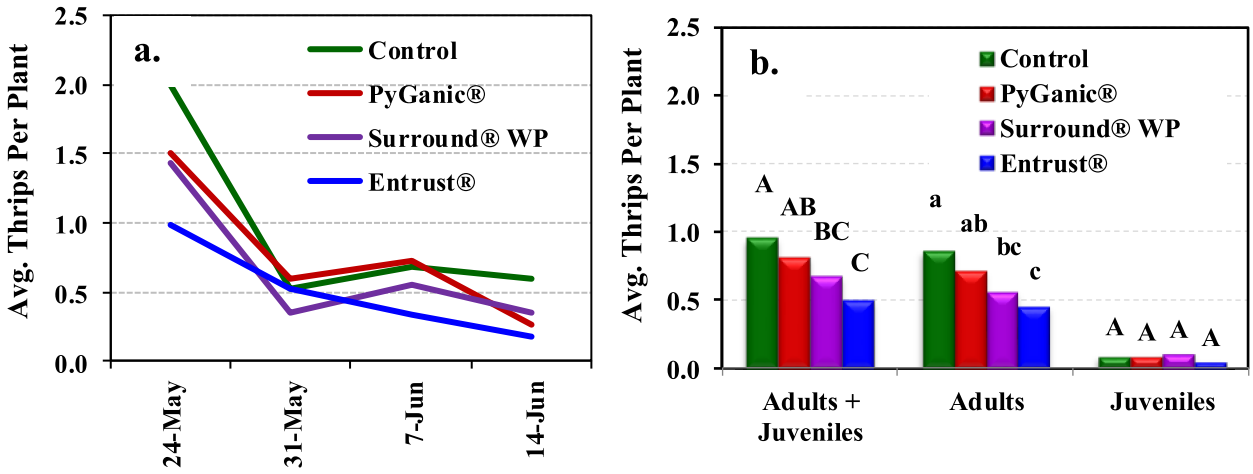

Figure 1. Effect of organic products on thrips abundances; a) Weekly thrips population dynamics, and b) Four-week average densities of adults, juveniles and total thrips.

Effect on Shoot and Root Lengths

Ten-week average data revealed that shoot lengths of plants in the Entrust®-treated plots exhibited significant stunting versus plants in Surround®-and PyGanic®-treated and untreated control plots (Fig. 2a), but root lengths (Fig. 2b) were statistically similar across treatments. Thrips feed on mesophyll intracellular materials, which results in surrounding epidermal collapse. More extensive injury completely disrupts leaf cellular structure, causing mesophyll and epidermal cell desiccation. Theoretically, high thrips densities should result in greater leaf damage, ultimately reducing photosynthesis in the infested plant, which, in turn, should negatively impact root and shoot growth. In conducting this experiment, thrips densities were observed to be relatively low, leading to the expectation of plant damage insufficient to produce statistically significant differences in cotton leaf chlorophyll content, which might, given greater observed thrips densities, have also impacted root and shoot lengths. Contrary to this extemporaneous expectation, while thrips densities in Surround® and Entrust®-treated plots were similar, the average height (shoot length) of Entrust®-treated plants was significantly lower than that of Surround®-treated plants, indicating a possible physiological effect of one of these two organic pesticide products on plant growth. This assertion needs further verification via a separate physiological study.

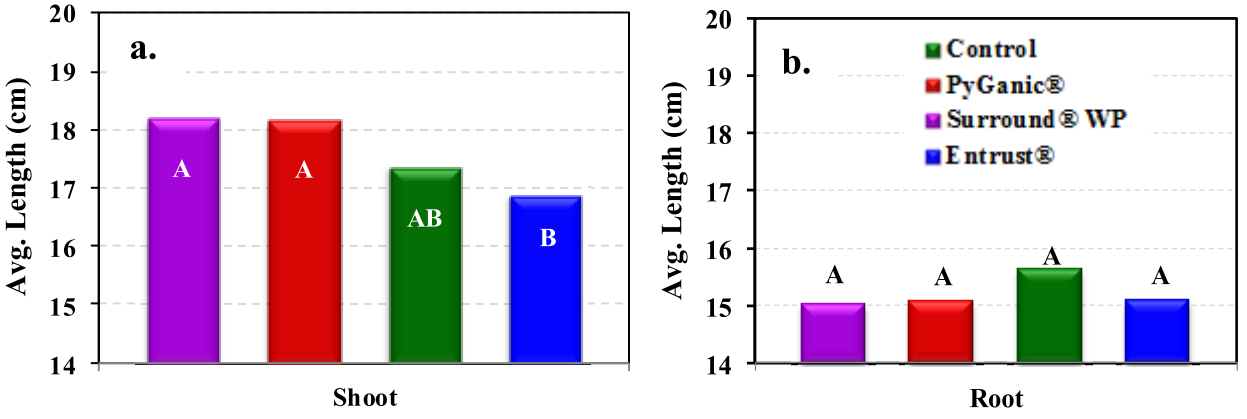

Figure 2. Average shoot (a) and root (b) lengths (n=10 weeks) as affected by selected treatments.

Effect on Leaf Area

Average leaf area per plant was low in all treatment plots for the first four weeks of growth (until mid-June), afterwards leaf area increased rapidly until mid-July. Leaf area increased most rapidly after mid-July (Fig. 3a). Generally, the first few weeks following cotton germination are considered to be the most critical thrips damage vulnerability window, but even during this well-known thrips-sensitive period, significant differences in plant leaf area between treatment plots were not observed. Average main-stem leaf size (Fig. 3b) was also statistically similar across all treatments. These results were found, likely, due to the relatively low (considerably below economic threshold) seasonal thrips population (Fig. 1a) and the associated negligible cotton crop damage. However, ten-week average data showed that average leaf areas in treated plots and untreated control plots differed significantly. Leaf area was significantly lower in control plots than in from PyGanic®- and Surround®-treated plots. Additionally, separation in average leaf area between treated and untreated plants was observed to have begun late during the season, specifically in July. With no observed treatment effect on leaf area during the first four weeks of cotton growth, late-season treatment effects were not expected, but data analysis clearly indicates that untreated plants exhibited lower average leaf area (Fig. 3). Residual organic pesticidal effects or peripheral effects resulting in plant physiological attenuation or enhancement are suspected contributors to this observation.

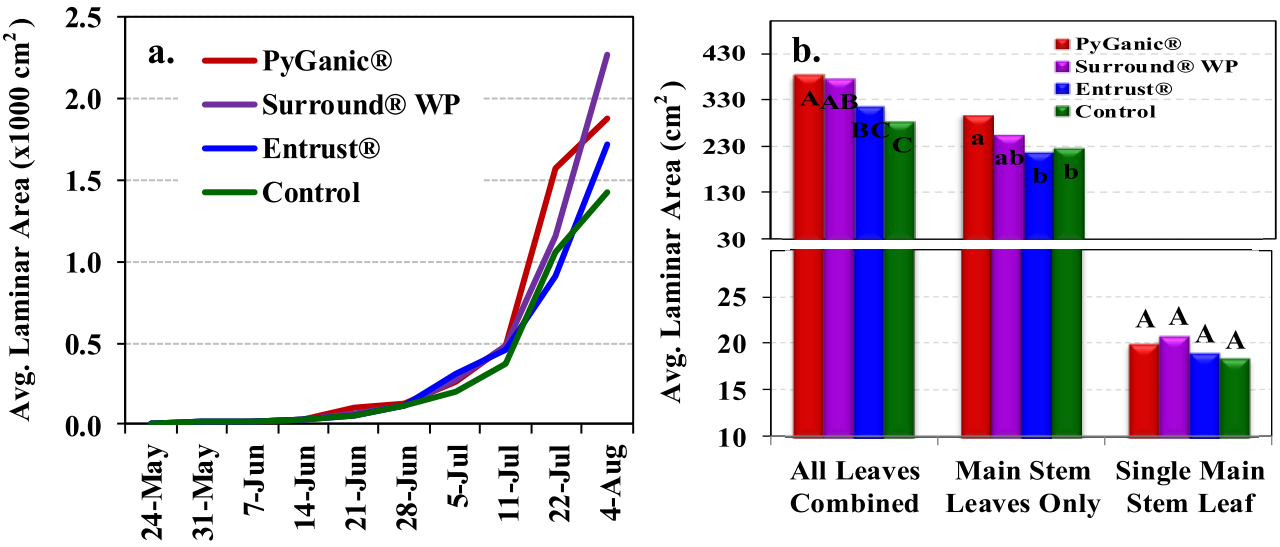

Figure 3. Influence of organic product application treatments on foliar growth;
a) Weekly average leaf area per plant, and b) Leaf area per plant averaged over ten weeks.

Effect on Plant Biomass

Plant biomass measurements, as assessed, were observed to have followed a pattern similar to that of leaf area. This was congruent with expectations, particularly given the importance of foliage in plant growth and development. For the first five weeks following seedling emergence, the rate of cotton plant biomass accumulation was slow, but began to increase rapidly at the end of June to a peak during the second week of July (Fig. 4a). Significant influence of organic product spray application treatments on cotton plant biomass accumulation were not observed until after eight weeks of crop growth, after which differences became obvious, approximately in late July and early August. Given an expectation of strong treatment effects, anticipating observed early-season differences would have been reasonable, but an observation of treatment effects only during the late season is difficult to explain. One possibility is that treatment effects may have been slight in magnitude but enduring in terms of plant growth, however; this hypothesis requires evaluation with formal experiments. Overall dry biomass (Fig. 4b) was significantly lower in plants from control plots than plants from Surround®-treated plots. Root, branch, leaf, and fruit biomasses were significantly lower in the control plots, likely due to higher thrips densities (Figs. 1 and 4), which may have

suppressed plant growth. Harsh environmental conditions in 2011 and concurrent, relatively low thrips densities inhibited proper evaluation of the effects of early thrips injury on plant parameters. This study will be repeated over the next two years to better elucidate these relationships.

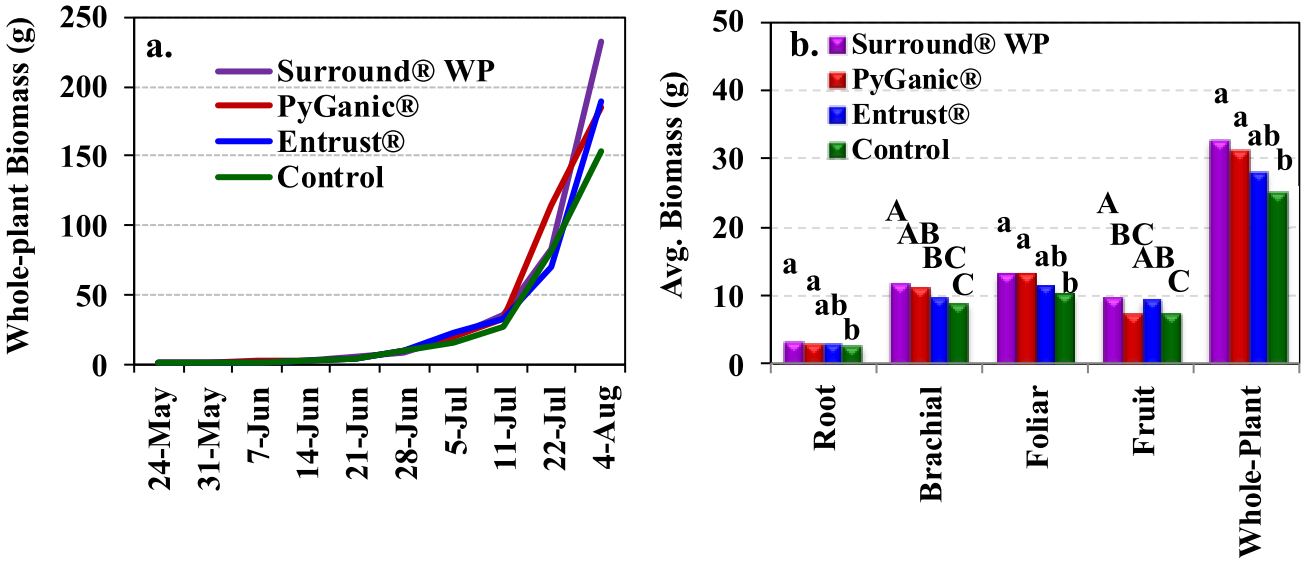

Figure 4. Influence of organic product treatments on cotton plant biomass; a) weekly average plant biomass, and b) ten week average biomass of different plant parts.

Summary

Record-breaking high temperatures and drought conditions during the 2011 growing season may have resulted in early-season western flower thrips densities considerably below those observed during more seasonable years, and certainly well below economic threshold levels. Higher densities would obviously have provided a more suitable situation for evaluation of differential thrips densities on cotton plant growth, development, and yield parameters. Nonetheless, despite seasonal drawbacks pertaining to the subject pest population, the average number of thrips (adult+larvae or adults only) was observed to be significantly higher in untreated control plots versus Entrust®- and Surround®-sprayed plots. Plants from Entrust®-treated plots were significantly stunted versus Surround®- and PyGanic®-treated plots. Total leaf area and overall dry biomass were significantly lower in plants from control plots than plants from Surround®-treated plots. Specifically, root, branch, leaf and fruit biomasses were significantly lower in the control plots, likely due to higher thrips densities in control plots. Under higher thrips densities, differences might have been more pronounced. Root length, average mainstem leaf size, and lint yields (data for which are not shown) were statistically similar across all treatments. More seasonable climatic conditions might have facilitated evaluation of the effects of early-season thrips injury on plant parameters. For more thorough investigation, two more years of study are planned.

Acknowledgements

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2011 FINAL REPORT

Cotton Incorporated Core Program

Project Number: 09-596

Evaluating Varietal Difference in COTMAN Compensation Capacity Value in Drip Irrigated Cotton Using *Lygus*-Induced Fruit Damage

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Evaluating Varietal Difference in COTMAN Compensation Capacity Value in Drip Irrigated Cotton Using *Lygus*-Induced Fruit Damage

Project Summary

A three-year (2009-2011) study was conducted in the Texas High Plains to quantify the lint yield compensatory abilities of two commercially available cotton cultivars, DP 104 B2RF (early maturity) and DP 161 B2RF (long-season), following pre-flowering insect-induced fruit loss or artificial (manual) square removal. Differential levels of pre-flower square loss were achieved by augmenting natural populations of western tarnished plant bugs [WTPB; *Lygus hesperus* (Knight)], with laboratory-reared nymphs released three times at weekly intervals or via manual fruit removal during the first three weeks of squaring. Treatments included: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray applications (spray control, SC), 5) artificial (manual) removal of pre-bloom first position squares (ASC_{1st}), and 6) artificial manual pre-bloom removal of all fruits (ASC_{all}). At the end of the fourth week of squaring, all treatment plots were sprayed with insecticide to eliminate further insect-related fruit losses. Subsequently, plots were managed for pests to the maximum possible extent.

The test was deployed in a 2 (cultivar) x 4 (insect-augmented and control treatments) x 4 (replications) factorial arrangement with a randomized complete block design (32 total plots). ASC_{1st} and ASC_{all} were merged into small areas within the eight SC control plots (2 cultivars x 4 reps). In all three study years, *Lygus* augmentation in pre-flowering cotton consistently resulted in significantly higher fruit loss percentages in both cotton cultivars than corresponding controls (UC and SC). Low, High, ASC_{1st}, and ASC_{all} significantly reduced first-position lint yields. Averaged across years and cultivars, SC, UC, Low, High, ASC_{1st}, and ASC_{all} produced 916, 928, 745, 779, 480, and 476 lbs/A first-position lint yields, respectively. Conversely, Low, High, ASC_{1st}, and ASC_{all} significantly increased lateral fruiting position lint yields. Averaged over three years, SC, UC, Low, High, ASC_{1st}, and ASC_{all} produced 298, 275, 385, 423, 768, and 631 lbs/A lateral position lint yields, respectively. In all cases, cotton plants were able to fully compensate for early-season fruit loss in terms of lint yields. This occurred largely via significantly increased overcompensation by lateral fruiting positions. It should be noted that, as with crop yields in general, the level of lint yield compensation is dependent upon the availability of input resources (e.g., water, plant nutrients, heat units) following early-season fruit losses. While our study clearly suggested cultivar differences related to micronaire and other lint quality parameters, three-year average micronaire values fell generally within or extremely near the normal, preferred range of 3.7-4.2, for both cultivars evaluated. These data indicate that the Texas Southern High Plains growing conditions generally do not incur significant quality compromise in compensatory lint following *Lygus*-induced early square loss. Nevertheless, it was apparent that all first position squares manually removed plots (ASC_{1st}) produced significantly lower mic cotton compared with spray control plots in both cultivars and in all years, demonstrating that excessive removal (100% first positions) of older first position pre-bloom squares induced the compensatory response of cotton plant to produce new squares that caused an increased proportion of less mature bolls during harvesting and ultimately reflected as low mic cotton.

Introduction

A previous three-year study evaluating the fruit loss compensation capacity of cotton grown in the Texas High Plains (THP), using pre-flowering artificial (manual) square removal treatments ranging from 0-100%, indicated that irrigated cotton can withstand 100% first-position pre-flowering square removal with no subsequent yield impact (Leser et al. 2004). Dryland cotton was observed to withstand 50% removal with no significant yield impact. Maturity was impacted substantially under the most severe treatments. Leser et al. (2004) concluded that irrigated cotton could tolerate 40% pre-flowering square loss with no associated yield or maturity impacts. However, these data were generated based on manual pinhead square removal. Previous researchers have indicated differential plant reactions to insect-induced fruit loss, likely due to insect feeding mechanisms, for example, as a result of insect secretion of enzymes and other chemicals in the process of feeding (Sadras 1995).

Our continuing research demonstrates cotton's capability to compensate for *Lygus hesperus*-induced early-season fruit loss, but at a lower level than has been observed following manual square removal. Higher crop inputs are required to achieve this effect, which has been observed to correlate strongly with soil moisture, other input variables, and overall plant vigor. Using *L. hesperus* nymphs, Barman et al. (2007) and Parajulee et al. (2008) demonstrated this phenomenon, wherein cotton was incapable of compensating for more than 25-30% of insect-induced fruit loss under limited irrigation. In the THP, water management is a critical crop management issue, but production has advanced to afford THP producers opportunities to use high-input resources to maximize production yields and profitability. Current research at the Helms Farm near Plainview, Texas demonstrates some of these advances, including high-yielding cultivars, near-100% irrigation efficiency (particularly sub-surface drip irrigation), aggressive insect management regimes, and proper nutrient management. Our research suggests that cultivars differ in their abilities to support plant bug infestations. This phenomenon holds true for both *Lygus* bugs and cotton fleahoppers. Expected differences in cultivar susceptibilities to plant bug injury and subsequent square loss demand characterization, especially as they pertain to the development of fruit loss-based plant bug economic thresholds.

The primary objective of this study was to quantify the yield-compensatory potentials of selected cotton cultivars (early maturity versus long-season) following exposure to differential levels of insect-induced fruit loss to facilitate economic threshold refinement. Improved and refined economic thresholds are expected to reduce the necessity for insecticide applications and, consequently, mitigate problems due to secondary pests. A secondary objective was to further quantify cotton's compensatory potentials following early-season insect-induced square loss versus early-season artificial (manual) square removal.

Materials and Methods

This three-year study was conducted at the Texas AgriLife Research & Extension Center farm located in Lubbock County near New Deal, Texas. A 5-acre sub-surface drip-irrigated field was used. Two cultivars, DP 104 B2RF (early maturity or short-season) and DP 161 B2RF (long-season), were evaluated. Test plots were planted on May 18, May 25 and May 11 in 2009, 2010 and 2011, respectively. Experimental plots were 12 rows wide and 75 ft long with 5-ft alleys. Row spacing was 40 inches. Four central rows were selected in each plot for plant bug treatment deployment and plant mapping. Pre-flowering *Lygus* bug-induced square loss treatment levels were

achieved by augmenting any naturally occurring plant bug populations with laboratory-reared 3rd-instar *L. hesperus* nymphs weekly during the first three weeks of squaring. Treatments included: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray application (spray control, SC), 5) pre-bloom artificial (manual) removal of all first-position squares and spray control (ASC_{1st}), and 6) pre-bloom manual removal of all fruits and spray control (ASC_{all}). In explanation for the stated ranges of bug augmentation (Low and High), it should be noted that following the 2009 season, the treatment release numbers were increased to the peaks of the stated ranges in 2010 and 2011 to encourage greater early-season fruit loss, facilitating a more complete evaluation of each cultivar's lint yield compensatory ability. The test was deployed in a 2 (cultivars) x 4 (treatments; High, Low, UC and SC) x 4 (replications) factorial arrangement with a randomized complete block design for a total of 32 experimental plots. The two manual removal treatments (ASC_{1st}, ASC_{all}) were overlaid into small areas within the eight SC control plots (2 cultivars x 4 reps). These treatments allowed for good comparisons of cotton plant responses to early-season insect-induced fruit loss to artificial manual fruit removal.

Insect augmentation began upon initial pinhead-sized square observation in all bug-augmentation treatment plots and consisted of 3 consecutive weekly releases of *L. hesperus* nymphs in the designated plots (Fig. 1). Plant bugs were aspirated from a laboratory colony (3rd instar) into 0.75-inch X 1.5-inch plastic vials. The bugs were carefully deposited on the mainstem terminal of each plant. The releases were conducted on July 2, 7, and 13 during 2009; July 1, 8, and 15 in 2010; and June 30, July 7, and 13 in 2011. Artificial square removal treatments followed the same schedule.

Immediately prior to each release date, fruit-set monitoring was conducted using the SQUAREMAN component of the COTMAN™ program in order to follow any differing plant growth or fruiting responses resulting from the variable levels of early-season fruit removal (Fig. 1). Pre-flower SQUAREMAN data were collected on July 1, 6, and 13 in 2009; June 30, July 7, and July 14 in 2010; and June 29, July 7 and 13 in 2011. Complete in-season plant mapping was conducted in all plots one week after the final *Lygus* release per study year, on July 21, July 26, and July 20 during 2009, 2010, and 2011, respectively. Immediately following, insecticides (Orthene, 2009; Carbine/Holster tank mix, 2010; Orthene, 2011) were applied to all treatment plots to minimize further fruit loss. Season-long monitoring followed in order to keep the plots generally pest-free until harvest.

COTMAN-SQUAREMAN plant mapping continued until physiological cut-out [Nodes Above White Flower (NAWF) =5], after which heat unit accumulations were monitored to aid decisions regarding harvest-aid application timing. Final complete plant mapping was conducted each study year immediately prior to harvesting (Fig. 1).

Harvest-aid chemicals were applied based on heat unit accumulations and percent open bolls for each cultivar. FirstPick® and DEF® 6 (boll opener/defoliation) were applied during 2009 in early-(DP 104 B2RF) and late-maturing (DP 161 B2RF) cultivars on September 30 and October 14, respectively. One week after boll opener/defoliant application in each cultivar, Gramoxone® was applied to finish crop preparation for harvest. Harvest dates for 2009 were October 15 (DP 104 B2RF) and 27 (DP 161 B2RF). For 2010, both cultivars were sprayed with a tank-mixture of Finish® plus DEF 6® on October 16, followed by an application of Gramoxone® on October 21. Both cultivars were harvested on October 27, 2010. In 2011, a tank-mix of Finish® 6 Pro and DEF®

6 was applied on September 21 and 29 on the DP 104 B2RF and DP 161 B2RF, respectively. A final treatment of Gramoxone® was then applied September 27 and October 7 on DP 104 B2RF and DP 161 B2RF, respectively. In 2011, the two cultivars were harvested separately on September 30th (early maturity DP 104 B2RF) and October 11th (full season DP 161 B2RF). Fiber samples were analyzed for lint quality parameters at the Cotton Incorporated Fiber Testing Laboratory (North Carolina).

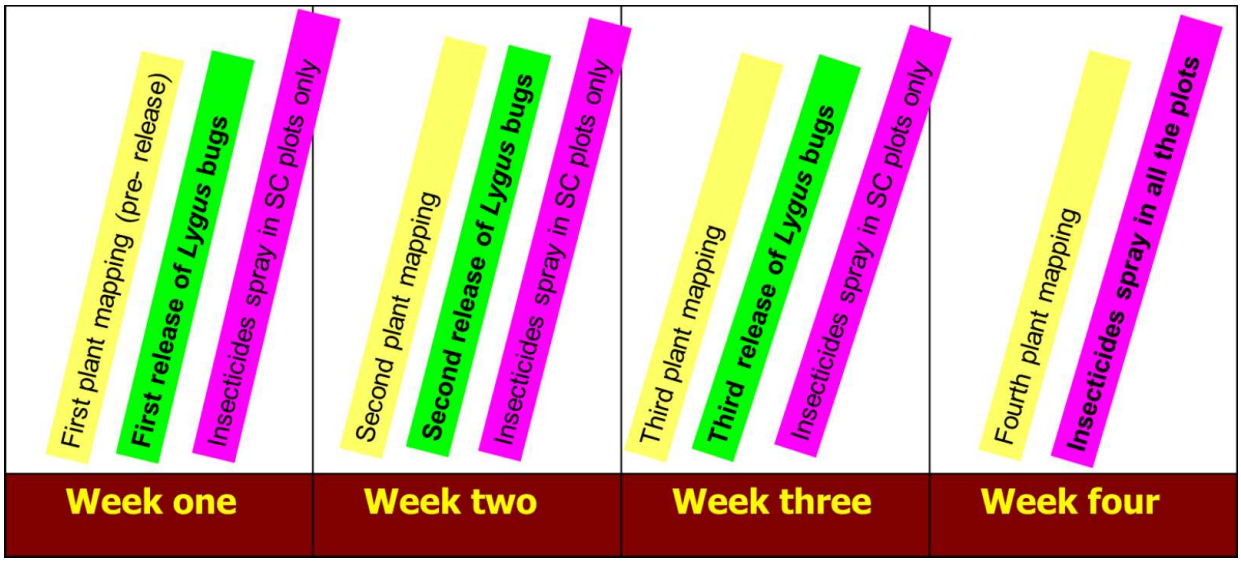

Figure 1. Experimental activity sequence related to the release of *Lygus* bugs and cotton fruit loss monitoring. Week one corresponds to first week of the pin-head squaring stage in each study year.

Results and Discussion

COTMAN™ Target Development Curve (TDC) and Fruiting

Figure 2 illustrates yearly cotton development and squaring initiation, which generally adhered to or preceded the COTMAN™ target development curve (TDC; black line), but peak fruiting was lower than the TDC in all years, particularly in control plots (blue and purple lines). Observed SQUAREMAN curves peaked 7-10 days later in DP 161 B2RF than in DP 104 B2RF, indicating cotton reproductive profile and crop maturity varietal differences. The crop maturity profiles of both cultivars varied among years. In 2009 and 2011, DP 104 B2RF reached cut-out (NAWF = 5) approximately 1 week earlier than DP 161 B2RF, whereas in 2010, both cultivars exhibited similar crop maturity timing. In 2009, *Lygus* augmentation treatments did not significantly affect the cotton crop maturity profile in either cultivar, whereas in 2010 and 2011, the *Lygus* augmentation treatments clearly caused significant delays in peak squaring and crop cut-out (NAWF = 5). In 2011, *Lygus* infestation delayed crop cut-out by approximately 3 weeks. Although cotton may compensate for early-season *Lygus*-induced fruit loss, a maturity penalty and potential lint quality discounts may be incurred. Possible lint quality penalties due to early-season cotton fruit loss will be discussed in more detail below.

Percent Fruit Loss

In all three study years, pre-flower *Lygus* augmentations in cotton consistently resulted in significantly higher fruit loss percentages in both cultivars than corresponding cultivar controls (UC

and SC) (Fig. 3). At four weeks into cotton squaring, the highest percent fruit losses were observed in *Lygus*-augmented treatments in 2011 (34-73%), followed by 2010 (45-58%) and 2009 (20-26%). Observations of elevated fruit loss in 2010 and 2011 likely resulted from *Lygus* augmentation increases in the Low and High treatments made in order to achieve higher early-season fruit loss percentages for better evaluation of the maximum crop compensation capacity in each cultivar.

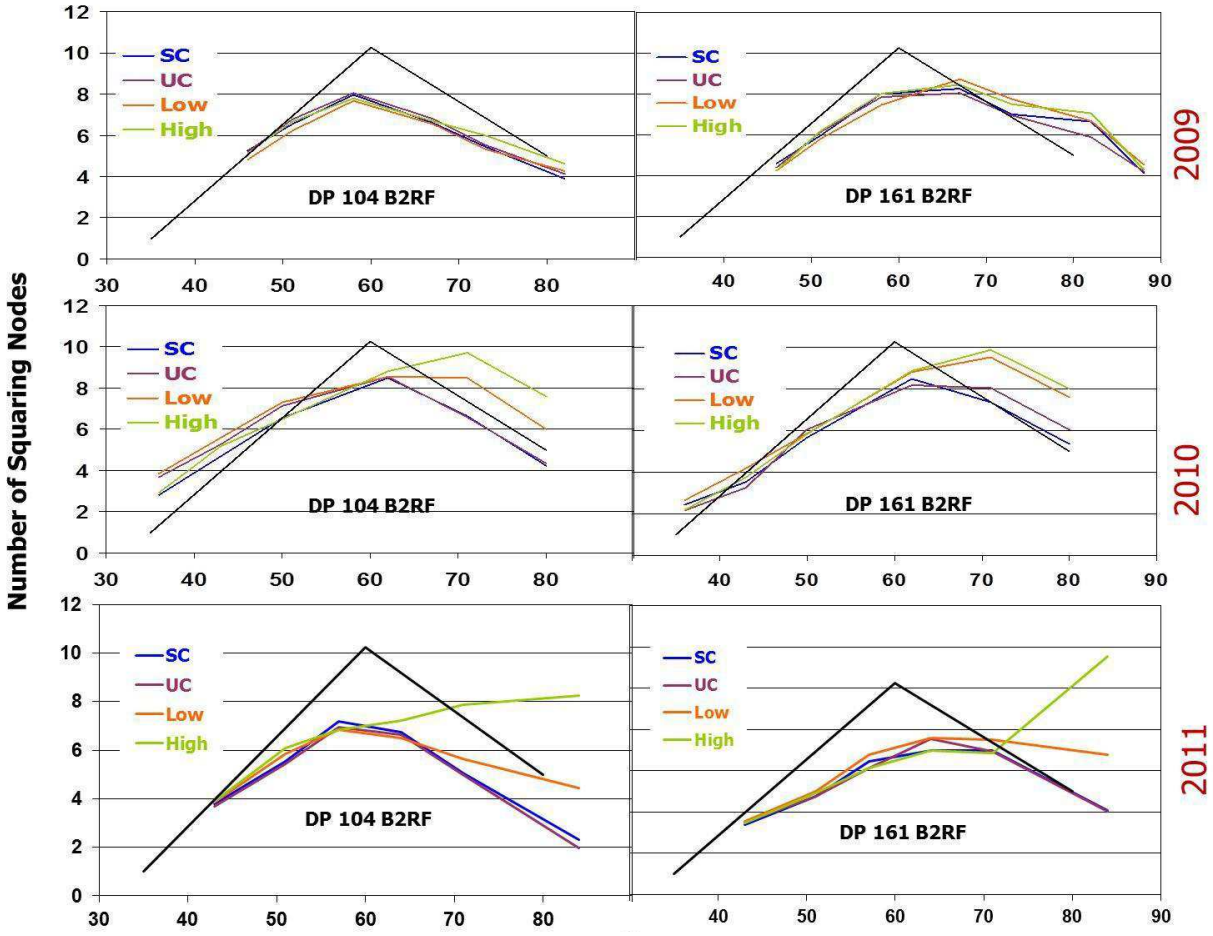

Figure 2. COTMAN^{TM} software cotton fruiting profiles as influenced by *Lygus*-induced fruit loss, and target development curves (black lines). For each year, two cultivars, DP 104 B2RF and DP 161 B2RF, were monitored. Lubbock, TX, 2009-2011.

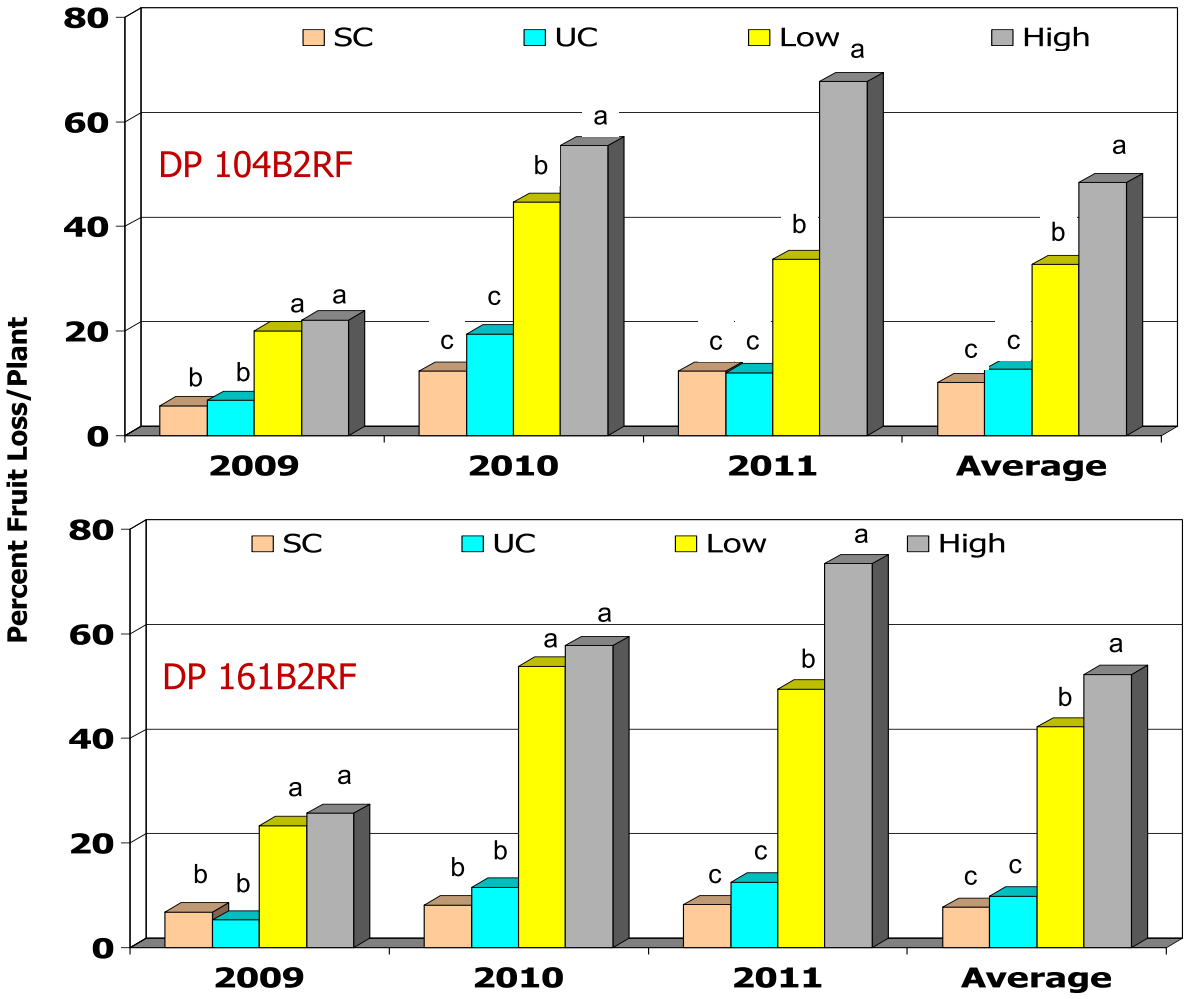

Figure 3. Observed percent cotton fruit loss one week after final *Lygus* release in DP 104 B2RF and DP 161 B2RF cotton cultivars. Within a single cultivar and year (or 3-yr average), treatment means with the same lowercase letter are not significantly different at P > 0.10. Lubbock, TX, 2009-2011.

Lint Yield

In all years, both cultivars compensated for *Lygus*-induced fruit loss (Fig. 4). Also in all years, although some numerical differences were observed in percent fruit loss values, both cultivars produced similar lint yields (Figs. 3 and 4). By determining the fruiting position-based lint yield contributions, it was revealed that insect-induced (Low and High) fruit removal treatments and manual fruit removal treatments (ASC_{1st} and ASC_{all}) significantly reduced the first-position lint yield contribution. Averaged across both years and cultivars, SC, UC, Low, High, ASC_{1st}, and ASC_{all} produced 916, 928, 745, 779, 480, and 476 lbs/A first-position lint yields, respectively. Conversely, both insect-augmented and manual removal treatments significantly increased lateral-position lint yield contributions. Treatments SC, UC, Low, High, ASC_{1st}, and ASC_{all} produced 298, 275, 385, 423, 768, and 631 lbs/A lateral-position lint yields, respectively. Interestingly, regardless of the manner of fruit removal, plants were able to fully compensate, in terms of final lint yield, for early-season fruit loss, largely by way of significant lateral fruiting overcompensation. In other words, cotton plants responded to pre-flower fruit loss by growing taller (adding more first-position

fruits) and wider (adding more 2+ position fruits on lateral branches). Again, it is important to note that the level of lint yield compensation realized is directly dependent upon the availability of adequate input resources (e.g., water, plant nutrients, heat units) following early-season fruit losses.

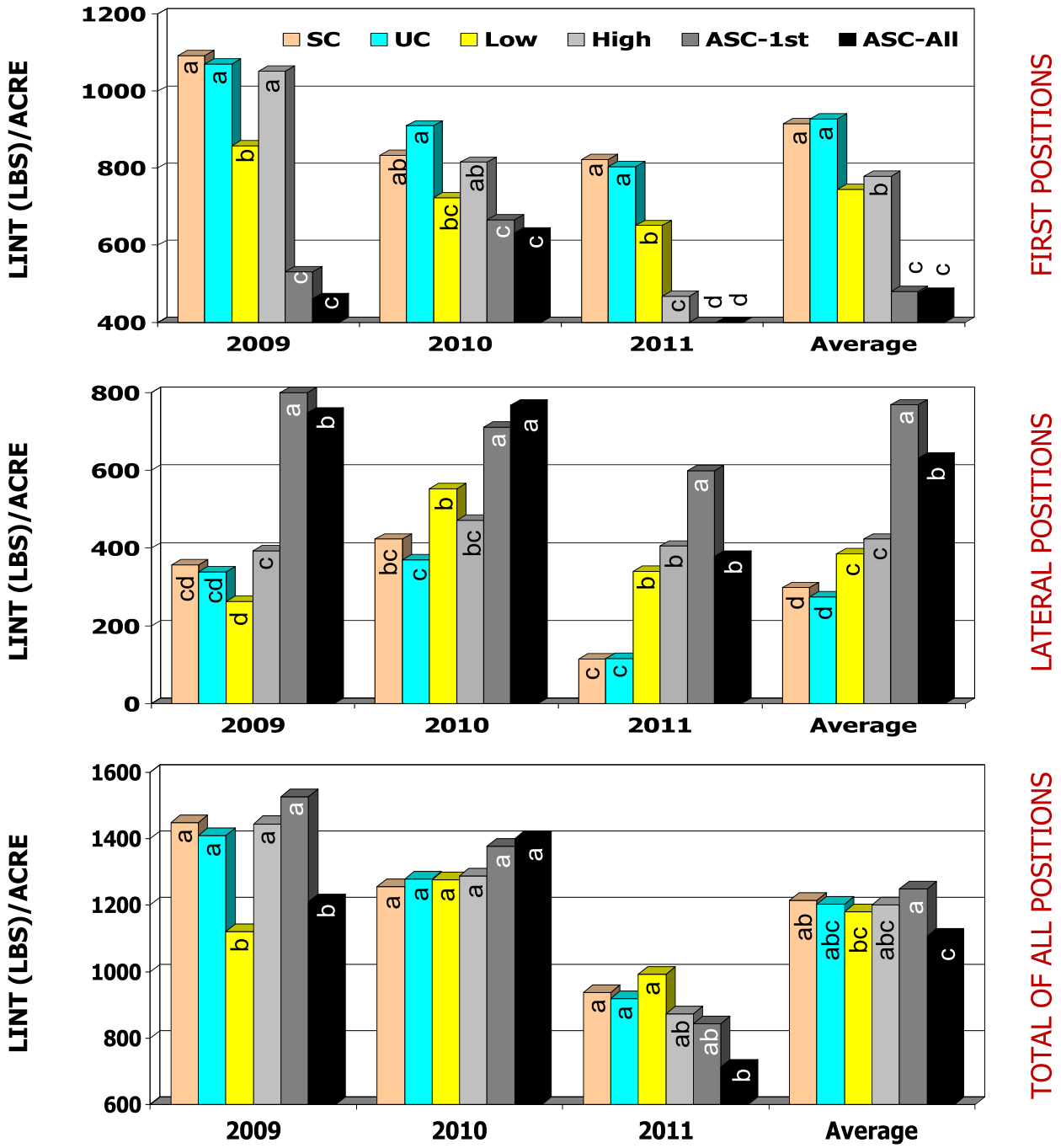

Figure 4. Yearly and 3-yr average lint yields (across two cultivars) from cotton plots receiving: 1) varied levels of *Lygus* augmentations (Low, High), 2) artificial (manual) square removal (ASC_{1st}, ASC_{all}), or 3), and controls (UC, SC). Yields were separated by fruiting positions (1^{st}, lateral, combined). Within a fruiting position type and year (or 3-yr average), treatment means with the same letter are not significantly different at $P > 0.10$. Lubbock, TX, 2009-2011.

Lint Quality

The fineness of individual fibers is measured by micronaire value. As cotton matures fiber become more course and the micronaire value increases. For the spinning industry, very fine cotton lint (low "mic" cotton) is not suitable for spinning since they cause more neps, poor dye absorption and high breakage so the optimum fineness range falls between a 3.7-4.2 micronaire values. On average, all cotton samples from these experiments were in this optimum fineness range except for a few exceptions. In general the DP 161 B2RF cultivar produced higher mic cotton than DP 104 B2RF (Figs. 5 and 6). The lateral position cotton lint had lower mic values compared to first position cotton. It was hypothesized that *Lygus* induced fruit loss can change the distribution of carbohydrate photosynthate to the maturing bolls within a plant and also induce new cotton boll formation which can increase the proportion of immature low mic cotton. A combined analysis of the three-year data showed *Lygus*-induced fruit loss in DP 104 B2RF did not significantly impact the micronaire values of both first position cotton and lateral position cotton (Fig. 5). In DP 161 B2RF, a full-season cultivar, the higher *Lygus*-infested plots produced significantly lower mic cotton lint compared to spray control and natural control plots, indicating the maturity delay in compensated bolls.

There was some year-to-year variation such as, in 2009, micronaire values were similar across treatments in DP 104 B2RF regardless of the fruiting position, but the insect-augmented treatments had significantly higher micronaire (premium range) in this cultivar in 2010. In DP 161 B2RF in 2010, the micronaire values were lower in insect-augmented treatments than in SC and UC plots, but these values were, again, in the premium range. Conversely, first-position DP 104 B2RF (2010) micronaire readings were significantly higher and the lateral position readings lint demonstrated the same, but less pronounced, numerical-only trend. Averaged across the three study years and regardless of fruiting positions, DP 104 B2RF micronaire values were statistically very similar between control treatments (SC and UC) and the *Lygus*-augmented treatments (Low and High), indicating a much lesser issue of lint quality compromise due to compensatory bolls in short-season cultivars. For DP 161 B2RF, the UC treatment had statistically higher three-year averaged micronaire readings than insect augmentation treatments. These observations clearly suggest cultivar differences related to micronaire, yet it should be noted that regardless of cultivar differences, with the exception of first-position DP 161 B2RF control treatments, all other observed three-year average micronaire values fell within or extremely near the normal, preferred range of 3.7-4.2. These data indicate that the Texas Southern High Plains growing conditions generally do not incur significant quality compromise in compensatory lint as influenced by *Lygus*-induced early square loss.

It was apparent that all first position squares manually removed plots (ASC_{1st}) produced significantly lower mic cotton compared with spray control plots in both cultivars and in all years. Manual removal of all older first position pre-bloom squares might have induced the compensatory response of cotton plant to produce new squares that caused an increased proportion of less mature bolls during harvesting and ultimately reflected as low mic cotton. In 2011, when the cotton plants were highly stressed due to extremely hot and dry weather, the higher level infestation of *Lygus* caused highly significant loss in micronaire value of the cotton (Figs. 5 and 6) compared to other more typical years. This indicates that when insect stress and environmental stresses are combined together, the stresses can synergistically negatively impact cotton fiber development.

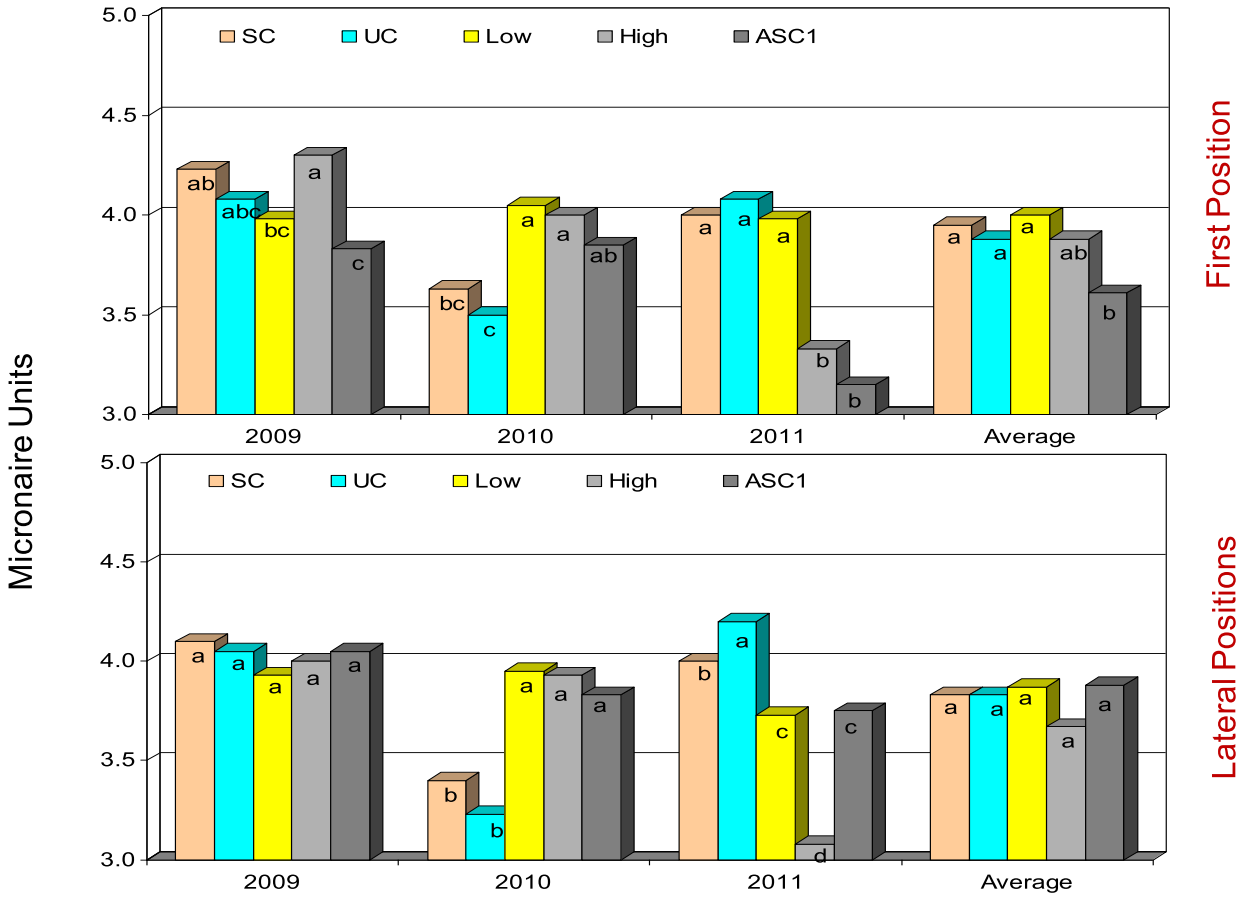

Figure 5. Average micronaire values of **DP 104 B2RF** cotton cultivar receiving one of the following square removal treatments: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray application (spray control, SC), 5) pre-bloom artificial (manual) removal of all first-position squares and spray control (ASC_{1st}). The top graph shows micronaire values of first position cotton bolls, while the lower graph depicts the micronaire values of lateral position cotton bolls. Columns within a year (or average) and fruiting position with the same lowercase letter are not significantly different ($P > 0.10$). Lubbock, TX, 2009-2011.

Undesirable cotton "neps" are often described as tightly tangled knot-like masses of unorganized fibers. A nep composed entirely of fibers is called a "fiber nep," while other neps can contain foreign matter (e.g. seed-coat fragments) entangled with fibers. Both create problems in textile mills and interfere with yarn quality. Fiber fineness and maturity determine, to a large degree, the amount of nepping that occurs during the ginning process. Immature, fine-fiber cottons tend to nep more readily than do mature, coarse fibers. Cotton lint quality is also determined by nep size and the number of neps per gram of fiber.

DP 161 B2RF cultivar not only produced higher mic cotton than DP 104 B2RF (Figs. 5 and 6) but also possessed consistently larger neps than DP 104 B2RF (lateral position fiber; Figs. 7 and 8).

Both *Lygus*-induced fruit loss and manual removal of cotton pre-bloom first position squares caused significant reduction in nep size (µm) in both first position and lateral position cotton in DP 161 B2RF cultivar (Fig. 8) and only in lateral position cotton in DP 104 B2RF (Fig. 7). The adverse cotton growing conditions in 2011 drastically reduced nep size compared with 2010 in DP 104 B2RF (Fig. 7) but nep size of DP 161 B2RF were similar between two years (Fig. 8). In 2010, significantly larger neps were found in lateral position cotton from SC and UC plots in DP 104 B2RF, whereas nep size in first position cotton remained unaffected by treatment.

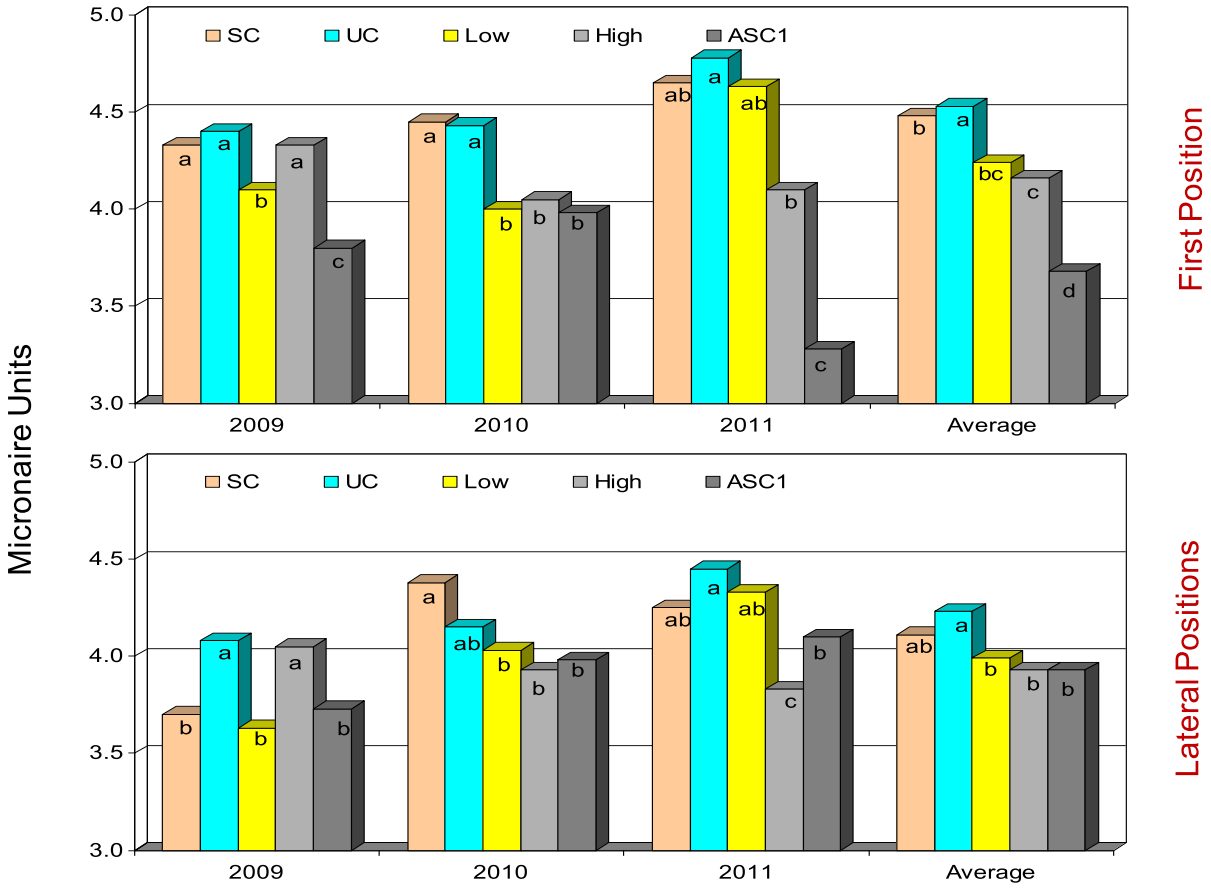

Figure 6. Average micronaire values of **DP 161 B2RF** cotton cultivars receiving one of the following square removal treatments: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray application (spray control, SC), 5) pre-bloom artificial (manual) removal of all first-position squares and spray control (ASC_{1st}). The top graph illustrates micronaire values of first position cotton bolls, while the lower graph depicts the micronaire values of lateral position cotton bolls. Columns within a year (or average) and fruiting position with the same lowercase letter are not significantly different ($P > 0.10$). Lubbock, TX, 2009-2011.

Two-year average nep sizes within first-position lint samples were statistically similar across treatments in DP 104 B2RF, while the lateral position cotton nep sizes varied among treatments. It was obvious that both *Lygus*-induced fruit loss and manual removal of first position fruits in DP 104

B2RF cultivar significantly reduced the nep size in the lateral fruiting position cotton. The 2011 data were much more variable and exhibited no clear trend, likely due to historical record-breaking temperatures and drought conditions coupled with a limited irrigation cotton cropping system.

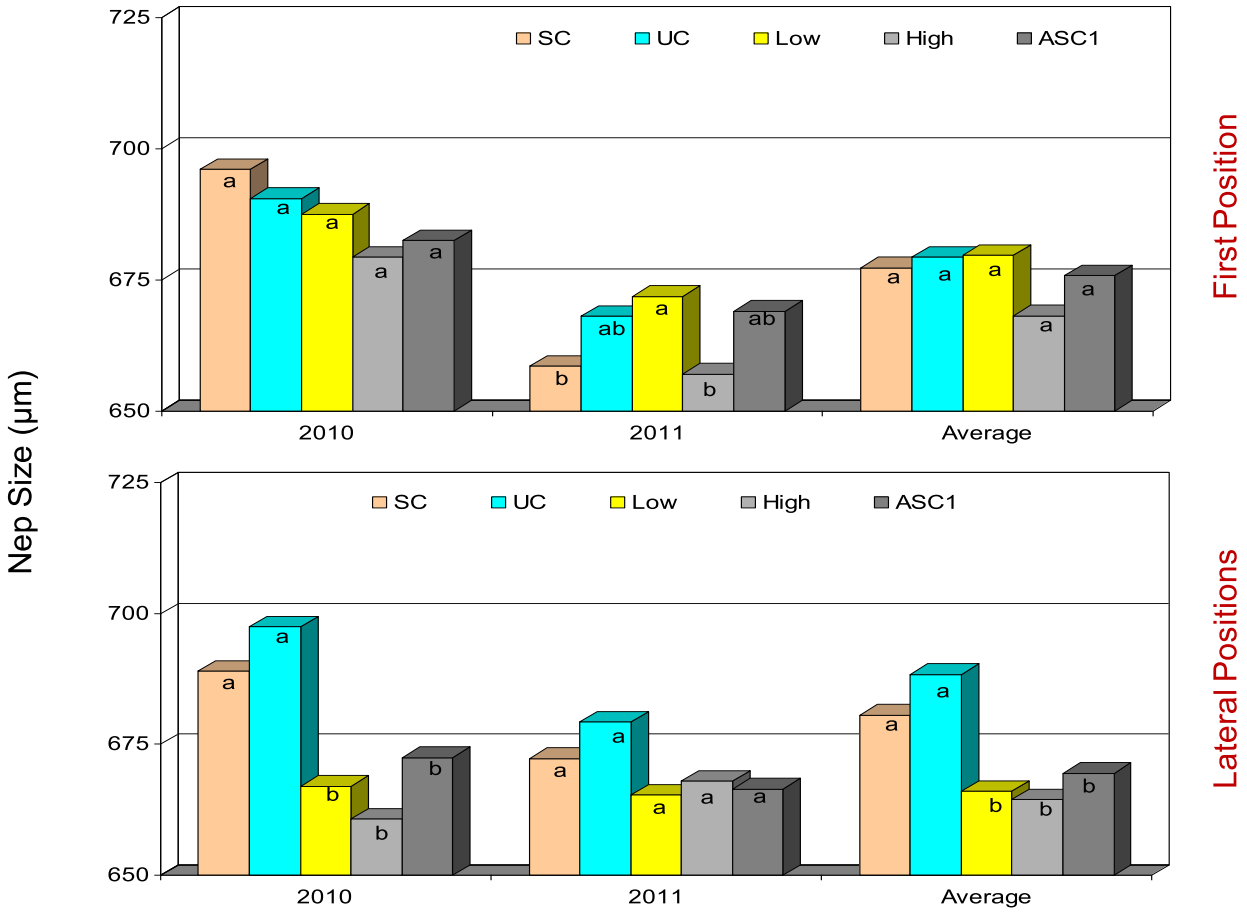

Figure 7. Average nep size of **DP 104 B2RF** cotton cultivar receiving one of the following square removal treatments: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray application (spray control, SC), 5) pre-bloom artificial (manual) removal of all first-position squares and spray control (ASC_{1st}). The top and bottom graphs illustrate the nep size values of fiber from first position and lateral position bolls, respectively. Columns within a year (or average) and fruiting position with the same lowercase letter are not significantly different ($P > 0.10$). Lubbock, TX, 2009-2011.

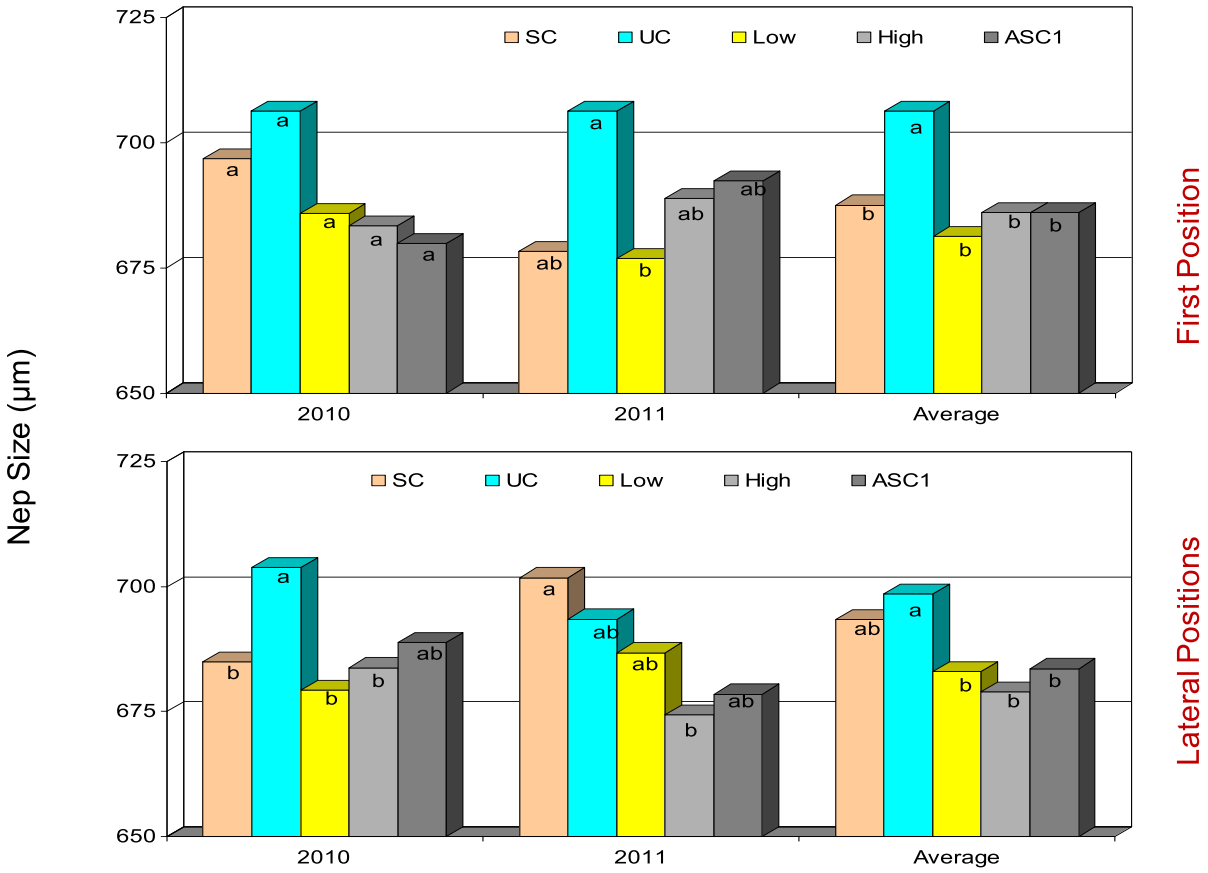

Figure 8. Average nep size of **DP 161 B2RF** cotton cultivar receiving one of the following square removal treatments: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray application (spray control, SC), 5) pre-bloom artificial (manual) removal of all first-position squares and spray control (ASC_{1st}). The top and bottom graphs illustrate the nep size values of fiber from first position and lateral position bolls, respectively. Columns within a year (or average) and fruiting position with the same lowercase letter are not significantly different ($P > 0.10$). Lubbock, TX, 2009-2011.

The numbers of neps in each gram of lint is also an important fiber quality parameter. In the textile mill, cotton lint with higher numbers of neps per gram result in lower quality fabrics. In general, both cotton cultivars produced similar numbers of neps per gram (Figs. 9 and 10). Averaged across the two study years, artificial removal of early-season, first-position squares (ASC_{1st}) resulted in significantly more neps in first-position lint in both cultivars. Compared with SC and UC plots *Lygus* infestations (both Low and High treatment) did not greatly affect the neps per gram in either cultivar.

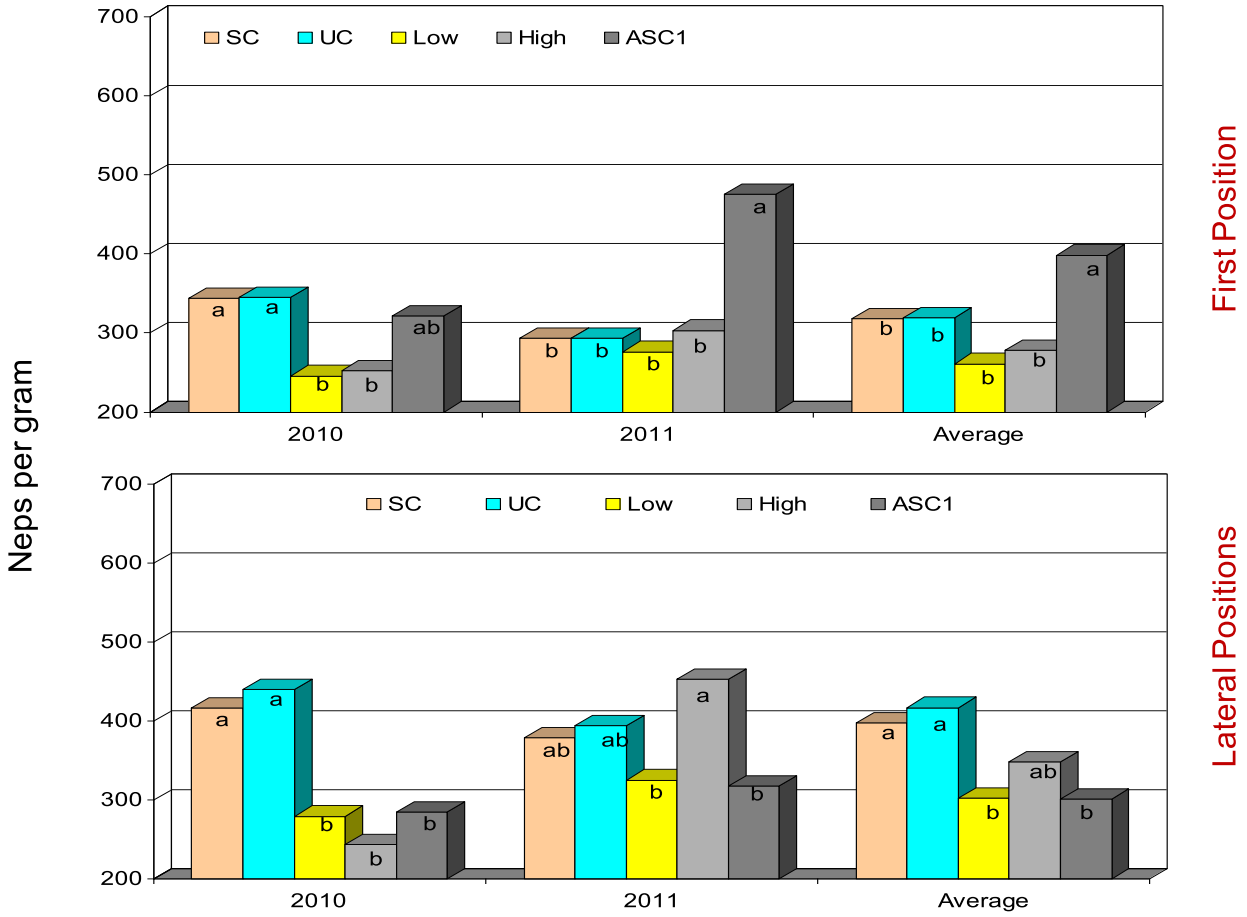

Figure 9. Average neps per gram of **DP 104 B2RF** cotton cultivars receiving one of the following square removal treatments: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray application (spray control, SC), 5) pre-bloom artificial (manual) removal of all first-position squares and spray control (ASC_{1st}). The top and bottom graphs illustrate neps per gram values of fiber from first position and lateral position bolls, respectively. Columns within a year (or average) and fruiting position with the same lowercase letter are not significantly different ($P > 0.10$). Lubbock, TX, 2009-2011.

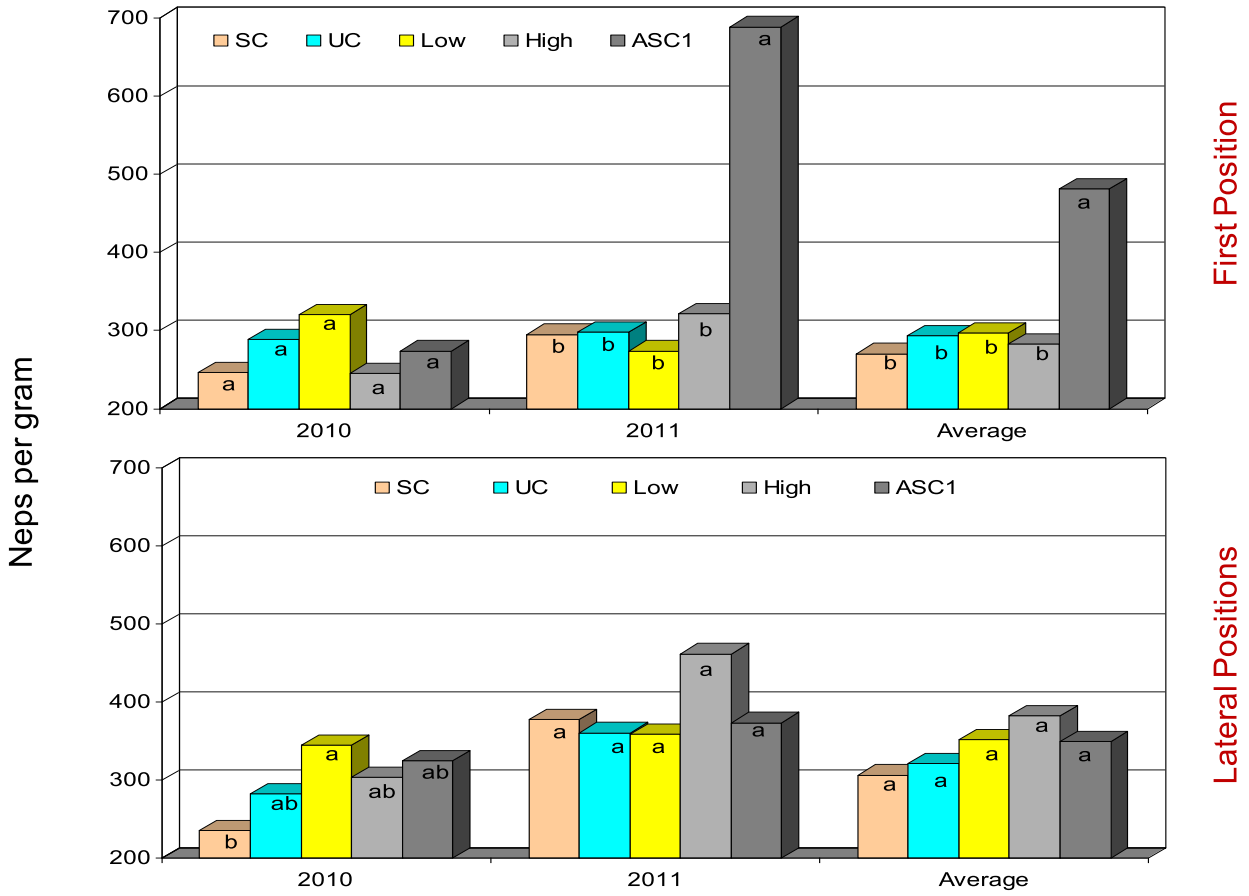

Figure 10. Average Neps per gram of **DP 161 B2RF** cotton cultivars receiving one of the following square removal treatments: 1) augmentation of 2-3 bugs per plant (Low), 2) augmentation of 4-6 bugs per plant (High), 3) 0 bugs augmented (untreated control, UC), 4) 0 bugs achieved through spray application (spray control, SC), 5) pre-bloom artificial (manual) removal of all first-position squares and spray control (ASC_{1st}). The top and bottom graphs illustrate the neps per gram values of fiber from first position and lateral position bolls, respectively. Columns within a year (or average) and fruiting position with the same lowercase letter are not significantly different ($P > 0.10$). Lubbock, TX, 2009-2011.

Acknowledgements

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2011 ANNUAL REPORT

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 has been ongoing 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 immediately 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. However, variation in residual nitrogen did not significantly affect 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. 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. No arthropod populations develop in 2011 due to extreme temperature and drought. A strong correlation was found between leaf chlorophyll content and lint yield. 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 four years, the zero-N treatment produced the lowest yield (912 lb/acre) and yield increased curvilinearly with each additional 50 lb N added, with highest average lint yield occurring in 150 lb N/acre treatment (1,288 lb/acre).

Introduction

A three-year study under a limited irrigation production system in Lamesa, Texas (Bronson et al. 2006) characterized 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. The specific goal of this project was to generate COTMAN™ parameter values in relation to nitrogen fertility in a high yielding cotton production system.

Materials and Methods

The study was conducted at the Texas 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, and 200 lb/acre) had been deployed to the same experimental units (Fig. 1) consistently for nine consecutive years to induce maximum discrimination among treatment plots through variation in soil residual nitrogen.

The study reported herein was conducted for four years of this project (2008-2011). 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. A high-yielding FiberMax cultivar, FM960B2R, was planted on May 13 (2008), May 20 (2009), and May 27 (2010), and DP104B2RF on June 14 (2011). However, the 2010 test was replanted with DP104B2RF on June 16 due to poor plant stand, whereas the 2011 planting was delayed until mid-June due to pre-planting drought. 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 (target rate of 56,000 seeds/acre) in 30-inch rows and was irrigated with a subsurface drip irrigation system.

Leaf area, plant height, and root length were measured on July 3 (2008), July 20 (2009), July 27 (2010), and July 15 (2011) to evaluate the influence of residual nitrogen on early plant growth patterns. In 2009, 2010, and 2011, 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), and July 21 to August 25 (6 weeks) in 2011. Soil samples were taken from the experimental plots on

July 14 (2008), July 6 (2009), March 25 (2010), and April 27 (2011) 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), and August 3 (2011) 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. 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 (Beerwinkle et al. 1997) to collect insects from upper-canopy foliage, beginning from square initiation and ending at crop cutout.

Cotton aphid populations did not develop in 2008, despite repeated applications of lambda-cyhalothrin @ 4 oz/A (July 25, August 8 and 22, September 5) intended to stimulate aphid population growth. Thus, neither aphid counts nor leaf nitrogen were monitored in 2008. In 2009, five sequential applications of lambda-cyhalothrin @ 4 oz/A were applied (August 5, 17, 31, September 7 and 14) to enhance cotton aphid activity in the field to evaluate the effect of nitrogen treatment, but the aphid densities remained well below treatment threshold throughout the season. Aphid counts were made on August 20, 27, September 3, 10, and 18, 2009. Leaf nitrogen was indirectly estimated by using *in situ* chlorophyll meter reading of the fifth mainstem node leaf throughout the growing season (July 30, August 7, 13, 20, 27, September 3, 10, 18, 25, October 6 in 2009). In 2010, cotton aphids were sampled on August 9, 18, 27, September 2, and 9. Chlorophyll measurement and leaf collections for nitrogen analyses were also conducted on aphid sampling dates. In 2011, four sequential applications of lambda-cyhalothrin @ 4 oz/A were applied (August 12, 19, 26, September 2) to enhance cotton aphid activity in the field to evaluate the effect of nitrogen treatment, but the aphid densities did not develop on this test. The 2011 summer was the hottest and driest on record, which prevented any cotton aphid development in the Texas High Plains region. Ten fifth mainstem-node leaves per plot were also collected simultaneously with the chlorophyll reading to measure actual leaf nitrogen in the laboratory by chemical analysis. Hand-harvested yield samples were obtained from each plot. Fiber samples were analyzed for lint quality parameters at the Cotton Incorporated Fiber Testing Lab (North Carolina).

Results and Discussion

In all study years, soil residual N levels were significantly higher in plots which received the two highest application rates of N fertilizer versus plots which received lower-rate N applications, excepting plots which received zero N in 2008 and 150 N treatment in 2011 (Fig. 2). In addition, soil in plots having received the two highest rates of N application treatment exhibited similar residual N amounts, except for 2011. Even though some variation in plant height, root length, and leaf area was noted early in the crop season, differential amounts of soil residual N generally did not influence early plant growth, except for zero N plots in 2010 (Figs. 3-5). In 2010, delayed planting and an entirely different cultivar compared with that in 2008 and 2009 might be attributed for this reduced plant growth (shorter plants and lower leaf area) at zero N plots.

Measured leaf chlorophyll content varied with nitrogen application level, and leaf chlorophyll content from cotton in those plots which received 0 lb N/acre or 50 lb N/acre were ascertained to be significantly lower than all others in all years (Fig. 6). Cotton in

plots which received the three highest nitrogen application rates (100, 150, and 200 lb N/acre) exhibited relatively consistent leaf chlorophyll readings (Figs. 6-7). 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 abundance was very low in 2008 and aphid monitoring was abandoned in that year. Cotton aphid activity began in late August in 2009 and early August in 2010, and densities peaked in 3-4 weeks following the initiation of the aphid activity. 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. 7). There were no significant differences in aphid densities across N augmented plots. While cotton aphid densities remained below economic threshold (50 aphids/leaf for two consecutive weeks) in 2009, aphid populations surpassed the economic threshold in all N-augmented plots in 2010 whereas aphids remained below 50/per leaf, except for 1 week, in zero-N plots. Aphid populations did not develop in 2011 due to unusually high temperature and drought throughout the crop growing season. Higher rates of applied N (>100 lbs/A) resulted in significantly higher leaf chlorophyll content compared to that in lower or zero N plots (Fig. 6-7). A strong correlation was found between leaf chlorophyll content and lint yield.

Nitrogen fertility level influenced fruiting profile (Fig. 8) and boll maturity. Plants essentially quit setting additional squares in zero and 50-lb applied N plots two weeks into flowering while higher N plots were actively producing squares. As a result, cotton in zero N plots cut-out while all N augmented plots had NAWF>5 at 79 and 73 DAP (days after planting) in 2009 and 2010, respectively (Fig. 8). However, crop cut-out occurred at around the same time in 2011 (60 DAP) for all treatments due to extreme temperature and drought.

For 2008 and 2009, cotton in zero N plots were terminated with harvest aid applications much earlier than did cotton in N augmented plots, although data depicting this are not shown here. 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. The zero N plots produced the lowest yield (1,236 and 1,049 lb/acre in 2008 and 2009, respectively) (Fig. 9). Yield increased curvilinearly with each additional 50 lb N added, with the numerically highest average yield (1,742 and 1,591 lb/acre in 2008 and 2009, respectively) occurring in augmented 150 lb N/acre treatment (Fig. 9). In both 2008 and 2009, yield increased curvilinearly with added N, but the yield did not significantly increase beyond 100 lb N/acre with additional N. Consistent numerical decline in yield beyond 150 lb N/acre between the two years suggests that N application beyond 150 lb/acre may be unfavorable for cotton yield.

As stated previously, the 2010 crop was replanted in mid-June and the 2011 crop planting was delayed until mid-June due to drought with a short season cultivar, thus making year-to-year yield comparisons a bit cautionary. Nevertheless, the relationship between nitrogen application rate and lint yield in 2010 and 2011 were similar to what was observed in the previous two years. That is, zero-N plots produced the lowest yield and yield increased curvilinearly with each additional 50 lb N added, with highest average

yield occurring in 150 lb N/acre treatment. Lint yield was similar across all treatments in 2011. Averaged across all five N treatments, lint yield values were 1540, 1418, 883, and 648 lbs/acre for 2008, 2009, 2010, and 2011, respectively. As stated earlier, 2008 and 2009 crops were planted in May with a full season cultivar that produced significantly higher yields than in 2010 and 2011, the years with delayed planting and short-season cultivars. Averaged over four years, lint yield values were 912, 1061, 1169, 1288, and 1248 lb/acre in zero, 50, 100, 150, and 200 N treatments, respectively. These data clearly demonstrated that the augmentation of N fertilizer >100 lb/acre did not significantly increase the lint yield.

Lint quality parameters were generally inferior in 150 and 200 lb/acre plots compared with those observed in lower N plots. Micronaire values for 2008-2010 crops, except for zero and 50 N in 2009, were all below normal range (Fig. 10). Nevertheless, micronaire values were higher in lower N augmented or zero N treatments, except for 2010. In 2011, micronaire values were significantly lower in the two highest N treatments compared with that in zero, 50, and 100 N treatments (Fig. 10). It is possible that the extreme temperature, drought, and limited season length affected the normal maturity process in treatments with excessive N augmentation. Averaged over four years, micronaire values were significantly lower in 150 and 200 N compared with that in zero and 50 N treatments, suggesting crop termination without full lint maturity at higher N.

Acknowledgments

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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

Fig. 1. Plot layout of the long-term nitrogen fertility rate study at Helms farm near Halfway, Texas, 2008-2011. Treatments consisted of 0, 50, 100, 150, and 200 lbs N applied per acre. Each of the 25 experimental units measured 16 rows wide X 120 ft in length.

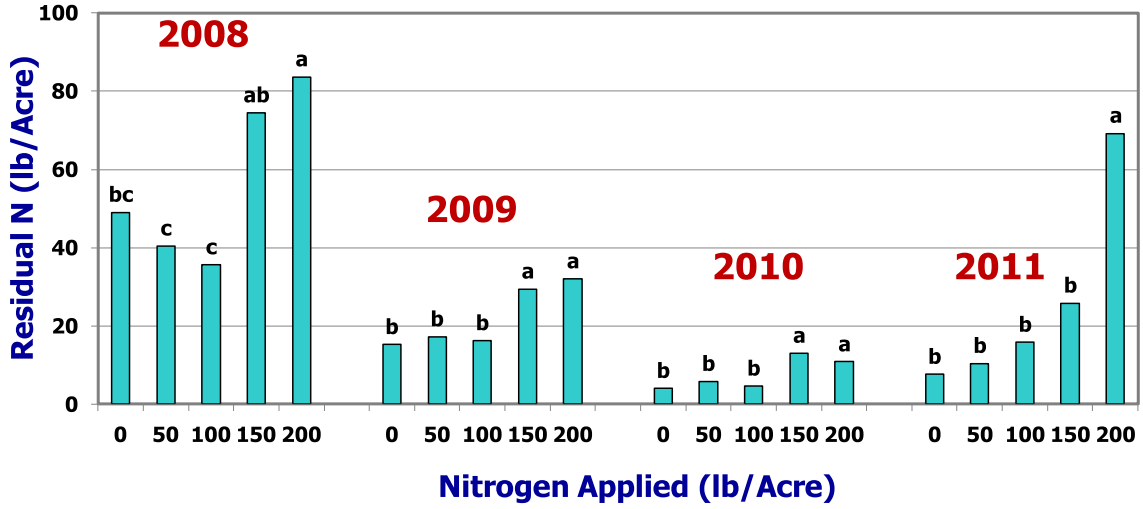

Fig. 2. Effect of nitrogen application rates on residual nitrogen after six (2008) to nine (2011) years of repetitive applications, 2008-2011.

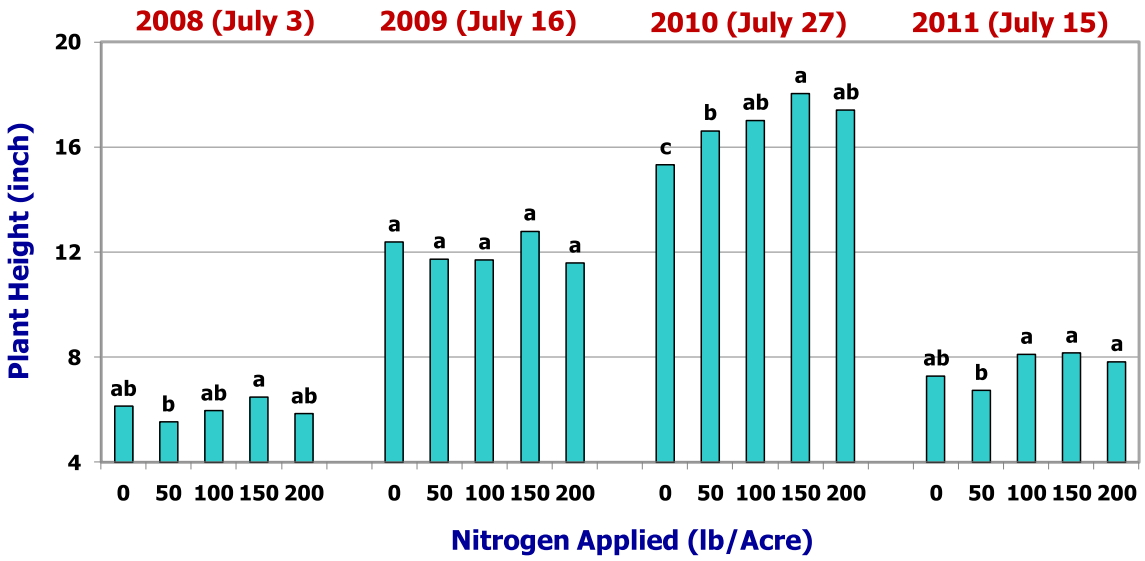

Fig. 3. Effect of residual soil nitrogen on early plant growth as measured by plant height, 2008-2011.

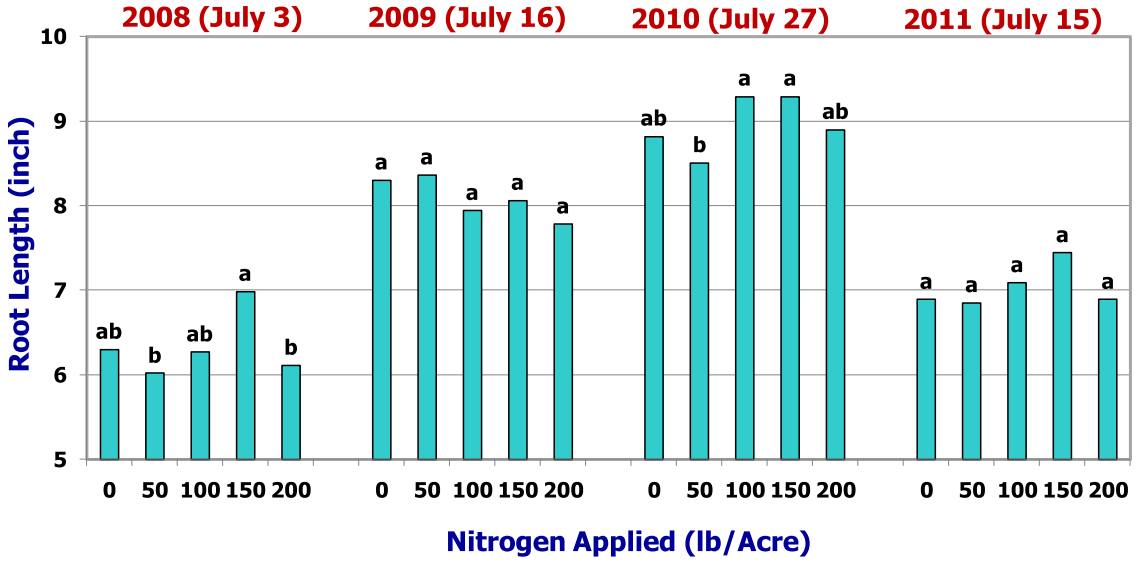

Fig. 4. Effect of residual soil nitrogen on early plant growth as measured by root length, 2008-2011.

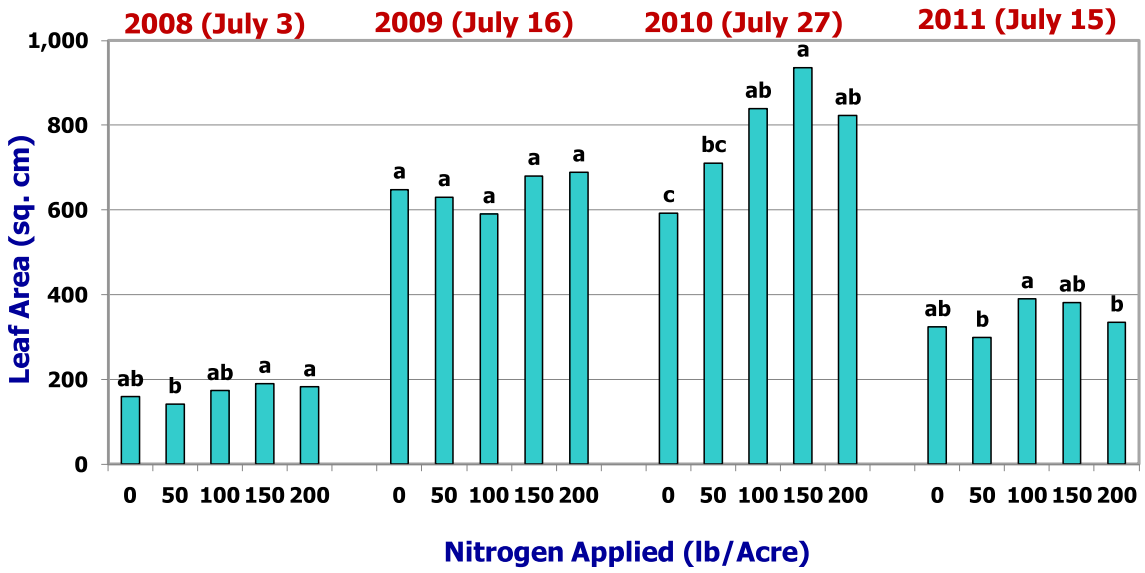

Fig. 5. Effect of residual soil nitrogen on early plant growth as measured by total leaf area, 2008-2011.

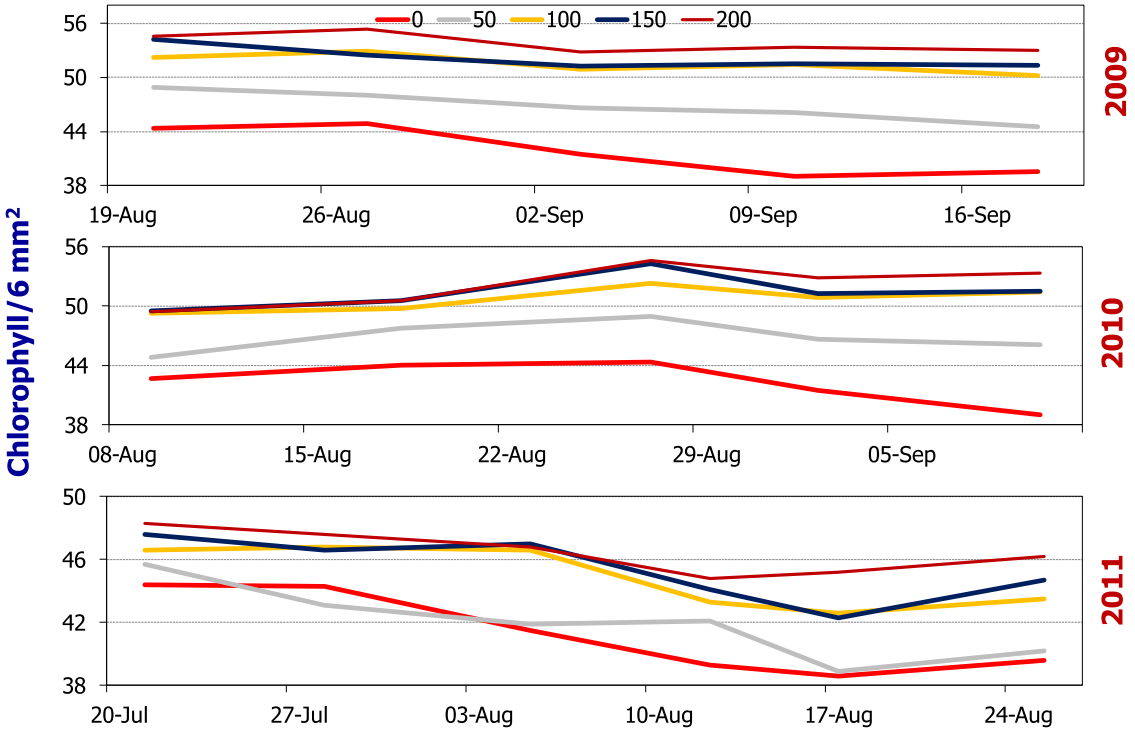

Fig. 6. Temporal dynamics of cotton leaf (5th main stem) chlorophyll content as affected by variable rates of nitrogen application into the soil of replicated experimental plots, 2009-2011.

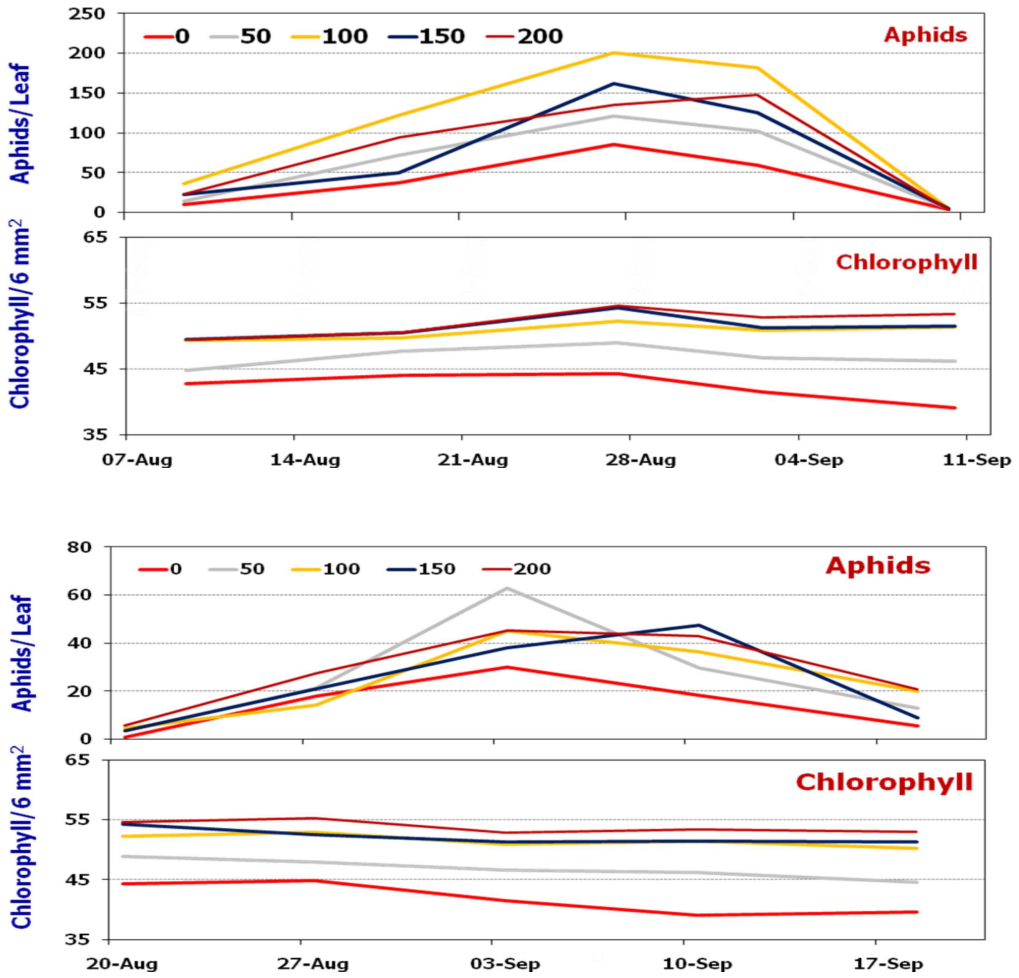

Fig. 7. Temporal dynamics of cotton aphid abundance in relation to cotton leaf (5th main stem) chlorophyll content as affected by variable rates of nitrogen application (top chart – 2009 and bottom chart – 2010).

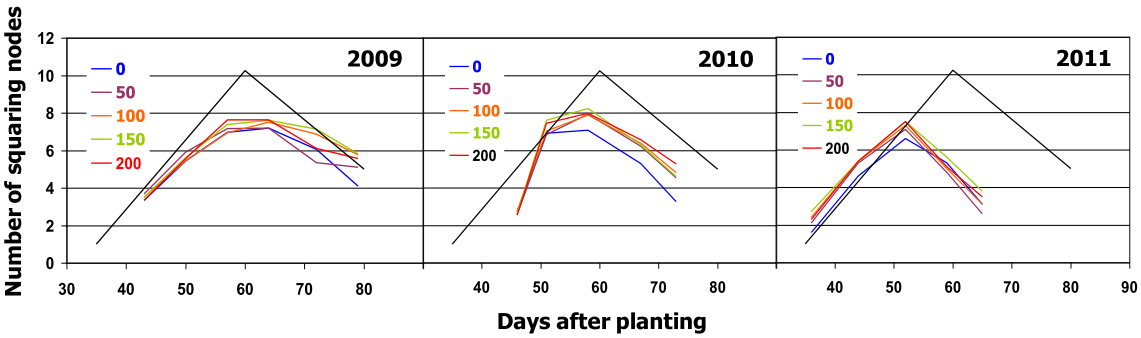

Fig. 8. Cotton fruiting profile as indicated by SQUAREMAP component of the COTMAN crop monitoring program as influenced by variable rates of nitrogen application in replicated experimental plots, 2009-2011.

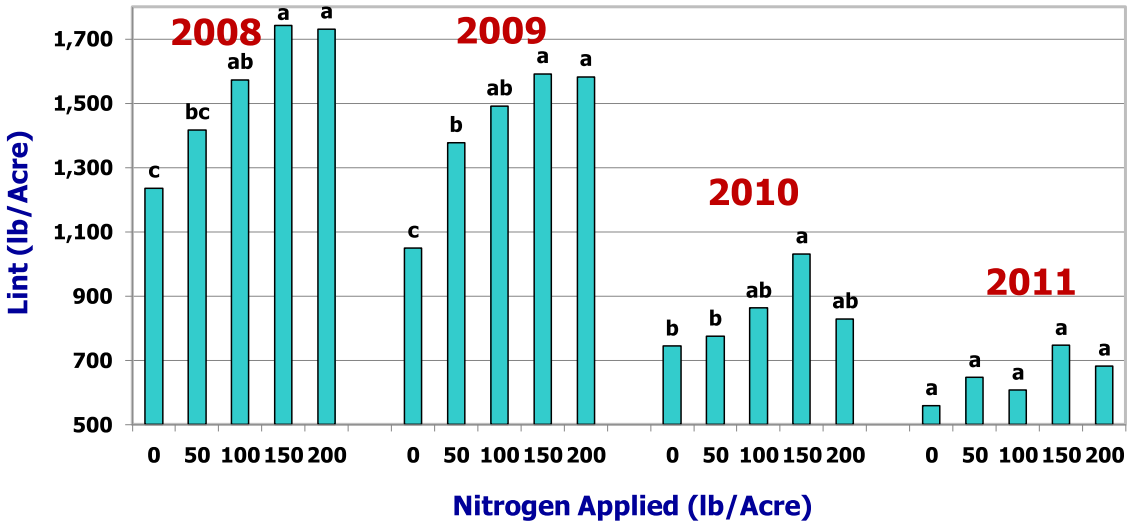

Fig. 9. Effect of variable rates of soil applied nitrogen on cotton lint yield, 2008-2011.

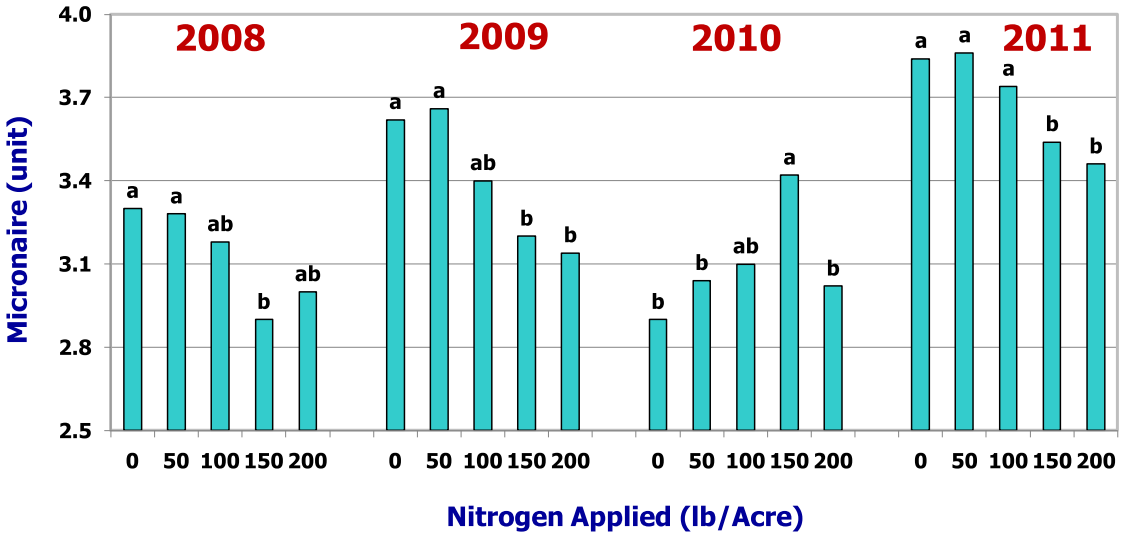

Fig. 10. Effect of variable rates of nitrogen application on cotton lint micronaire values, 2008-2011.

2011 FINAL REPORT

Cotton Incorporated/State Support Program

Project Number: 08-305TX

Evaluation of Western Tarnished Plant Bug as a Late Season Pest of Cotton

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Evaluation of Western Tarnished Plant Bug as a Late Season Pest of Cotton

Project Summary

Several species within the *Lygus* genus are important cotton pests in Texas and other U.S. cotton growing states. *Lygus* adults and nymphs feed upon cotton reproductive tissues, causing fruit abortion and impacting lint yields and fiber quality. Insecticides often provide adequate *Lygus* control, but extensive insecticide use can produce undesirable ecological and financial impacts. Informed decision-making with the goal of minimizing insecticide use is critical to mitigating such impacts. The goal of this study was to develop a late-season insecticide application termination guideline for Texas High Plains cotton growers. Optimal insecticide application termination timing may facilitate pesticide load reduction in the agro-ecosystem. Field studies evaluating the cotton boll-damaging potentials of *Lygus* adults and nymphs at various levels of cotton boll maturity were conducted from 2007 to 2011. Individual bolls were caged and grouped by assigned heat unit (HU) accumulation thresholds (150, 250, 350, and 450 HU) and were subjected to three treatments: *Lygus* adult feeding for 48 h, late-instar *Lygus* nymph feeding for 48 h, and caged control bolls (zero infestation). Per-boll sizes, weights, carpel wall hardness, internal and external damage, and lint and seed yields were recorded. Assessed parameters were compared with those of corresponding controls. *Lygus* adults and nymphs both cause external lesions on bolls throughout boll development. However, generally speaking, *Lygus* were unable to inflict internal damage to bolls exceeding 350 accumulated heat units. With 4-year data, it is clear that *Lygus* late-instar nymphs pose a greater threat to maturing cotton bolls than *Lygus* adults, especially one week-old bolls. On a field basis, insecticide intervention to manage *Lygus* is unnecessary beyond 350 HU (15-20 days) post-cut-out (NAWF=5). For cultivar ST 4554 B2RF, *Lygus* could not cause internal damage to the cotton bolls bigger than ~3 cm diameter. Thus is the justification for the Pesticide Termination Rule whereby applications end when most of the harvestable cotton bolls become equal or bigger than 3 cm.

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. In Texas, approximately five million acres of cotton have been planted annually in recent years, and Texas is the largest cotton producing state (Williams 2011). In 2010, a 3.91% reduction in U.S. cotton yields was attributable to arthropod pests – 0.68% due to *Lygus*, which was ranked third among other yield-reducing pests and also cost more per infested acre, requiring more applications per treated acre, than any other arthropod pest (Williams 2011). In Texas, over 2 million acres were infested by *Lygus* in 2010 (Williams 2011). Although advancements in agricultural technology continue to increase cotton productivity, cotton growers face the challenge of boosting yields in order to meet the demands of an increasingly competitive global cotton market.

In the Texas High Plains (THP), the most intensive and concentrated cotton-producing region in the world, cotton production may be increased by mitigating losses due to insect pests. Approximately one third of THP cotton acres were infested by *Lygus* in 2010. Insect pests such as *Lygus* impact yields and impose major costs in terms of crop protection expenses, pest monitoring, and ecological impacts due to environmental pesticide load and pollution. Following the introduction of *Bt*-technology (Bollgard and Bollgard II cotton), outbreaks of lepidopteran pests have been drastically reduced, and in recent years, secondary piercing-sucking pests such as *Lygus* are of increasing concern in the THP (Parajulee *et al.* 2008).

Lygus bugs typically feed on young squares, flowers, and cotton bolls. The feeding damage results in fruit abortion, anther damage, or internal boll injury (developing seed and lint damage), negatively impacting lint yield and fiber quality (Godfrey *et al.* 2009). Since reliable *Lygus*-resistant or tolerant cotton cultivars are unavailable, cotton producers rely on chemical 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 levels. Once an above-threshold *Lygus* population is detected, growers generally continue pesticide applications, perhaps arbitrarily, until cotton bolls have opened. In addition to being expensive, chemical insecticides often produce undesirable ecological impacts. Thus, in addition to reducing management costs, reducing such pesticide applications would minimize associated environmental implications and secondary pest problems.

Oosterhuis and Kim (2004) reported that cotton bolls that accumulated 350-450 Heat units were safe from any piercing sucking insect. In this project, it was hypothesized that *Lygus* may be unable to damage cotton bolls once a certain boll maturity level has been reached, after which pesticide applications would be superfluous. The actual cotton boll damaging potential of *Lygus* 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 goal of this study, in evaluating the theorized hypothesis, was development of a pesticide termination rule based on *Lygus* cotton boll damaging potential. In order to reach this goal, study objectives included: 1) Develop indicators for determining when cotton bolls are safe from western tarnished plant bug (*L. hesperus*) feeding injury, and 2) Compare the cotton boll damage potential of adult and late-instar western tarnished plant bugs. In addition to its importance in reducing pesticide loads in the agroecosystem, such a rule, if applied, could save cotton growers substantially in terms of insecticides and costs associated with their use. Further, an understanding of differences in the *Lygus* cotton boll damaging potential, as it relates to boll age (HU accumulations), could improve establishment and interpretation of *Lygus* economic thresholds.

Materials and Methods

A 4-year (2007-2011) study was conducted in an irrigated cotton field at the Texas AgriLife Research and Extension Center research farm located near Lubbock, Texas. Cotton cultivar ST 4554 B2RF was planted on beds with 40-inch row-spacing. Four treatments and two blocks were deployed in a randomized, complete block design. Experimental workload and quality control considerations demanded, for practical reasons, that no more than two blocks be deployed. Treatments consisted of groups of cotton bolls (cohorts) of designated heat unit (HU; [(daily min °F temp + daily max °F temp) / 2] – 60°F) accumulation thresholds (150, 250, 350, and 450 HU) subjected to a 48 h infestation (single *Lygus* bug). A fifth, caged but uninfested cohort served as the control. The five treatments were assigned to randomly selected individual rows (plots) in each block. One hundred new, first-position white flowers were individually caged in each plot. Thus, the experiment consisted of 1,000 (2 block x 5 treatments x 100 cages) caged fruits. Individual flower caging was achieved using 8-oz polystyrene beverage containers modified as tubes enclosed in sheer cloth sleeves. Daily HU accumulations were monitored from initial caging and caged bolls were infested upon achievement of the designated treatment HU thresholds.

The experiment was conducted exclusively using *Lygus* adults in 2007 and *Lygus* nymphs in 2011. Studies conducted in 2008, 2009, and 2010 were split-plot randomized block designs with *Lygus* stage (adults or nymphs) as the main plot factor and the 5 treatments as sub-plot factors. *Lygus* adults (one week into adulthood) and 4th-instar *Lygus* nymphs were used. *Lygus* insects were laboratory-reared using fresh green beans as an initial establishment substrate, and then trained with cotton reproductive tissues during the week preceding field deployment. For 6 h prior to release, the *Lygus* test insects were not allowed to feed, based on the assumption that starvation would stimulate feeding voracity. Prepared *Lygus* insects were deployed to appropriate boll cages to feed for 48 h at each treatment HU accumulation threshold, and then removed. No insects were released in control plots, and only final yield and fiber quality parameters were collected from control bolls.

From the treatment plots, an initial set of 30 bolls were collected from each plot at the end of the 48-h *Lygus* exposure and measured for size, weight, and carpel wall hardness. Carpel wall hardness of each boll was measured by recording the peak exocarp puncture force using a Wagner Force One^{TM} Model FDIX (Wagner Instruments, Greenwich, CT) with a 1-mm testing needle and mounted to a Mark-10 Model ESM force test measurement stand. From a second set of 30 bolls, *Lygus*-induced external lesions (dark, sunken punctures) and internal lesions (discolored wart formations observable on the interior exocarp wall) were quantified via visual examination and subsequent dissection. The remaining 40 bolls were left in the field until hand-harvesting, after which yield, seed damage, and fiber quality parameters were assessed. Data were analyzed via analysis of variance (ANOVA) using PROC GLM, SAS 9.2 (SAS Institute, 2008). Means were separated using least square means (LSMEAN) procedure at $\alpha = 0.1$.

Results and Discussion

Cotton Boll Growth and Development

There was significant year to year variation in boll diameter (df=4, 4; f=153.7; p<.0001), weight (df=4, 4; f=160.9; p<.0001), and carpel wall hardness (df=4, 4; f=44.8; p<.001). This was expected because of major variations in annual weather patterns. For example, record-breaking hot, dry weather in 2011 facilitated more rapid cotton boll HU accumulations versus other study years. Cotton boll sizes and weights were significantly lower in 2011 than equivalent HU stages in other years due to significant plant-stresses and accelerated maturation processes. Conversely, the prevalence of cool, wet, cloudy days in 2007 retarded HU accumulation, delayed maturation, and resulted in larger, heavier cotton bolls versus other study years. In all years, cotton boll diameters increased continuously as they accumulated HU from 150 HU to 350 HU (Fig. 1A). There was a significant effect of boll maturity on the boll diameter (df=3, 15; f=447.7; p<.0001), boll weight (df=3, 15; f=546.6; p<.0001) and boll hardness (df=3, 15; f=184.1; p<.0001). The rate of increase was initially high (from 150 to 350 HU), then leveled-off, as there was no significant change in average boll diameter from 350 HU to 450 HU (Fig. 1B). Cotton boll weight (Fig. 2B) and carpel wall hardness (Fig. 3B) followed similar growth patterns as boll diameter, although boll weight and hardness continuously increased (significantly) as bolls became older, yet the rate of increase in boll weight and hardness decreased greatly after bolls reached the 350 HU maturity level and beyond. Cessation of boll growth at 350 HU, as indicated by diameter, weight and carpel wall hardness observations, signals a shift in cotton boll physiological processes at that stage. It is demonstrated that this shift, whether physical, chemical, nutritional, or the combination of several factors, reduces *Lygus* feeding ability and/or attraction to cotton bolls.

Lygus Cotton Boll Feeding Preference

Lygus boll feeding preference cage studies were conducted with adults in 2007-2010 and nymphs in 2008-2011. The boll feeding preference was determined by evaluating the proportion of bolls injured or damaged by *Lygus* adults and nymphs. In all years, *Lygus* adults and nymphs caused one or more external lesions on more than 80% of the bolls offered at the four treatment maturity levels. Since *Lygus* may externally injure bolls at any of the selected maturity levels, successful determination of boll feeding preference required quantification of internal warts, or *successful* punctures, rather than solely by examination of external lesions caused by random probing.

The boll feeding preference of both *Lygus* life stages, measured as the percentage of bolls with one or more internal warts, showed both adults (df=3, 12; f=16.0; p<.0002) and nymphs (df=3, 12; f=83.2; p<.0001) clearly and significantly favored young bolls – those having accumulated 150 or 250 HU versus 350 or 450 HU (Figs. 4A-B). Cotton bolls exhibiting external lesions may not exhibit corresponding internal warts. Likewise, bolls with internal warts may not exhibit concomitant seed damage. More than 80% of all bolls exhibited external lesions, but less than 40% of bolls had damaged seeds. As cotton bolls grew and matured, the probability of exhibiting *Lygus*-induced seed damage decreased significantly.

The proportion of bolls with damaged seeds was decreased significantly for both adult (*df*=3, 12; *f*=22.3; *p*<.0001) and nymphs (*df*=3, 12; *f*=19.8; *p*<.0001) as bolls matured. The proportion of bolls with damaged seed was significantly lowest with bolls at the 350-450 accumulated HU (Figs. 4C-D). This indicates a *Lygus* feeding preference for the seeds in younger bolls (<350 HU). When bolls exceed 350 accumulated HU, the probability of seed damage by nymphs decreased significantly. The proportions of cotton bolls with internal warts and damaged seeds were higher in the case of *Lygus* nymph infestation versus adult infestation, indicating that *Lygus* nymphs could be more injurious to cotton bolls than adults.

Damage Potential of *Lygus*

Regardless of *Lygus* developmental stages (adults or nymphs), the numbers of external lesions, internal warts, and damaged seeds per boll decreased with boll maturation, particularly between 150 and 350 HU bolls (Fig. 5). Thus, cotton boll susceptibility to injury due to *Lygus* feeding decreases with increasing boll maturity. Average external lesions, internal warts, and damaged seeds per boll were significantly lower at 350 and 450 HU than at 150 or 250 HU (Fig. 5). Cotton bolls exceeding 350 accumulated HU (such as those having accumulated 450 HU) exhibited no *Lygus* adult-induced internal damage whatsoever, and only negligible (insignificant in terms of lint yield impact) *Lygus* nymph-induced internal damage (Figs. 5C-D). Although there was year-to-year variation in the number of external lesions, internal warts and damaged seed per boll, for simplicity, only results from combined analysis of all years' data have been discussed.

There was a significant difference (*df*=3, 12; *f*=27.5; *p*<.0001) among the average number of external lesions caused by adult *Lygus* feeding on bolls at various maturity levels. The number of lesions caused by adult feeding was highest in bolls infested at 150 HU followed by those infested at 250 HU, and then 350 to 450 HU, (350 and 450 treatments did not differ from each other). Average numbers of lesions were 9.3, 5.93, 4.54, and 3.43 per boll when infested with an adult at 150, 250, 350, and 450 HU stages, respectively (Fig. 5A). *Lygus* nymphs also caused a similar pattern of external injury to maturing cotton bolls. *Lygus* nymphs caused significantly (*df*=3, 12; *f*=46.6; *p*<.0001) highest number of external lesions in the bolls infested at 150 HU followed by that at 250, and then the 450 and 350 HU treatments, both of which did not differ significantly from each other. Average numbers of lesions were 11.4, 8.07, 5.24, and 4.10 per boll when infested at 150, 250, 450, and 350 HU stages, respectively (Fig. 5B).

Significant differences (*df*=3, 12; *f*=25.8; *p*<.0001) were observed among the average number of internal warts caused by adult *Lygus* feeding on bolls at various maturity levels. The number of warts caused by adult feeding was significantly highest in bolls infested at 150 HU, followed by 250 HU, and 350 and 450 HU treatments which again did not differ from each other. For the adults, average numbers of warts were 2.55, 1.21, 0.41, and 0.02 per boll when infested at 150, 250, 350, and 450 HU stages, respectively (Fig. 5C). *Lygus* nymphs also caused similar pattern of internal warts to maturing cotton bolls. *Lygus* nymphs caused significantly (*df*=3, 12; *f*=51.7; *p*<.0001) higher numbers of external lesions in the bolls infested at 150 HU, 250 HU, then followed by the similar 350

and 450 HU treatments. Average numbers of lesions were 3.81, 2.92, 0.69, and 0.22 per boll when infested at 150, 250, 350, and 450 HU stages, respectively (Fig. 5D).

There were significant differences (df=3, 12; f=49.8; p<.0001) among the average number of damaged seeds caused by adult *Lygus* feeding on bolls at various maturity levels. The number of damaged seeds caused by adult feeding was highest in bolls infested at 150 HU, then those infested at 250 HU, and followed by the 350 and 450 HU treatments which, as before, did not differ from each other. Average numbers of damaged seeds per boll were 1.41, 0.42, 0.02, and 0.01 when infested at 150, 250, 350, and 450 HU stages, respectively (Fig. 5E). *Lygus* nymphs also caused a similar pattern of seed damage with respect to boll age. *Lygus* nymphs caused a significantly (df=3, 12; f=17.8; p<.0001) highest number of seed damage in the bolls infested at 150 HU followed by that at 250, and lowest numbers at 350 and 450 HU. Average numbers of seed damage per nymph per boll in a 48-h period were 1.28, 0.88, 0.44, and 0.38 when infested at 150, 250, 350, and 450 HU stages, respectively (Fig. 5F).

Adults versus Nymphs

Only in 2009 and 2010 were *Lygus* adults and nymphs evaluated simultaneously in a large-scale field study. In both years there were significant differences in number of warts or lint and seed yield per boll observed between adult and nymphal infestation treatments at lower HU accumulating bolls (Table 1), with nymphs inflicting greater injury to the maturing bolls. In 2009, *Lygus* nymphs also induced significantly more external lesions per boll to more mature (350 and 450 HU) bolls than adults (Table 1). As stated earlier, both adults and nymphs were able to cause external injury to bolls of all ages, but the number of external lesions per boll (or the attempted internal feeding) was significantly higher for nymphs compared with that in adult. Also, the success of the external probing to cause internal injury correlated with the number of attempts. That is, *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 (younger; 150-250 HU) bolls (Jubb and Carruth 1971). It may be that the rapid development of late-instar nymphs creates a greater demand for food, or that some fitness change occurs once nymphs transition to adulthood.

Impact of *Lygus* Injury on Cotton Yield

Cotton yield responses to *Lygus* injury were recorded only in 2009 and 2010. Average cotton lint yields were significantly higher in 2010 than in 2009. Both adults and nymphs significantly (df=4, 16; f=24.8; p<0.0001) reduced lint yield when infested at 150 and 250 HU compared with that in control cohort (Fig. 6A-B). It was also observed that late-instar nymphs were able to induce greater damage in terms of lint yield than adults, specifically at the 150 HU stage. Lint yield was decreased by damage caused by *Lygus* adults on 150 and 250 HU aged bolls, although to a lesser extent than was seen from nymphs on 150 HU aged bolls. It may be that a brief period of intense feeding by late-instars might have produced such damage, while adult feeding produced less intense damage over a greater period of time. Lint yields of infested 350 and 450 HU bolls were statistically similar to corresponding controls and apparently not impacted by minor observed seed damage.

Cotton seed and cotton lint response data were similar. Average cotton seed per boll was higher in 2010 than in 2009. *Lygus* adults and nymphs caused similar seed damage at each HU accumulation threshold except 150, wherein the impact of greater nymphal damage was realized in terms of significantly higher average seed yield in adult-infested bolls. Average cotton seed yields of infested 350 and 450 HU bolls were similar to corresponding controls (Fig. 6C-D), indicating no seed yield impacts from *Lygus* damage at these thresholds. However bolls having accumulated 150 and 250 HU incurred significant (df=4, 16; f=44.7; p<0.0001) seed yield impacts due to both adult and nymphal *Lygus* infestation.

Young cotton bolls, specifically those having reached 150 or 250 HU, are susceptible to seed and lint yield impacts upon exposure to 48 hours of *Lygus* infestation. As such, cotton crops bearing relatively immature (<350 HU) fruit loads should be closely observed for and protected from *Lygus* damage. As the crop fruit load, predominantly, accumulates >350 HU, safety from *Lygus* damage can be expected. At that point, control measures, including insecticidal intervention, are not economically justified.

Relationships between *Lygus* Damage and Cotton Boll Characteristics

Individual boll data from 2007 to 2011 were averaged by insect stage, treatment (HU), and block for regression and correlation analyses. Correlation analyses of damaged seed counts, cumulative heat units, boll diameters, boll weights, and boll hardness showed significant negative relationships between the average numbers of damaged seed with all other variables (Table 2). Increasing cumulative heat units correlated with decreased seed damage (Fig. 7A); as cotton bolls grew in size, the number of *Lygus*-damaged seeds decreased (Fig.7B). Similarly, as cotton boll weight increased, the average number of *Lygus*-damaged seed were decreased (Fig.7C); and as the carpel wall hardness increased, the number of *Lygus*-damaged seeds decreased (Fig.7D).

From among the four variables tested, regression analyses with seed damage data revealed cotton boll diameter as the most effective ($R^2 \approx 60\%$) and external lesions as the least effective ($R^2 \approx 36\%$) predictors of potential seed damage imposed by *Lygus* feeding. Boll diameter is a superior indicator of boll maturity to predict potential seed damage due to a *Lygus* infestation. Furthermore, boll diameter was the easiest measurement to obtain for use in timing pesticide termination and formulating recommendations for cotton growers. It should be recognized that there will be some inherent varietal differences in regard to boll size versus boll maturity; therefore, some slight varietal specific refinements will possibly be needed to be accurately judge boll maturity across differing varieties.

Relationships between *Lygus* Damage and Cotton Yield

Individual boll data from 2009 to 2011 were averaged by insect stage, treatment (HU), and block for regression and correlation analyses. Correlation analyses of lint yield, seed yield, external lesions, internal warts, and damaged seed data showed significant negative relationships between lint yield or seed yield with number of warts per boll and number of damaged seeds per boll. Correlations between lint yield or seed yield and number of external lesions were insignificant (Table 2), indicating that external lesions induced by *Lygus* feeding is not a valid predictor of lint or seed yield impacts. Regression analyses

revealed the number of damaged seeds per boll as a superior predictor of lint or seed yield impacts, as the R^2 value for seed damage data exceeded that of internal/external lesions when regressed with lint and seed yield (Fig. 8). Though time-consuming and a practical concern, counting damaged seeds appears to be the best method for estimating potential cotton yield impacts due to *Lygus* feeding. Potential yield losses cannot be estimated by counting external lesions or via field *Lygus* sampling.

Summary

Lygus adults and nymphs both externally injure cotton bolls throughout boll development. Internal wart and damaged seed due to a *Lygus* (adult or nymph) infestation were reduced as cotton bolls accumulated higher numbers of heat units. With four-year data, it is clear that *Lygus* late-instar nymphs and adults are capable of causing generally similar damage to maturing bolls and reducing lint yield up to 250 HU, with nymphs inflicting significantly greater injury than adults. However, *Lygus* are generally unable to internally damage bolls exceeding 350 accumulated HU. Insecticide intervention to manage *Lygus* is unnecessary beyond 350 HU (15-20 days) post-cut-out (NAWF=5). *Lygus* generally do not cause internal damage to the cotton bolls larger than ~3 cm diameter. Thus is justified to terminate pesticide applications when most of the harvestable cotton bolls become equal or bigger than 3 cm after crop cut-out.

Acknowledgments

We thank our funding agencies including Cotton Incorporated State Support Program and Plains Cotton Growers, Inc. The authors are indebted to Mahendra Adhikari, Kamala Adhikari, Swagat Parajulee, Poma GT, Anup Bastola, Diwash Neupane, Michele Fekete, Andrew Stevens, and the late Anabel Reid, whose technical assistance in the field was invaluable.

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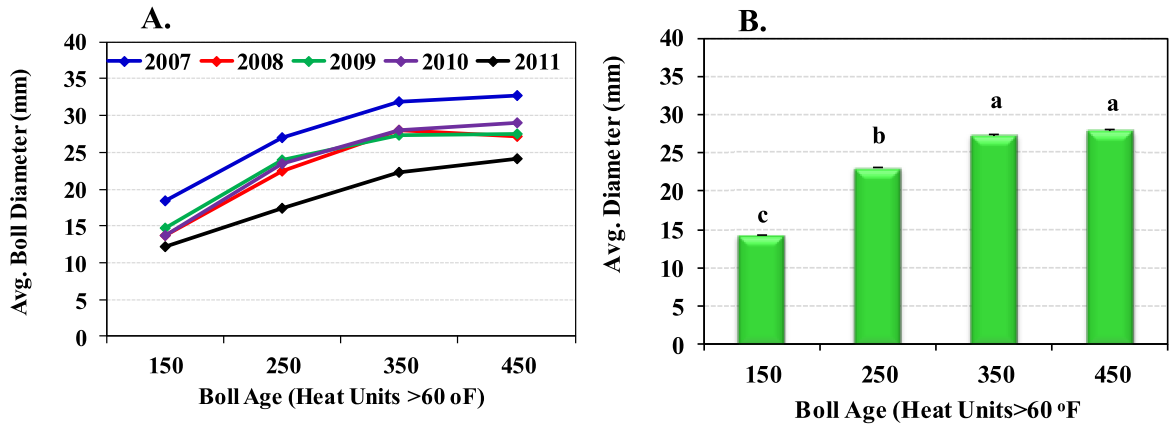

Figure 1. Relationships between cotton boll diameter and cumulative heat unit thresholds, A) Yearly data for five years (2007 to 2011), and B) Five-year average data. Different alphabets above bars indicate significant differences in average boll diameter between heat unit thresholds. Means were separated using LSMEAN procedure at α=0.1.

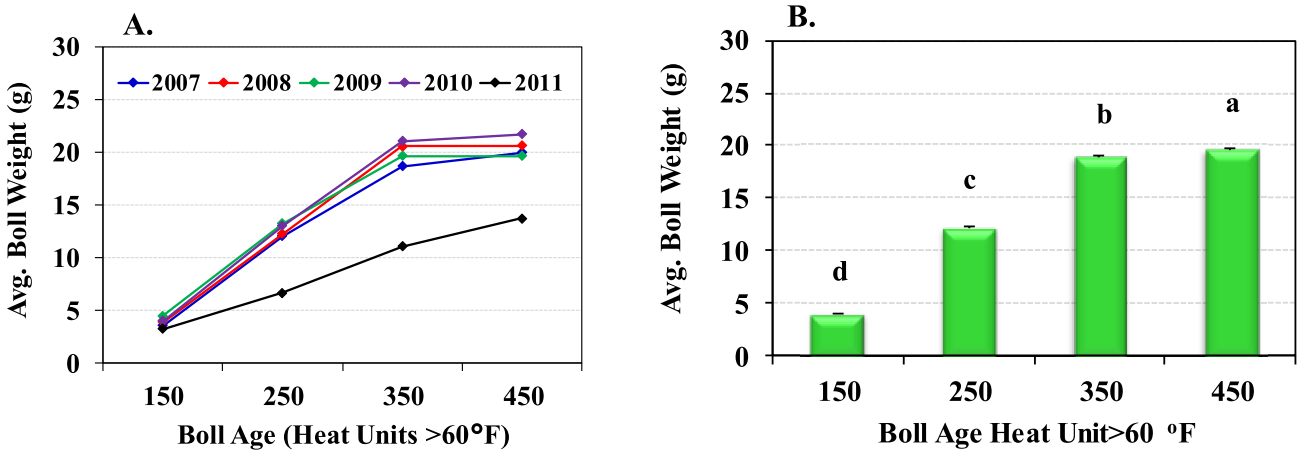

Figure 2. Relationships between cotton boll weight and cumulative heat unit thresholds. A) Yearly data for five years (2007 to 2011), and B) Five-year average data (right). Different alphabets above the bars indicate significant differences in average boll weight between heat units. Means were separated using LSMEAN procedure at α=0.1.

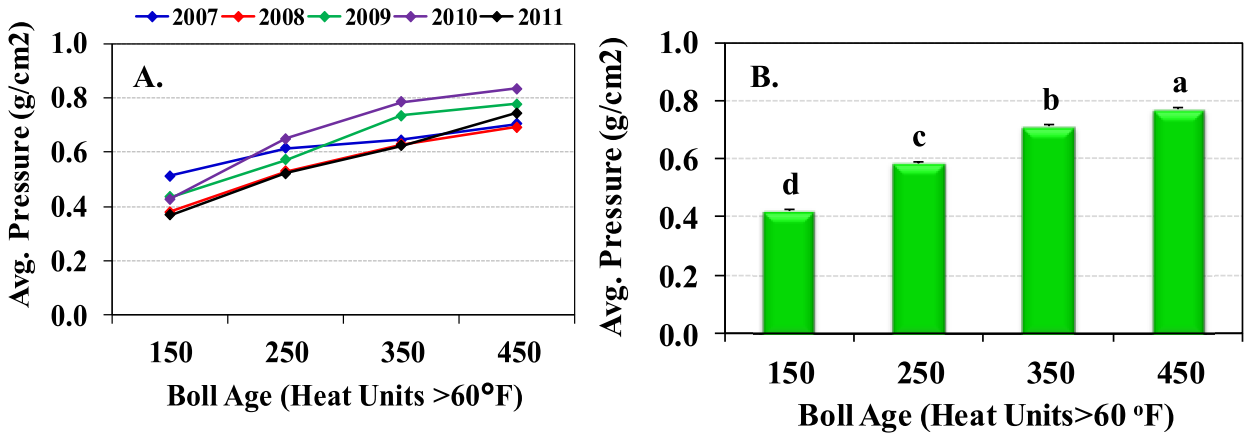

Figure 3. Relationships between carpel wall hardness and the cumulative heat unit, A) Yearly data for five years (2007 to 2011), and B) Five-year average data. Different alphabets above the bars indicate significant differences in average boll weight between heat units. Means were separated using LSMEAN procedure at α=0.1.

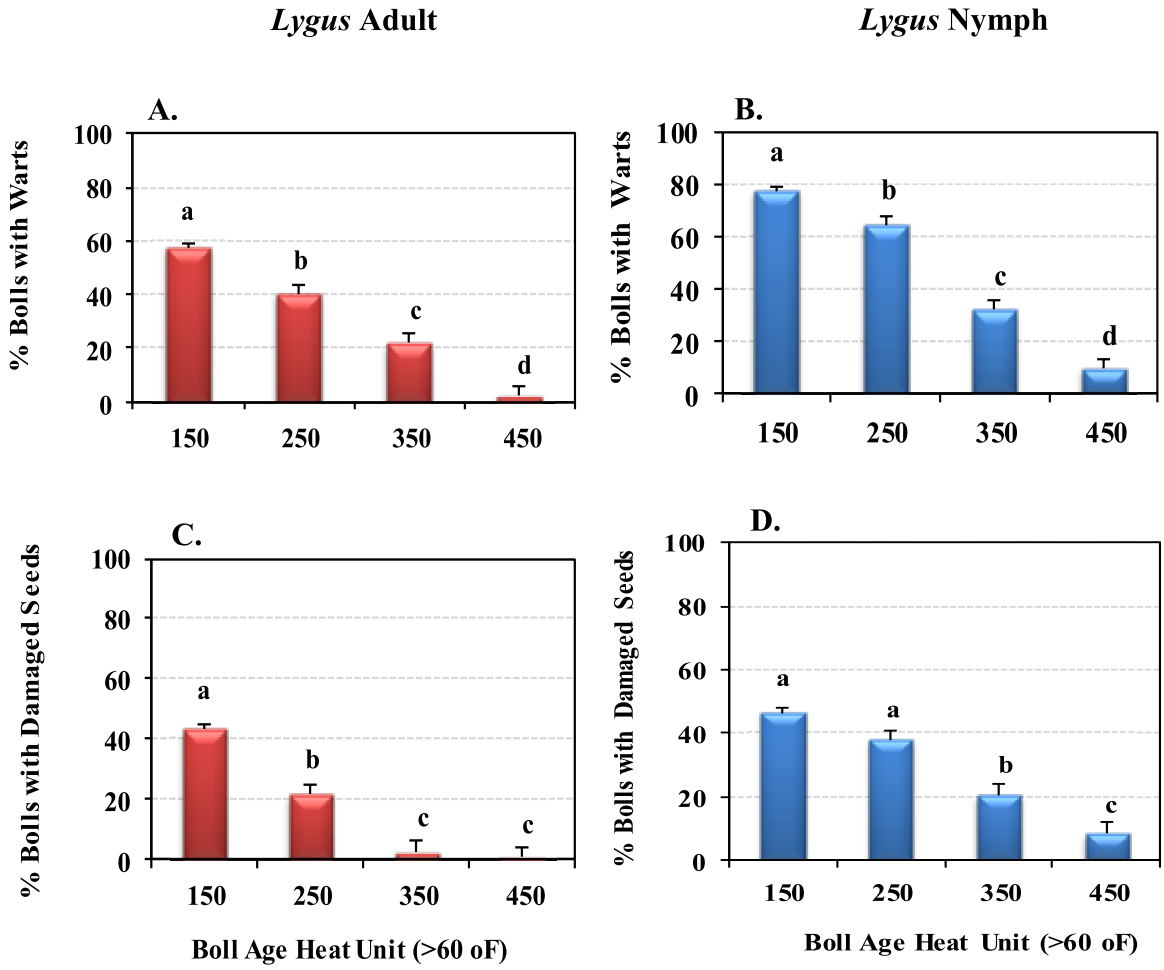

Figure 4. Cotton boll susceptibility to *Lygus* adults and nymphs at various levels of boll maturity, A) Average percentage of bolls with one or more internal warts caused by adult feeding, B) Average percentage of bolls with one or more internal warts caused by nymph feeding, C) Average percentage of bolls with one or more damaged seeds caused by adult feeding, and D) Average percentage of bolls with one or more damaged seed caused by nymphal feeding. Data for adult *Lygus* were collected from 2007 to 2010, whereas data for nymphs were collected from 2008 to 2011. *Lygus* bugs were allowed to feed on a caged boll for 48 hours. Different alphabets above the bars indicate significant differences in variables among heat units. Means were separated using LSMEAN procedure at α=0.1.

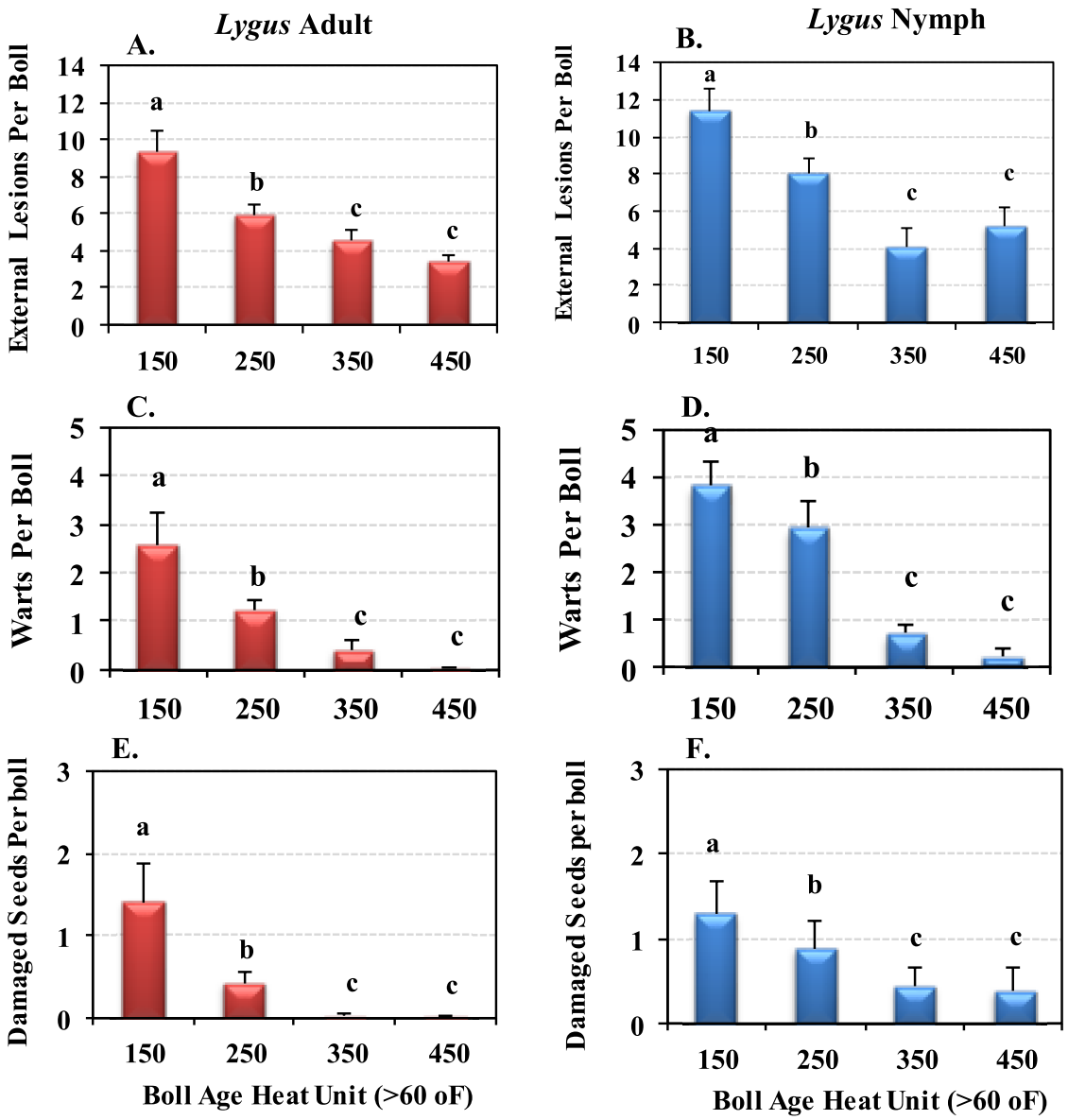

Figure 5. Cotton boll damage potential of *Lygus* adults and nymphs as measured by quantity of boll injury at various maturity levels, A) Average number of external lesions per boll caused by a single adult, B) Average number of external lesions per boll caused by a single nymph, C) Average number of internal warts per boll caused by a single adult, D) Average number of internal warts per boll caused by a single nymph, E) Average number of damaged seeds per boll caused by a single adult, and F) Average number of damaged seeds per boll caused by a single nymph. Data for adult *Lygus* were collected from 2007 to 2010, whereas data for nymphs were collected from 2008 to 2011. *Lygus* bugs were allowed to feed on a caged boll for 48 hours. Different alphabets above the bars indicate significant differences in variables among heat units. Means were separated using LSMEAN procedure at α=0.1.

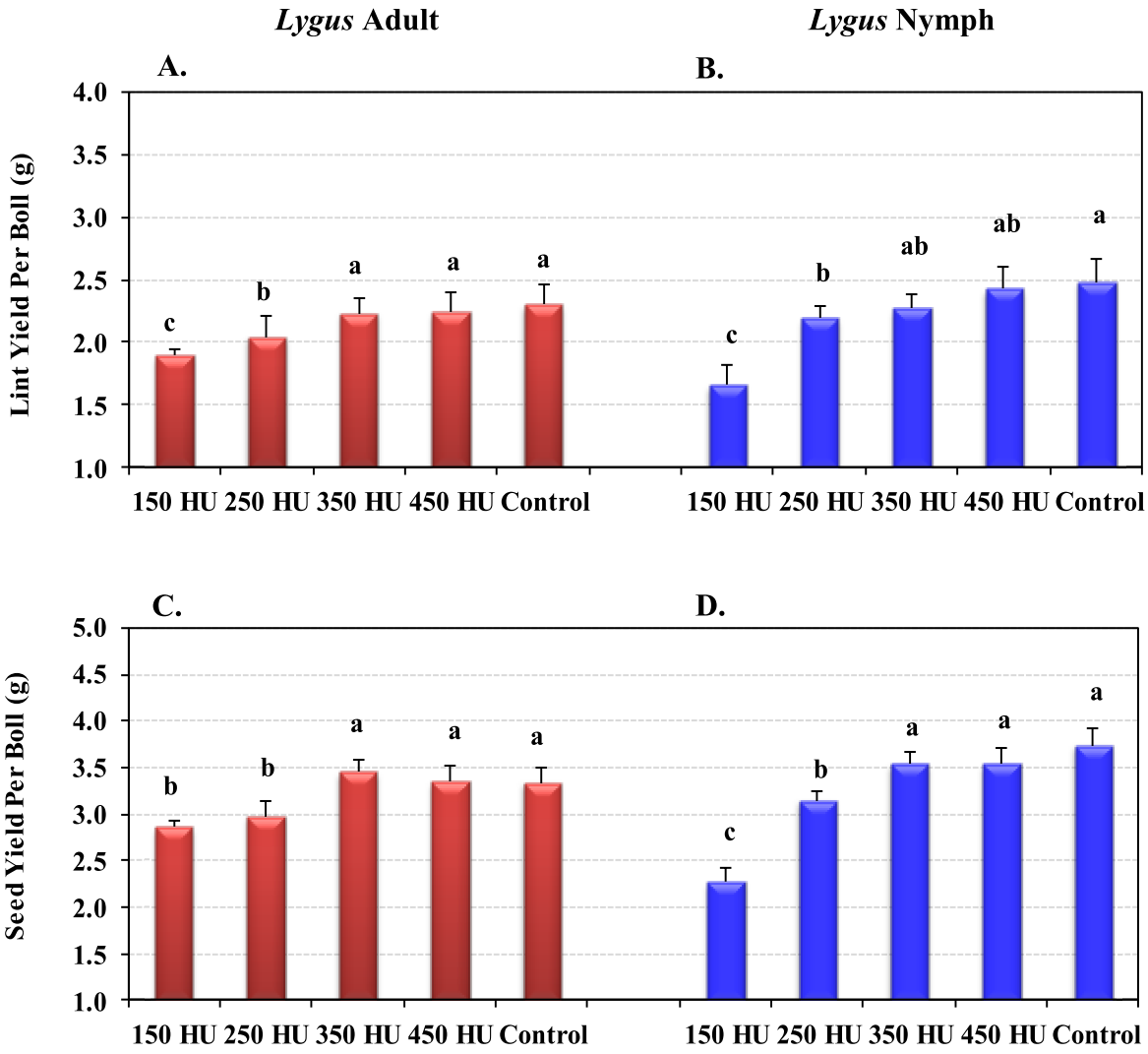

Figure 6. Cotton yields affected by *Lygus* adults and nymphs injury to bolls of varying maturity levels, A) Average lint yield per boll affected by single adult feeding for 48 hours, B) Average lint yield per boll affected by a single nymph feeding for 48 hours, C) Average seed yield per boll affected by a single adult *Lygus* feeding for 48 hours, and D) Average seed yield per boll affected by a single nymph feeding for 48 hours. Different alphabets above the bars indicate significant differences in average lint or seed yields among heat units. Means were separated using LSMEAN procedure at α=0.1.

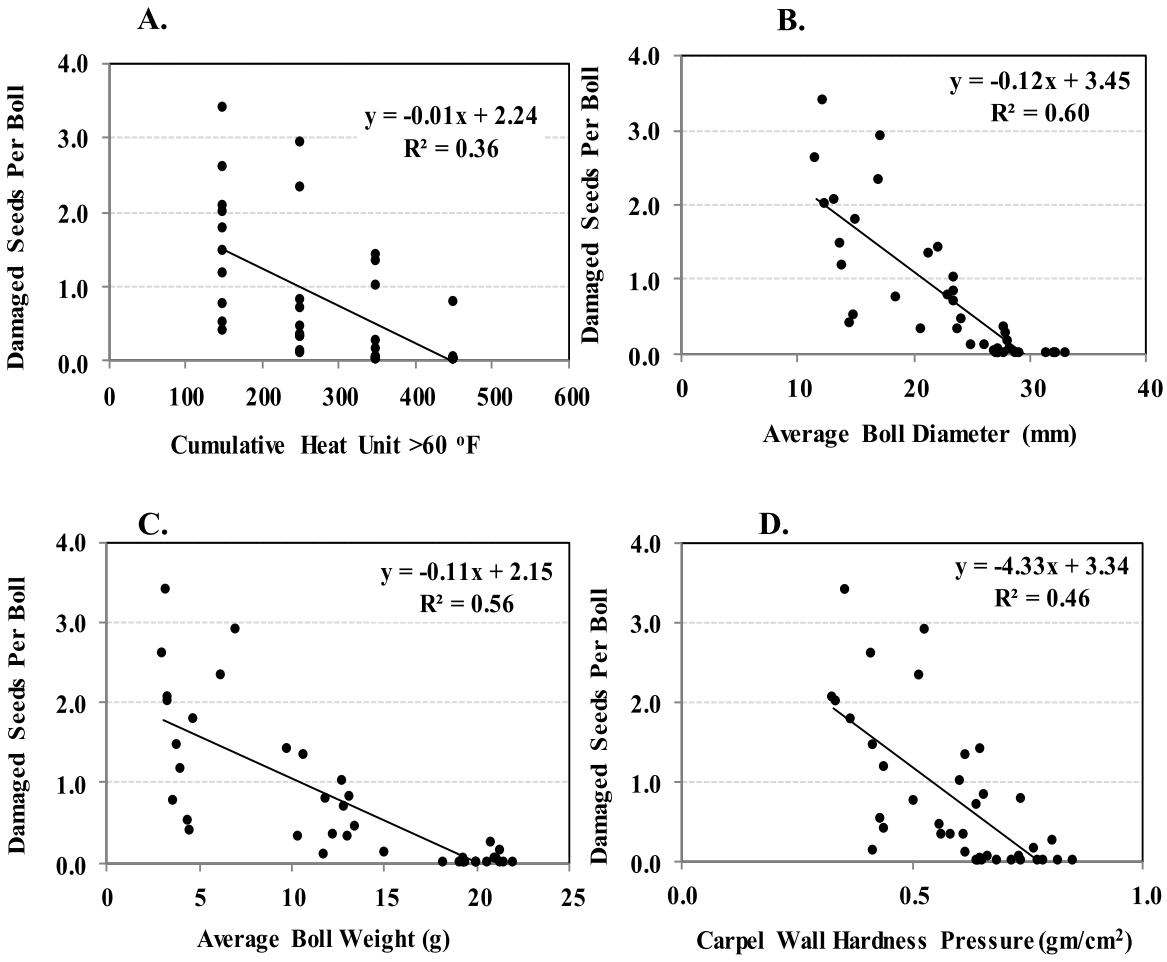

Figure 7. Relationships between average numbers of *Lygus* damaged seed and four boll growth and developmental variables including: A) Average number of damaged seed predicted by cumulative heat units, B) Average number of damaged seed predicted by boll diameter, C) Average number of damaged seed predicted by boll weight, and D) Average number of damaged seed predicted by carpel wall hardness (maximum pressure required to puncture the boll).

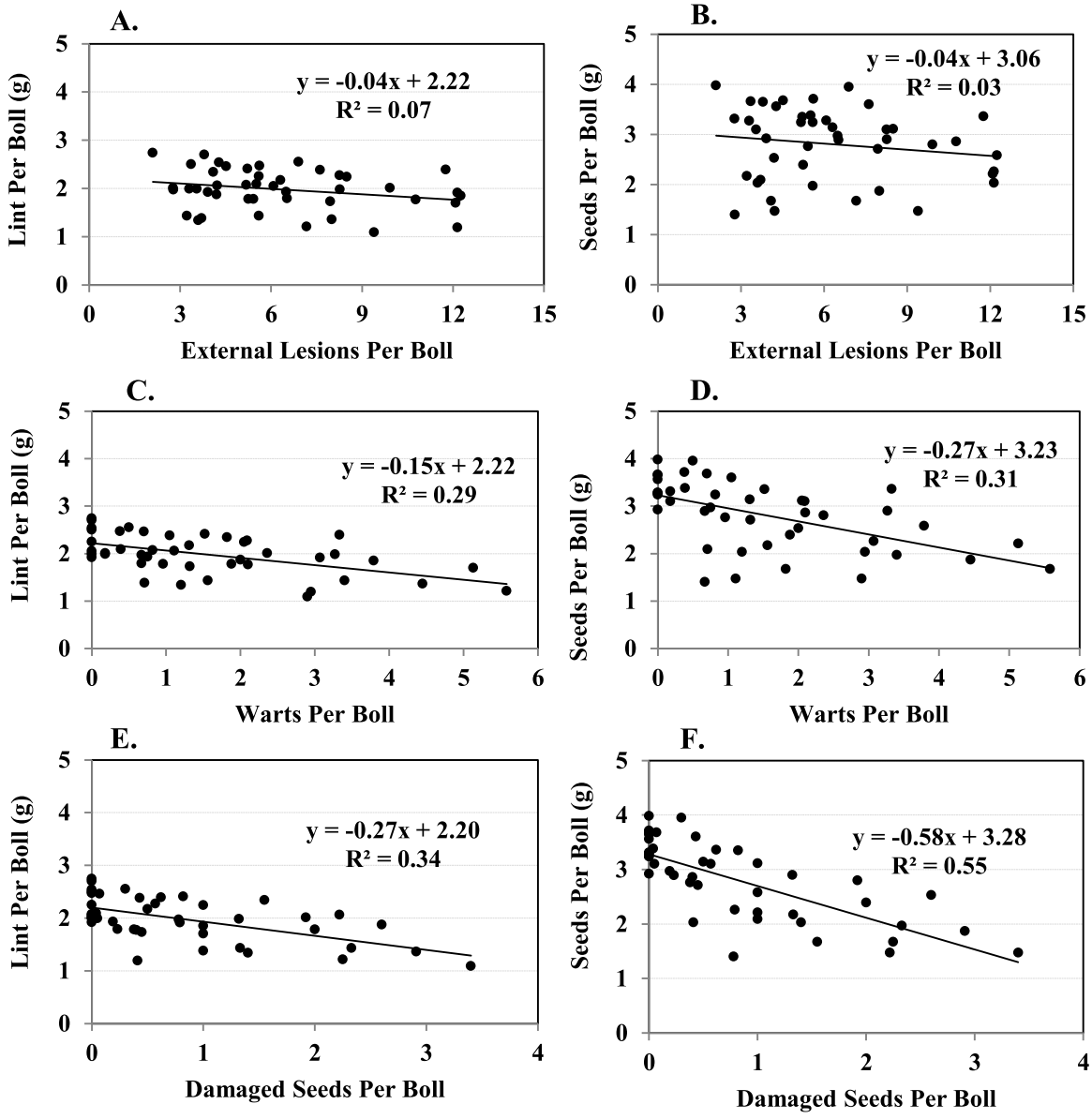

Figure 8. Relationships between average cotton yields (lint and seeds) and three types of boll injuries caused by *Lygus* infestation including: A) Lint yield predicted by external injury lesions, B) Seed yield predicted by external injury lesions, C) Lint yield predicted by internal warts, D) Seed yield predicted by internal warts, E) Lint yield predicted by number of damaged seeds, and F) Seed yield predicted by number of damaged seeds.

Table 1. Effect of Cotton Boll Cumulative Heat Units on Various Yield and *Lygus* Injury or Damage Variables (Mean ± SE) in 2009 and 2010 and Comparison between Damage Potential of *Lygus* Adults and Nymphs.

		2009				2010			
Variables	Heat Unit	Adult		Nymph		Adult		Nymph	
External lesions	150	8.65 ± 2.12 a	B	12.14 ± 0.00 a	A	9.10 ± 0.83 a	B	12.17 ± 0.08 a	A
	250	6.69 ± 1.27 ab	A	6.41 ± 0.10 b	A	6.86 ± 1.64 b	B	10.01 ± 1.75 ab	A
	350	3.16 ± 0.39 b	B	5.35 ± 0.17 c	A	5.07 ± 0.55 c	A	7.26 ± 0.36 b	A
	450	3.61 ± 0.31 b	B	5.84 ± 0.24 bc	A	3.82 ± 0.46 c	A	2.95 ± 0.85 c	A
Internal warts	150	1.39 ± 0.72 a	B	3.01 ± 0.06 a	A	2.82 ± 0.46 a	B	4.46 ± 0.67 b	A
	250	1.14 ± 0.18 ab	A	1.03 ± 0.28 b	A	1.79 ± 0.26 b	B	2.71 ± 0.62 b	A
	350	0.18 ± 0.00 bc	A	0.61 ± 0.22 bc	A	0.54 ± 0.16 c	A	0.78 ± 0.28 a	A
	450	0.00 ± 0.00 c	A	0.00 ± 0.00 c	A	0.00 ± 0.00 c	A	0.00 ± 0.00 a	A
Damaged seeds	150	0.32 ± 0.09 a	A	0.60 ± 0.19 a	A	1.62 ± 0.30 a	A	1.00 ± 0.00 a	A
	250	0.42 ± 0.04 a	A	0.35 ± 0.16 a	A	0.91 ± 0.09 b	A	0.60 ± 0.03 b	A
	350	0.03 ± 0.03 b	A	0.02 ± 0.02 b	A	0.04 ± 0.04 c	B	0.37 ± 0.07 c	A
	450	0.00 ± 0.00 b	A	0.00 ± 0.00 b	A	0.00 ± 0.00 c	A	0.00 ± 0.00 d	A
Lint yield	150	1.78 ± 0.01 b	A	1.55 ± 0.36 b	A	2.00 ± 0.01 c	A	1.78 ± 0.08 c	B
	250	1.76 ± 0.03 b	A	2.05 ± 0.12 a	A	2.34 ± 0.08 b	A	2.32 ± 0.06 b	A
	350	2.00 ± 0.01 a	A	2.08 ± 0.01 a	A	2.47 ± 0.01 ab	A	2.47 ± 0.08 b	A
	450	1.96 ± 0.04 a	A	2.15 ± 0.10 a	A	2.52 ± 0.02 a	A	2.72 ± 0.02 a	A
Seed yield	150	2.88 ± 0.02 ab	A	2.14 ± 0.12 c	B	2.85 ± 0.05 c	A	2.40 ± 0.19 b	B
	250	2.74 ± 0.02 b	A	3.06 ± 0.08 b	A	3.23 ± 0.12 b	A	3.23 ± 0.13 a	A
	350	3.21 ± 0.11 a	A	3.31 ± 0.07 a	A	3.70 ± 0.01 a	A	3.78 ± 0.18 a	A
	450	3.10 ± 0.18 ab	A	3.26 ± 0.02 a	A	3.61 ± 0.05 a	A	3.82 ± 0.17 a	A

Means followed by different small letters were significantly different (LSD, at $\alpha=0.1$) among bolls with different maturity levels (heat units) within a particular year and stage of *Lygus* for a particular variable measured; whereas means followed by different capital letters show the mean comparisons between adult and nymph within a particular heat unit in a particular year for the measured variable.

Table 2. Correlation coefficients (*r)* and in parenthesis *P* values showing relationships between boll growth/developmental parameters and *Lygus* damaged seeds (a); and *Lygus* injury or damages and cotton yield parameters (b).

	Heat Unit	Diameter	Weight	Pressure
a) Damaged seeds	-0.46 *(<0.0001)*	-0.71 *(<0.0001)*	-0.67 *(<0.0001)*	-0.59 *(<0.0001)*
	External Lesions	Internal Warts	Damaged Seeds	-
b) Lint yield	-0.261 *(0.0869)ns*	-0.54 *(0.0002)*	-0.58 *(0.0001)*	-
Seed yield	-0.16 *(0.2909)ns*	-0.56 *(<.0001)*	-0.74 *(<.0001)*	-

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

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Abstract

From 2002-2011, adult (moth) flight patterns of the cotton bollworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), and beet armyworm (Spodoptera exigua (Hübner) were monitored by using pheromone traps in three Texas High Plains (THP) counties. During the first four years, moth captures were monitored approximately weekly during all months in three counties which represent the northern (Hale), central (Lubbock), and southern (Gaines/Dawson) regions of the THP. Weekly monitoring has continued in Lubbock County since 2006. Yearly and historical flight profiles are provided and discussed for each of the three counties.

Introduction

In 2002, an ongoing trapping study was initiated to investigate the weekly and seasonal flight activity patterns of the cotton bollworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), and beet armyworm, *Spodoptera exigua* (Hübner) moths in the southern Texas High Plains (THP). Insect pheromone traps were used to measure the seasonal abundance of these pests. Previous research on two of these species includes Parajulee et al. (2004) report on a 14-year (1982-1995) study of monitoring THP bollworm and tobacco budworm populations. In the neighboring Texas Rolling Plains, Parajulee et al. (1998) report on a similar 15-year trapping study which included both weekly and daily trap service intervals.

These three species are significant cotton pests in the THP, which is widely recognized as the most intensive cotton growing area in the world. In the THP, the cotton bollworm is classified as an important economic pest of cotton and other regional crops, while the tobacco budworm and beet armyworm are classified as occasional pests. Seed from genetically modified cotton is available with Bollgard (*Bt*) technology which provides excellent crop protection from these pests. It is important to continue monitoring these pest populations due to the significant amount of cotton acreage that is not planted with this technology, particularly lower input dryland acres which account for approximately 40-50% of the THP cotton acreage. The Bollgard technology was adopted in less than 5% of the THP cotton acreage by 2004, but its adoption since then has increased significantly to the current level of nearly 80% in irrigated acreage due to superior agronomic characteristics in most cultivars with Bt technology. There is also an interest in determining whether the widespread adoption of Bt technology in crops such as cotton and corn will bring about an overall decrease in lepidopteron pest populations across local and neighboring regions. Continued long term monitoring of these pest populations will hopefully help address questions of this type.

Materials and Methods

During the first four years (2002-2005), nine (3 monitored species x 3 replications) pheromone traps were placed in each of three selected counties representing northern (Hale), central (Lubbock) and southern (Gaines/Dawson) regions of the southern Texas High Plains. Monitored species included the cotton bollworm, tobacco budworm and beet armyworm. In each county, three sites (replications) were selected and one trap for each pest species was placed at each site, then baited and monitored approximately weekly throughout the year (2002-2005). Traps originally located in Gaines County (southern county) were moved to neighboring Dawson County after the second year of the study to facilitate more frequent monitoring. Beginning in 2006, traps with the same protocols and sites were serviced only in Lubbock County during the early spring to late fall period.

Trap types used to capture the adult moths included the Texas pheromone trap (Fig. 1A, Hartstack et al. 1979) for bollworm and budworm moths and green bucket traps (Fig. 1B) for beet armyworm moths. Pheromone for all three species was secured from a single source (Trece™, Inc., Adair, OK). The cotton bollworm and tobacco budworm traps were re-baited approximately twice monthly and the beet armyworm pheromone was changed monthly. The bucket (capture container) on beet armyworm traps also contained a 1-inch x 1-inch toxicant strip to kill the moths soon after capture. Exact locations of all trapping sites were determined using a hand-held Garmin® GPS device.

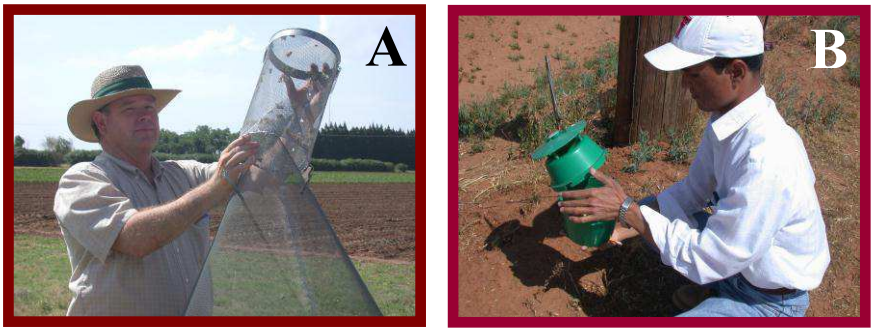

Figure 1. Texas pheromone traps (A) are commonly used to monitor moth populations such as the cotton bollworm and tobacco budworm, while the green bucket trap (B) is commonly recommended for beet armyworms.

Results and Discussion

Cotton Bollworm

Figure 2A illustrates the calculated historical bollworm flight profiles (based upon pheromone trap captures) across year for the three counties. Bollworm flight activity was low or non-existent during the period of 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 fruit is vulnerable to damage. Within this extended period of activity, the highest numbers of moths responded to traps from early August to mid-September.

Four (2002-2005) individual yearly bollworm moth flight patterns for each county are shown in Fig. 3. For study years 2002-2005, the within-year county trap response patterns for the three counties were relatively similar to each other, and between years the patterns were also similar except for differences in cotton bollworm abundance. Overall population levels detected in Lubbock County were highest in 2002 with peaks of approximately 2,000 moths/trap/10-day period and lowest in 2004. Yearly flight profiles and moth abundance were relatively similar to each other in other years of the survey.

Tobacco Budworm

Figure 2B shows the historical flight activity for tobacco budworms by county across year. Small numbers of budworms started responding to traps in late April while peak numbers were observed from early June to early October. Hale County (northern area) peaked one month later than the more southern areas and also had an abbreviated period of flight activity. After each county's peak activity period, numbers fell quickly with essentially no moth activity detected by late October. Lubbock County had the highest number of tobacco budworms responding to pheromone traps (Fig 2B) but this was likely skewed by the much higher Lubbock County counts in 2002 and 2003 (Fig. 4).

Within the years 2002-2005, tobacco budworm trap responses had similar patterns in all counties although moth numbers were notably higher in Lubbock County during the active periods of 2002 and 2003 (Fig. 4). With the exception of 2004, the period of highest flight activity for Hale County typically reached its peak approximately one month later than the more southern counties (Fig. 4).

Beet Armyworm

The averaged historical trap response profile (Fig. 2C) indicates that beet armyworm populations on the Texas Southern High Plains displayed two peak periods of flight activity during the study. The first peak typically occurred in mid-April followed by an extended peak of moth activity during the period of late August to late November.

Based upon the 2002-2005 yearly data (Fig. 5), the two peak periods of annual beet armyworm flight activity reflected in the historical profile (Fig. 2C) can be easily seen in most of the individual annual flight profiles. Although beet armyworms can be captured during all months of the year, they are primarily active during the period of early March to early December. Figure 5 illustrates the similarities of the county moth activity patterns within years and at the same time shows how vastly different overall moth abundance can be between individual years.

Moth Flight Behavior during the Last 10 Years

Figures 6-8 highlight the moth flight behavior during the last 10 years for these three significantly important caterpillar species in the Texas High Plains. The last 10 years of moth survey in the Lubbock County encompass the timeframe with boll weevil free cotton production enterprise in THP along with the adoption of Bollgard technology that confers resistance to these caterpillar species. Figure 6 depicts the average number of bollworm moths/trap/week in Lubbock County during the years immediately following boll weevil eradication and beginning of Bollgard adoption (2002-2004), increased Bollgard adoption years (2005-2007), and Bollgard adoption peak years (2008-2010), compared with the current year (2011). It is evident that

bollworm moth abundances have generally declined as the adoption of Bollgard technology increased. Also, the peak moth activity has shifted earlier during 2008-2010 that coincided with Bollgard adoption rate of >50%. While closer scrutiny is warranted to ascertain this observation, it is plausible that the overall bollworm pressure could have been lower in THP during the recent years compared with that in 5-6 years when the adoption of Bollgard technology was <30%. The 2011 moth survey data did not show a clear peak and the densities were lower than in previous years, but it appears that the perceived peak was much earlier than those in 2002-2007. A similar trend is also observed in tobacco budworm moth activity patterns, but the overall tobacco budworm moth activity declined significantly that coincided with increased adoption rate of Bollgard technology (Figure 7). While a clear trend of peak moth activity was not evident for beet armyworm moths, the overall beet armyworm moth activity declined as with bollworm and tobacco budworm moths, except for 2011. The unusual drought and record temperatures could be attributed for increased beet armyworm moth abundance in 2011. We intend to continue this survey work in 2012 and in future years.

Acknowledgements

Funding for this study was provided by Texas AgriLife Research Hatch Project 8810 and Plains Cotton Growers, Inc.

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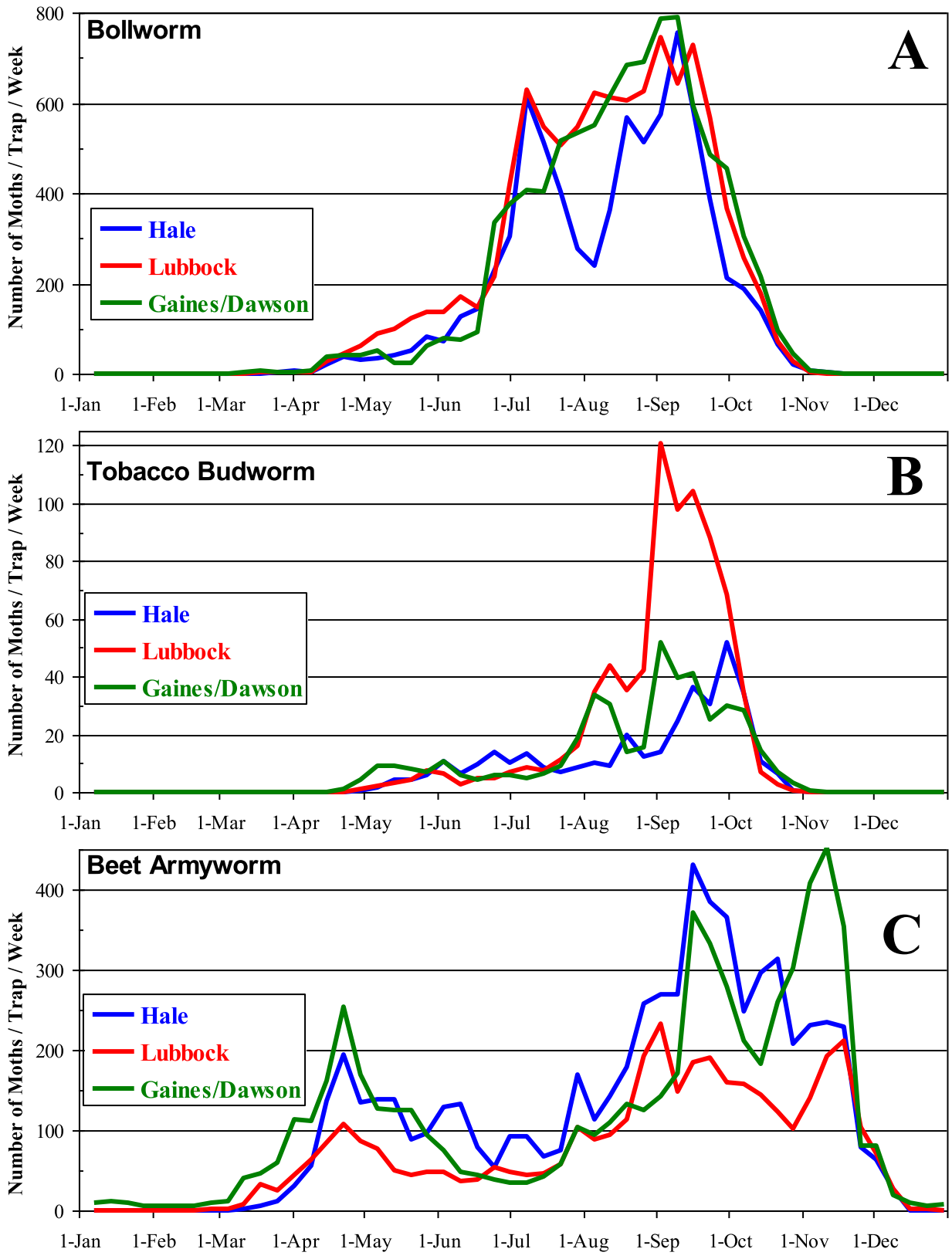

Figure 2. Historical flight profiles (weekly trap captures averaged across all four years) for the cotton bollworm (A), tobacco budworm (B), and beet armyworm (C). For each of the three cotton pest species, county flight profiles are given so that comparisons can be made for areas roughly representing the northern (Hale), central (Lubbock) and southern (Gaines/Dawson) regions of the southern Texas High Plains region, 2002-2005.

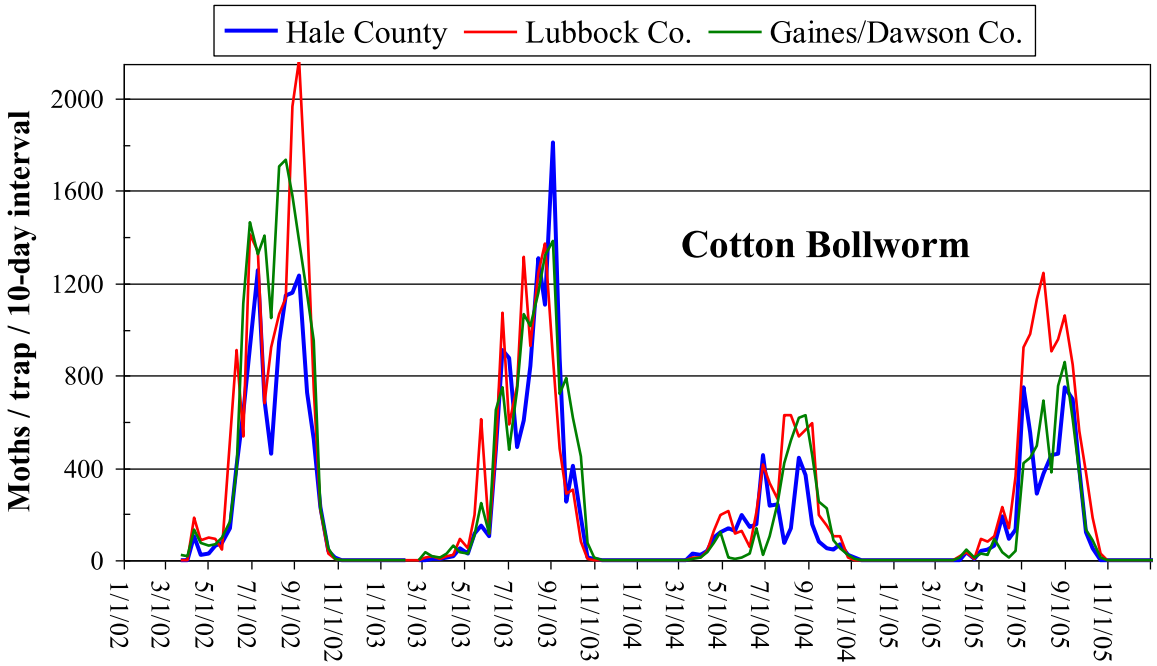

Figure 3. Average number of cotton bollworm moths/trap/10-day period in selected southern Texas High Plains counties, including Hale, Gaines/Dawson, and Lubbock County, 2002-2005.

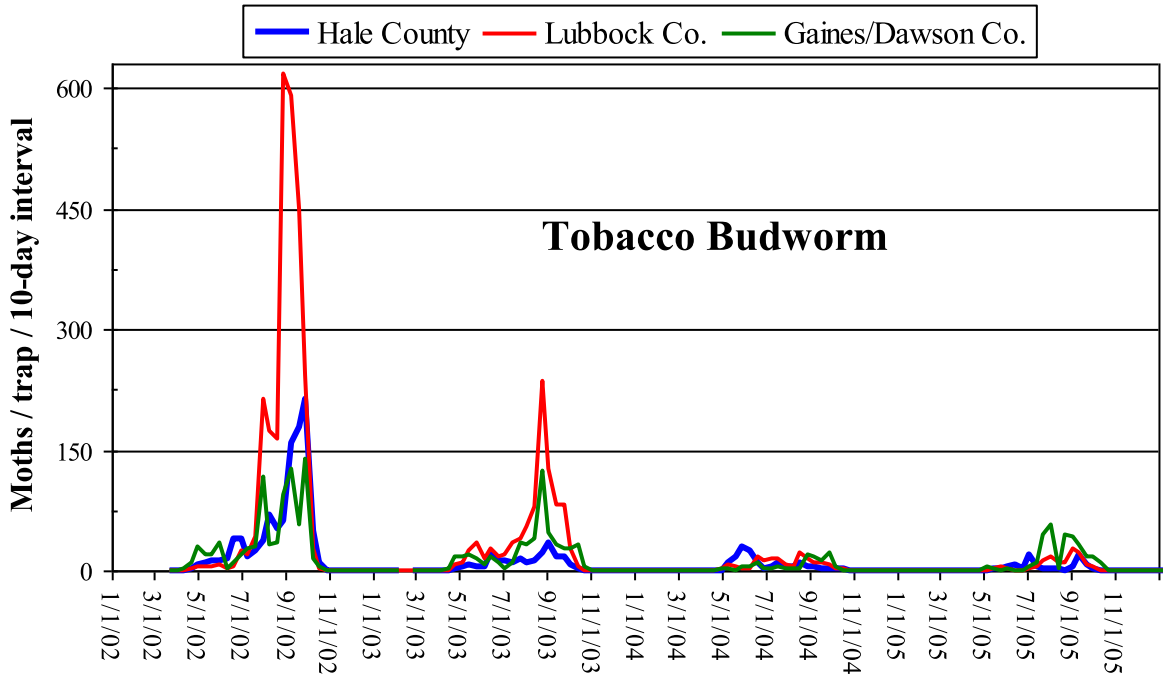

Figure 4. Average number of tobacco budworm moths/trap/10-day period in selected southern Texas High Plains counties, including Hale, Gaines/Dawson, and Lubbock County, 2002-2005.

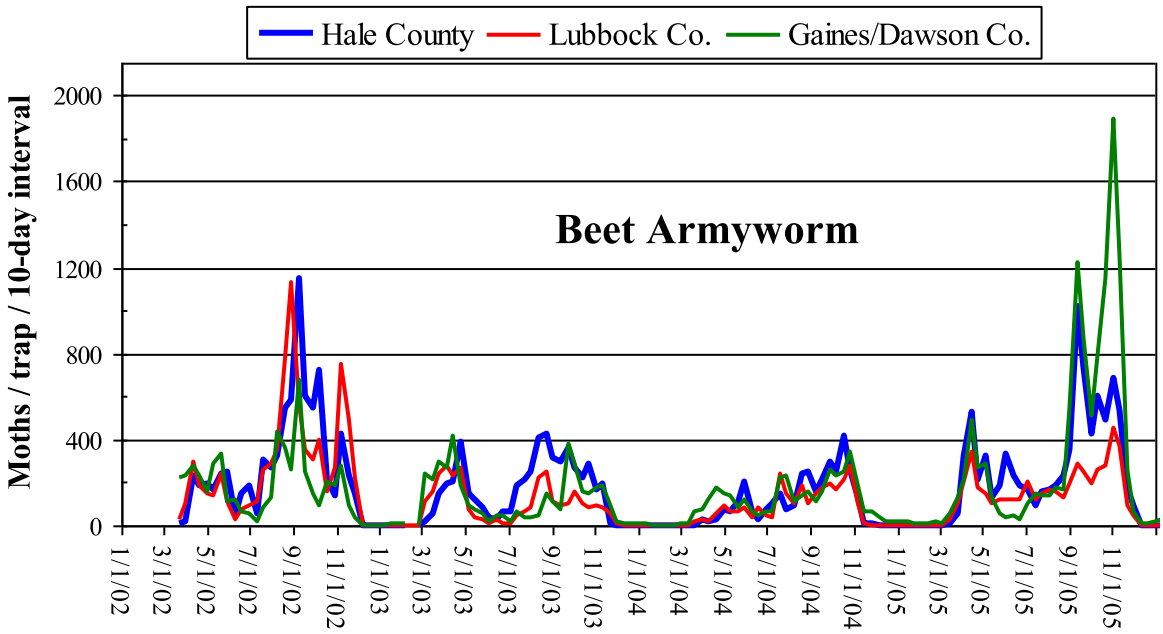

Figure 5. Average number of beet armyworm moths/trap/10-day period in selected southern Texas High Plains counties, including Hale, Gaines/Dawson, and Lubbock County, 2002-2005.

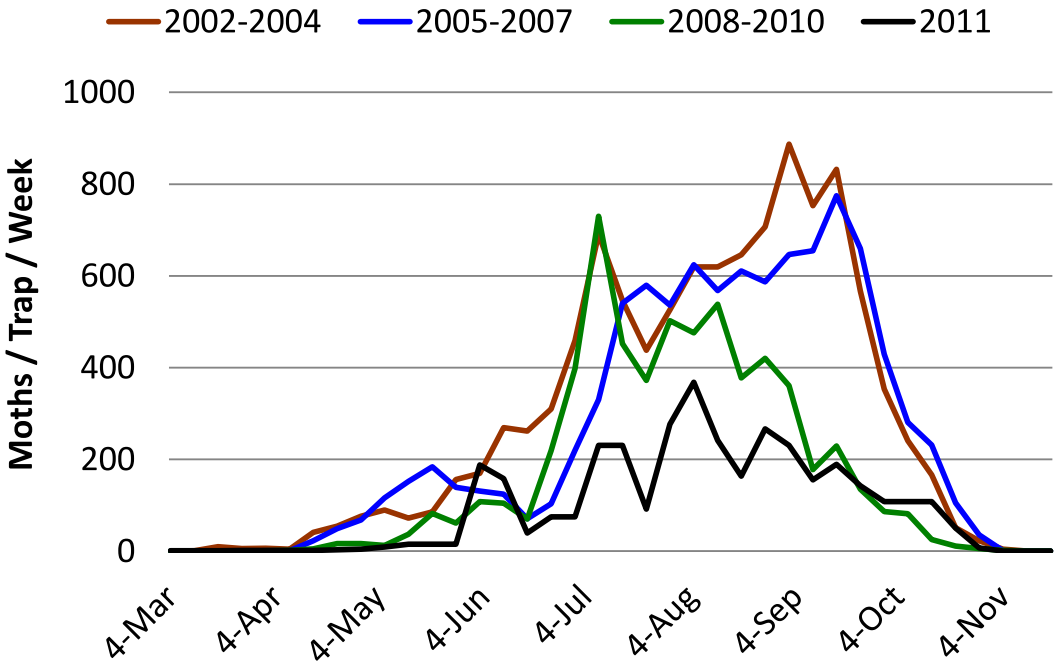

Figure 6. Average number of bollworm moths/trap/week in Lubbock County, depicting the years immediately following boll weevil eradication and beginning of Bollgard adoption (2002-2004), increased Bollgard adoption years (2005-2007), and Bollgard adoption peak years (2008-2010), compared with the current year (2011).

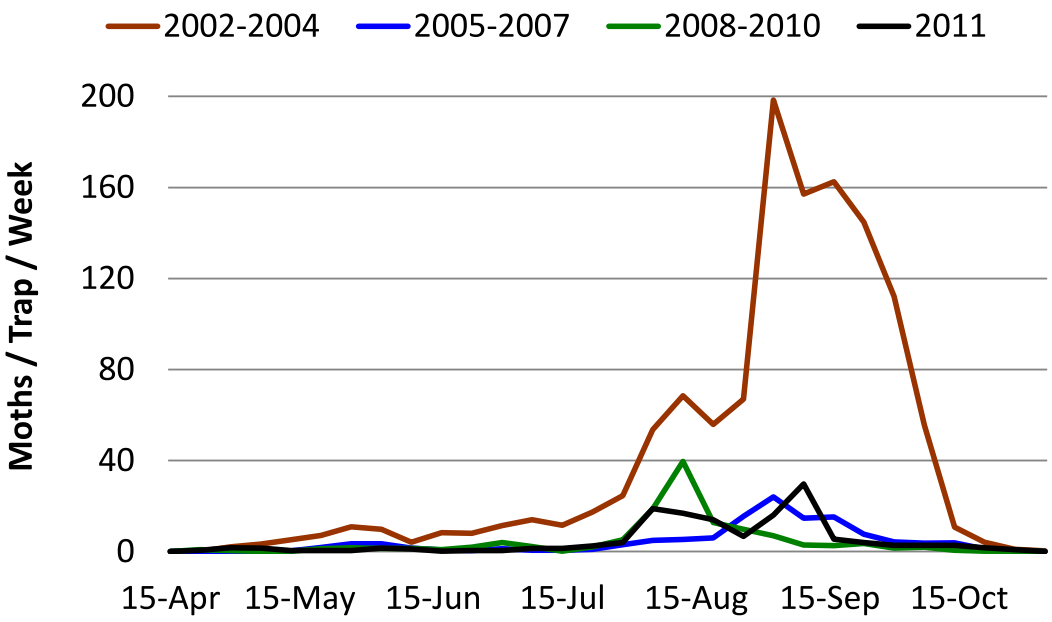

Figure 7. Average number of tobacco budworm moths/trap/week in Lubbock County, depicting the years immediately following boll weevil eradication and beginning of Bollgard adoption (2002-2004), increased Bollgard adoption years (2005-2007), and Bollgard adoption peak years (2008-2010), compared with the current year (2011).

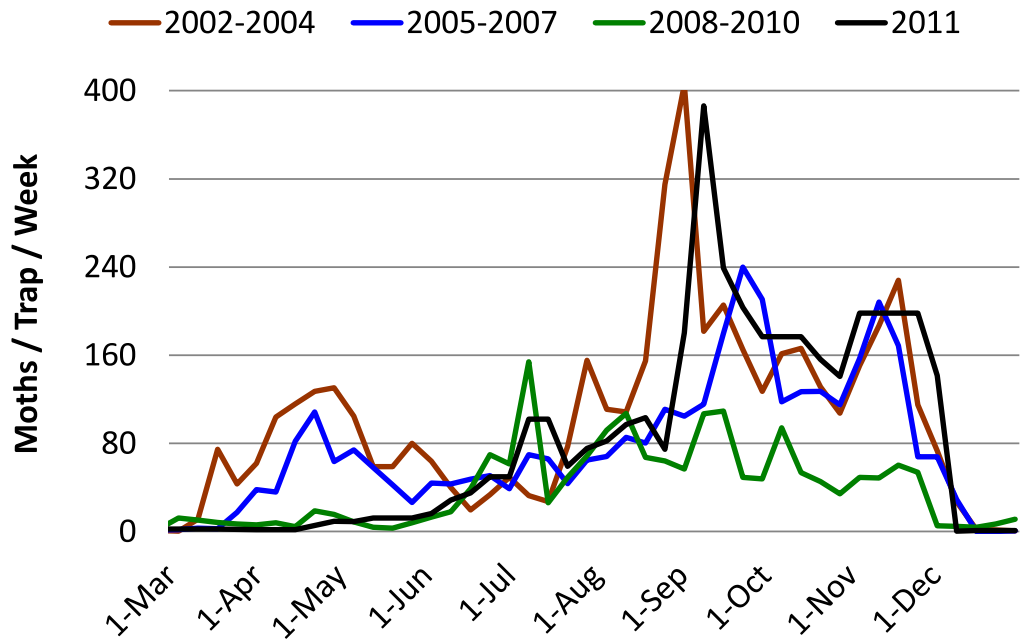

Figure 8. Average number of beet armyworm moths/trap/week in Lubbock County, depicting the years immediately following boll weevil eradication and beginning of Bollgard adoption (2002-2004), increased Bollgard adoption years (2005-2007), and Bollgard adoption peak years (2008-2010), compared with the current year (2011).

Geographic pattern of host-associated differentiation in the cotton fleahopper, *Pseudatomoscelis seriatus*

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Abstract

Host-associated differentiation (HAD) is the occurrence of genetically distinct, host-associated lineages. Most of the cases of HAD in phytophagous insects have been documented in specialist insects inhabiting feral ecosystems or in generalist parthenogens in agroecosystems. Herein we report HAD in the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), a native, generalist, non-parthenogenetic insect feeding on native wild hosts [horsemint, *Monarda punctata* L. (Lamiaceae) and woolly croton, *Croton capitatus* Michx. (Euphorbiaceae)] and on cotton [*Gossypium hirsutum* L. (Malvaceae)] in the USA. Examination of genome-wide genetic variation with AFLP markers and Bayesian analyses of *P. seriatus* associated with three different host plant species at five locations in Texas revealed a geographic pattern of HAD. The geographic pattern of HAD corresponded with differences in precipitation among the locations studied. In three locations, two distinct lineages of *P. seriatus* were found in association with horsemint and cotton/woolly croton, whereas in two other locations, populations associated with the different host plants studied were panmictic. We suggest that precipitation differences among locations translate into heterogeneity in vegetation distribution, composition, and phenology, which altogether may contribute to the observed geographic pattern of HAD.

Introduction

Research on ecological speciation of herbivorous insects over the past four decades has suggested that host plants may play an important role in starting the process of genetic differentiation (Berlocher & Feder, 2002; Dres & Mallet, 2002; Bethenod et al., 2005). Host plants can exert strong natural selection, which may promote reproductive isolation and further radiation of insect lineages on different host plant species (Ehrlich & Raven, 1964; Mopper, 1996; Martel et al., 2003; Miller et al., 2003; Diegisser et al., 2009; Funk, 2010), resulting in host-associated lineages (Dobler & Farrell, 1999; Stireman et al., 2005; Peccoud et al., 2009). Insects that use different host plant species across their geographic distribution are not only likely to experience divergent selection pressures by host plants but also variable climatic conditions at different locations (Via, 1991; Thompson, 1994; Sword et al., 2005). Particularly, selection pressure may vary if host plant densities and/or the communities in which the insect and its host plants are situated (i.e., predators, competitors, alternative host plants, diseases, etc.) differ across the insect's geographic distribution.

Variation in the availability and/or abundance of host plant species within an herbivore's distribution range may generate differences in the pattern of host plant specialization or adaptation (Kuussaari et al., 2000). Herbivore populations of the same species may be specialized on different host plant species at different locations and yet the species may be characterized as a generalist when its entire geographic distribution is considered (Fox & Morrow, 1981). Therefore, it is always more realistic to examine host-associated differentiation (HAD) throughout the entire geographic distribution of a species (Toju, 2009).

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However, fine scale heterogeneity in vegetation composition and other ecological factors are often overlooked when studying interspecific interactions.

A growing number of studies have documented HAD of insect herbivore species (Guttman et al., 1981; Carroll & Boyd, 1992; Emelianov et al., 2001; Nason et al., 2002; Brunner et al., 2004; Stireman et al., 2005; Conord et al., 2006; Ohshima, 2008; Dorchin et al., 2009). Although the majority of these examples have studied unmanaged or wild systems composed of perennial plant species, there are some examples of HAD of herbivores in agro-ecosystems (Via, 1991; DeBarro et al., 1995; Ruiz-Montoya et al., 2003; Vialatte et al., 2005; Alvarez et al., 2007). These examples show that HAD in herbivore insects is not uncommon in agricultural systems, which are mostly managed and prone to relatively high levels of anthropogenic disturbances.

In the present study we report HAD in the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae). *Pseudatomoscelis seriatus* has numerous host plant species, native wild hosts as well as introduced crop species. Previous studies indicated that several aspects of *P. seriatus* biology such as host plant preference, total developmental time, nymphal mortality, and fecundity are differentially influenced by their host plant species (Gaylor & Sterling, 1976; Beerwinkle & Marshall, 1999). The objectives of the present study were (1) to detect whether or not HAD was present in *P. seriatus* populations associated with three selected host plant species, and (2) to assess the geographic structure of HAD at a regional scale (i.e., the state of Texas, USA).

Materials and methods

Sample collection

Insects were collected from Lubbock, San Angelo, College Station, Weslaco, and Corpus Christi, all in Texas (Figure 1, Table 1). All five locations are in areas under intensive cotton cultivation and belong to distinct agro-ecological zones based on precipitation, elevation, soil type, and vegetation composition (http://www.tpwd.state.tx.us). We collected fleahoppers associated with cotton, horsemint, and woolly croton from several fields (2–5 fields per host plant species) within each location. In two study locations, Lubbock and San Angelo, we did not find woolly croton.

Pseudatomoscelis seriatus adults were collected during the peak fleahopper activity on each host plant (which is the flowering stage of each plant) by using a standard sweep net and a motorized blower also known as a 'keep-it-simple' sampler (Beerwinkle et al., 1997). The identity of *P. seriatus* collected from horsemint, woolly croton, and

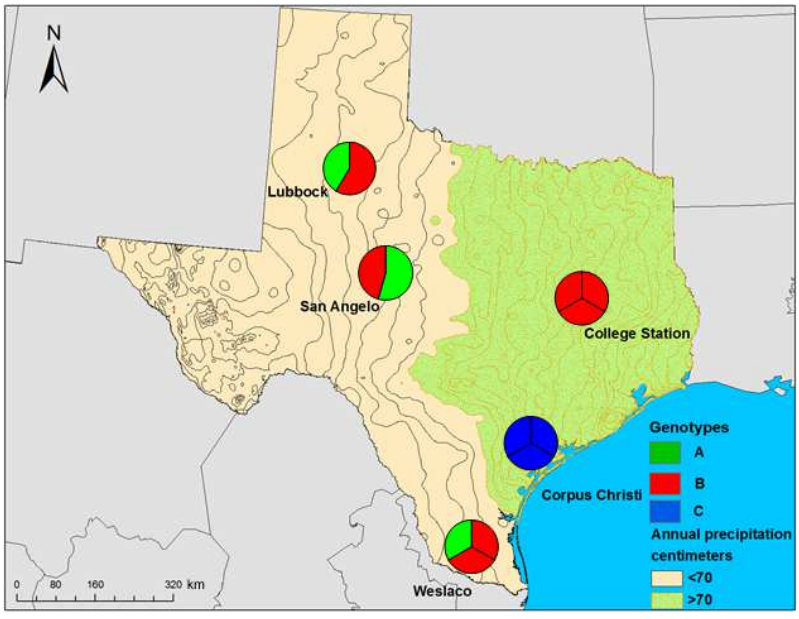

Figure 1 Study locations along with the genetic population structure of *Pseudatomoscelis seriatus* in an annual precipitation map of Texas. Presence and proportion of the three genotypes (A–C) of *P. seriatus* are shown in a pie diagram for each location. Genotype A comprises fleahoppers from horsemint, genotypes B and C were collected from horsemint, cotton, and woolly croton. The rainfall map was generated in ArcGIS from available annual rainfall data for the state of Texas.

Table 1 Collection location, host plant, population code, and geographic information (latitude, longitude, elevation) of *Pseudatomoscelis seriatus* populations used in the study

Location (TX, USA)	Host plant	Population code	Latitude (N)	Longitude (W)	Elevation (m a.s.l.)
Lubbock	Horsemint	LH	33.571	101.804	967
			33.489	101.619	874
	Cotton	LC	34.155	101.950	1066
			33.949	101.695	975
			33.981	102.078	1005
			33.641	102.079	1017
San Angelo	Horsemint	SH	31.661	100.330	618
			31.852	100.292	532
			32.064	100.305	617
			32.388	100.378	682
	Cotton	SC	31.422	100.140	358
			32.093	101.353	849
			31.328	100.161	579
			31.381	100.332	571
			31.414	100.073	555
College Station	Horsemint	CH	31.423	96.242	58
			30.842	96.617	85
			30.535	96.444	72
			30.692	96.515	70
			30.706	96.565	79
	Cotton	CC	30.535	96.444	72
			30.692	96.515	70
			30.399	96.269	62
			30.706	96.565	79
	Woolly croton	CW	30.392	90.350	58
			30.546	96.506	73
			30.844	96.622	83
Weslaco	Horsemint	WH	26.935	98.134	39
			26.799	98.412	72
	Cotton	WC	26.137	97.958	20
			26.385	98.253	44
			26.290	98.333	52
	Woolly croton	WW	26.137	97.958	20
			26.079	98.078	25
			26.119	97.968	20
Corpus Christi	Horsemint	TH	29.253	96.179	30
			29.442	97.101	102
			28.950	96.208	15
	Cotton	TC	27.969	97.713	32
			29.209	96.228	30
			27.848	97.644	24
	Woolly croton	TW	27.627	97.793	21
			27.610	97.753	16
			27.952	97.684	22

cotton was confirmed by a mirid systematist (Dr. Joseph C. Schaffner, Texas A&M University). Insects were preserved in 85% ethanol at 4 °C until used for DNA extractions. We used 12–20 fleahoppers per host plant species at each location for genetic analyses.

Insects

Pseudatomoscelis seriatus is a native insect of North America. It is considered a generalist herbivore reported to feed on ca. 160 host plant species belonging to 35 plant families (Snodgrass et al., 1984; Esquivel & Esquivel, 2009). In the

1920s, heavy yield losses of cotton were attributed to *P. seriatus* in various regions of Texas (Reinhard, 1926). Presently, *P. seriatus* occurs in several cotton growing regions in the USA, mostly in Texas, Oklahoma, Arkansas, Mississippi, and Louisiana. *Pseudatomoscelis seriatus* is a hemimetabolous insect, which completes 8–9 generations per year and feeds externally using its sucking mouthparts on tender stems or flowering structures of its host plants. It hibernates as eggs, which hatch during March–April depending on the rainfall and temperature. After progressing through five nymphal instars, *P. seriatus* remains as an adult for about 12–15 days. A female lays about 10–15 eggs under the epidermal layer of the stem of its host plants. Adults are not active fliers but they may disperse long distances either through wind or by anthropogenic dispersal.

Host plants

For this study, we selected three host plant species of *P. seriatus*: *Monarda punctata* L. (Lamiaceae) commonly known as horsemint, cultivated cotton, *Gossypium hirsutum* L. (Malvaceae), and *Croton capitatus* Michx. (Euphorbiaceae) commonly known as woolly croton. These three species were selected because they are the most common fleahopper host plants at our study sites and they persist for a relatively long time during the spring, summer, and fall, respectively, maximizing their period of interaction with *P. seriatus* (Almand et al., 1976; Holtzer & Sterling, 1980). Depending on local climatic conditions, *P. seriatus* spends 3–5 generations in association with each of these three host plant species in our study areas. The three chosen host plant species carry different suits of defensive chemicals (Scora, 1967; Schmidt & Wells, 1990; Williams et al., 2001). The host plants are available to *P. seriatus* at different times of the year, with some overlapping periods. For instance, in College Station (Brazos County), in a typical year, the native spring wild host, horsemint becomes available for *P. seriatus* at the beginning of the growing season (April–June), whereas woolly croton becomes available from May until October. Cotton becomes suitable for fleahopper feeding 40 days after planting. However, the timing of cotton cultivation varies by location within the state of Texas. For example, near College Station, cotton is normally planted during mid-April, whereas in Lubbock and San Angelo, planting of cotton is typically optimal in mid-May. Due to the difference in climate among eco-regions, the phenologies of the fleahopper host plants vary in their degree of overlap among some of our sampling locations.

Genetic methods

Genomic DNA was extracted from randomly chosen individual specimens using DNeasy® kits (Qiagen, Valencia, CA, USA) following the manufacturer's recommended protocol for animal tissue. DNA concentration and quality were measured for each specimen using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA was eluted in 100 μl of Qiagen AE buffer.

Amplified fragment length polymorphism (AFLP) markers were generated using the protocol proposed by Vos et al. (1995) with slight modifications. Samples were randomly arranged in 96-well plates for AFLP analyses. Three to four samples were repeated within each plate and the same samples were repeated in all the plates used to check the reproducibility of our analysis. Restriction digestion and ligation steps were performed by adding 5.5 μl of genomic DNA to 5.5 μl of a master mix containing 1.1 μl 10× T4 DNA ligase buffer, 1.1 μl 0.5 M NaCl, 0.55 μl diluted bovine serum albumin (1 mg ml^{-1}), 0.05 μl *Mse*I [NEB R0525M (New England Biolabs, Ipswich, MA, USA)], 0.05 μl *Eco*RI (NEB R0101T), 0.03 μl T4 DNA ligase (NEB M0202M), 1 μl *Mse*I and 1 μl *Eco*RI adaptors [ABI 403077 (Applied Biosystems, Foster city, CA, USA)], and 0.61 μl ultra pure water (18.2 MΩ cm^{-1}). The entire reaction was left overnight at room temperature for adequate digestion. The next morning, 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). Pre-selective PCR amplification was performed in a (20 μl) reaction containing 4 μl of the diluted restricted/ligated DNA and 16 μl of a mixture of 1 μl of *Eco*RI and *Mse*I AFLP pre-selective primers mix (ABI 403078) and 15 μl of AFLP core mix (ABI 402005). The PCR protocol for the pre-selective 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 until subsequent procedure. 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 pre-selective PCR product was added with 15 μl platinum super mix [Invitrogen 11306016 (Life Technologies, Grand Island, NY, USA)], 1 μl of primers *Eco*RI-ACT (ABI 402045) or *Eco*RI-AAC (ABI 4303053), and 1 μl of *Mse*I-CAT (ABI 402018) or *Mse*I-CTC (ABI 402016). 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 (ABI 402985), 9 μl of HiDi formam-

ide, and 1 μl of selective PCR amplification product. Samples were analyzed in an ABI 3130 genetic analyzer (Applied Biosystems, Forest City, CA, USA). Results from capillary electrophoresis were analyzed by using GeneMapper® 4.0 (Applied Biosystems). Fragments within 50 and 400 bp with 100 or more relative florescent units were considered.

Statistical analysis

The SESim statistic (Medina et al., 2006) was used to assess the adequateness of the number of individuals and AFLP markers used to detect genetic population structure. A SESim value lower than 0.05 indicates that a given combination of markers and individuals is sufficient to detect genetic structuring at the geographic scale considered.

Data obtained from two primer pairs (E/ACT–M/CAT and E/AAC–M/CTC) were combined and analyzed as a single matrix. Population genetic information (% polymorphic loci, expected heterozygosity) was obtained after analyzing the AFLP matrix with GenAlEx 6.3 (Peakall & Smouse, 2006). Principal coordinate analysis (PCA) was performed by using Nei's genetic distance matrix (Peakall & Smouse, 2006) to visualize the relatedness of different populations in a two-dimensional coordinate system. Genetic differentiation was estimated by calculating F_{ST} values (Wright, 1969) for host-associated populations at each location and also by calculating an overall F_{ST} using ARLEQUIN v3.1 (Excoffier et al., 2005). Bayesian clustering of individual genotypes was performed in STRUCTURE 2.3.1 (Pritchard et al., 2000; Falush et al., 2007). The STRUCTURE run followed an admixture model, with 20 replicates for each K assuming K = 1–7. A total of 100 000 burn-in and 50 000 replications were used. The best estimate of K was determined by the method 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. Analysis of molecular variance (AMOVA) was carried out using ARLEQUIN v3.1 to partition the genetic variation among and within populations. Individuals were grouped in three ways and analyzed with AMOVA to understand the effect that location and host plants have on genetic variation. The three groups were (1) overall (host plant and locality combinations, i.e., 13 groups accounting for five locations and three host plants), (2) among locations (five groups accounting for five locations), and (3) among host plants (three groups accounting for three host plant species).

Results

We obtained DNA of adequate concentration (on average 63 ng μl^{-1}) and quality (2.1, 260/280 ratio) from individual *P. seriatus* DNA extractions. AFLP analysis of 196 individuals with two primer combinations (E/ACT–M/CAT and E/AAC–M/CTC) produced 432 bands. The AFLP scoring error rate was 3.2%. A SESim statistic of 0.028 indicated that this number of bands and individuals were sufficient to describe *P. seriatus* genetic population structure at the scale of this study (Medina et al., 2006). The percentage of polymorphic loci in the 13 purported *P. seriatus* populations (refer to Table 2 for annotated population information) varied from 39.4% (CH population) to 59.3% (TC population). The overall F_{ST} value for the *P. seriatus* populations sampled was 0.11, which was

Table 2 Genetic diversity indices based on AFLP data among *Pseudatomoscelis seriatus* populations, coded according to location and host plant origin

Location	Host plant	Population code	% polymorphic loci	Expected heterozygosity ± SE
Lubbock	Horsemint	LH	48.15	0.079 ± 0.006
	Cotton	LC	46.06	0.082 ± 0.006
San Angelo	Horsemint	SH	52.55	0.077 ± 0.006
	Cotton	SC	49.07	0.080 ± 0.006
College Station	Horsemint	CH	39.35	0.075 ± 0.006
	Cotton	CC	45.14	0.081 ± 0.006
	Woolly croton	CW	45.83	0.079 ± 0.006
Weslaco	Horsemint	WH	47.92	0.082 ± 0.006
	Cotton	WC	45.83	0.076 ± 0.006
	Woolly croton	WW	43.06	0.079 ± 0.006
Corpus Christi	Horsemint	TH	53.94	0.111 ± 0.007
	Cotton	TC	59.26	0.113 ± 0.007
	Woolly croton	TW	58.33	0.115 ± 0.007

significantly different from zero (P = 0.0001). Within-population variability, as indicated by the mean expected heterozygosity was consistent over all the populations ($H_E \approx 0.08$), with the exception of Corpus Christi, in which populations from each of the three host plants showed a higher value ($H_E \approx 0.11$) (Table 2). Pairwise F_{ST} values show that the Corpus Christi population, including individuals feeding on horsemint (TH), cotton (TC), and woolly croton (TW), was the most genetically distinct when compared with populations from the other four locations (Lubbock, San Angelo, College Station, Weslaco) (Table 3).

Principal coordinate analysis of all 13 purported populations collected from the three host plant species in the five locations considered in this study revealed that *P. seriatus* in Texas was grouped into three distinct clusters (Figure 2). PCA shows that at three sampling locations (Lubbock, San Angelo, and Weslaco), *P. seriatus* populations associated with horsemint (i.e., LH, SH, and WH) grouped together into a distinct horsemint-associated cluster, regardless of their geographic origin. On the other hand, *P. seriatus* populations from Corpus Christi (TH, TC, and TW) were grouped together in a unique cluster regardless of their host plant association. Finally, a third cluster was formed by a mixture of populations from all three host plant species from various locations (i.e., Lubbock, San Angelo, Weslaco, and College Station).

Similarly, the Bayesian clustering analysis performed in STRUCTURE 2.3.1 revealed that there are three (K = 3) distinct genetic populations of *P. seriatus* in Texas (Figure 3). The STRUCTURE output for K = 3, revealed a pattern of HAD in *P. seriatus*. In three locations (Lubbock, San Angelo, and Weslaco) horsemint-associated populations (i.e., LH, SH, and WH) were found. In contrast, populations in two locations (College Station and Corpus Christi) were not differentiated based on their host plant associations (Figure 3). There was no HAD in *P. seriatus* from Corpus Christi. However, *P. seriatus* from Corpus Christi represented a geographically distinct genotype, differentiated from the rest of the populations at the four other locations.

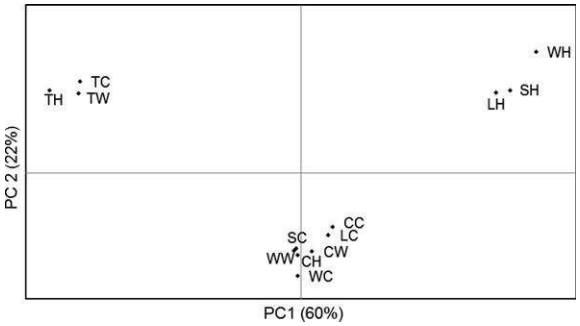

Figure 2 Principal coordinate analysis of *Pseudatomoscelis seriatus* populations. The distance matrix of 13 populations is projected in a two-dimensional space formed by principal coordinate 1 (PC1), explaining 60% of the variation, and PC2, explaining 22% of the variation. See Table 1 for an explanation of the population codes.

Analysis of molecular variance of all 13 purported *P. seriatus* populations also revealed that genetic variation was structured (Table 4). When the data were grouped by location or by host plant alone, there was no significant

Table 3 Pairwise comparisons of *Pseudatomoscelis seriatus* populations. Values below the diagonal are F_{ST} value and values above the diagonal are Nei's genetic distance. Values in bold represent significant F_{ST} for the respective pair of populations compared (P<0.05)

	Nei's genetic distance												
	LH	LC	SH	SC	CH	CC	CW	WH	WC	WW	TH	TC	TW
Pairwise F_{ST} value													
LH		0.012	0.006	0.015	0.016	0.012	0.013	0.006	0.017	0.015	0.037	0.033	0.033
LC	**0.098**		0.013	0.004	0.005	0.002	0.004	0.018	0.005	0.003	0.021	0.018	0.019
SH	0.026	**0.120**		0.018	0.017	0.013	0.016	0.007	0.018	0.016	0.040	0.035	0.035
SC	**0.139**	0.000	**0.172**		0.005	0.004	0.004	0.020	0.005	0.004	0.020	0.018	0.018
CH	**0.142**	0.014	**0.158**	0.019		0.005	0.004	0.021	0.004	0.005	0.020	0.020	0.018
CC	**0.098**	0.000	**0.118**	0.009	0.022		0.004	0.017	0.005	0.004	0.021	0.018	0.019
CW	**0.111**	0.000	**0.137**	0.001	0.017	0.000		0.019	0.003	0.003	0.020	0.019	0.017
WH	0.020	**0.157**	0.037	**0.191**	**0.197**	**0.149**	**0.166**		0.022	0.022	0.043	0.040	0.039
WC	**0.132**	0.000	**0.151**	0.003	0.016	0.000	0.000	**0.180**		0.004	0.021	0.020	0.019
WW	**0.128**	0.000	**0.150**	0.009	0.028	0.000	0.000	**0.193**	0.000		0.019	0.018	0.017
TH	**0.240**	**0.138**	**0.270**	**0.141**	**0.148**	**0.145**	**0.131**	**0.277**	**0.140**	**0.131**		0.009	0.008
TC	**0.203**	**0.104**	**0.229**	**0.116**	**0.126**	**0.110**	**0.106**	**0.242**	**0.114**	**0.107**	0.019		0.008
TW	**0.215**	**0.117**	**0.238**	**0.123**	**0.131**	**0.120**	**0.103**	**0.254**	**0.120**	**0.105**	0.009	0.010	

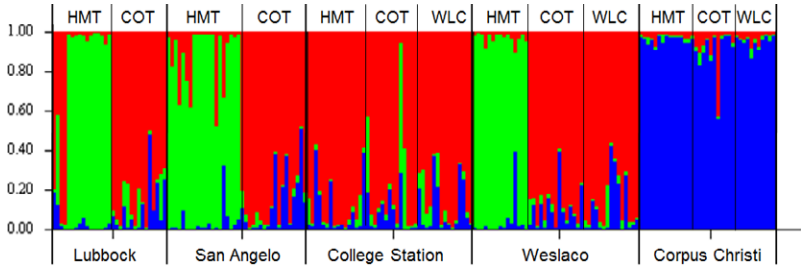

Figure 3 STRUCTURE analysis of *Pseudatomoscelis seriatus* populations inferred from AFLP markers. Individuals are organized according to the location and within each location, by each host plant (HMT, horsemint; COT, cotton; WLC, woolly croton). Three inferred genetic clusters (K = 3) are represented by the colors light gray (green), dark gray (red), and black (blue). Each individual is represented by a narrow vertical column, divided in segments in proportion to inferred membership of each of the three clusters.

Table 4 Analysis of molecular variance results for *Pseudatomoscelis seriatus* populations from three host plants (horsemint, cotton, woolly croton) in five locations in Texas, USA (Lubbock, San Angelo, College Station, Corpus Christi, Weslaco). Variation was partitioned in (a) overall (host plants × location), (b) grouped by location, and (c) grouped by host plant

	Source	d.f.	SS	Variance	% variation	F	P-value
(a)	Overall	12	925.85	3.34	11.07	0.111	<0.05
	Individuals within overall	183	4912.56	26.84	88.93		
(b)	Among locations	4	435.65	1.19	3.92	0.039	>0.05
	Host plants within locations	8	490.20	2.313	7.62	0.079	<0.05
	Individuals within location × host plant	183	4912.56	26.84	88.45		
(c)	Among host plants	2	225.84	0.68	2.23	0.022	>0.05
	Locations within host plants	10	700.01	2.87	9.44	0.097	<0.05
	Individuals within host plant × location	183	4912.56	26.84	88.33		

population structure. However, host plant groups within a locality or location groups within host plants showed significant population structure.

Discussion

The results of this study show HAD in *P. seriatus*. Interestingly, HAD is not uniformly distributed throughout the geographic distribution of this pest in Texas. In three of five study locations (i.e., Lubbock, San Angelo, and Weslaco), *P. seriatus* shows HAD. In College Station and Corpus Christi *P. seriatus* populations were not genetically differentiated based on their host plant association. Further, *P. seriatus* populations in Corpus Christi were geographically differentiated from the populations in the rest of the locations we sampled. Our results indicate a geographic pattern of HAD.

Several ecological studies have reported a geographic mosaic pattern of host plant use in phytophagous insects (Thompson & Cunningham, 2002; Berenbaum & Zangerl, 2006; Conord et al., 2006; Rich et al., 2008; Toju, 2009; Brunner & Frey, 2010; Craft et al., 2010), which is the basis of the geographic mosaic theory of co-evolution (Thompson, 2005). Our results show what can be called a geographic mosaic of HAD. In the case of *P. seriatus*, the observed geographic differences in the pattern of HAD could be due to the heterogeneity of the landscape and associated ecological factors across the geographic distribution of this insect. We observed a lower abundance of horsemint near Lubbock, San Angelo, and Weslaco than in College Station and Corpus Christi. Lubbock, San Angelo, and Weslaco receive an average annual rainfall of about 52.5 cm. In contrast, College Station and Corpus Christi receive an average annual precipitation of 87.5 cm (Figure 1), which correlates with a high abundance of horsemint. Areas with abundant horsemint have relatively large populations of *P. seriatus* in neighbouring cotton fields. In areas receiving low rainfall, horsemint patches are small and mainly restricted to low-lying areas or ditches where plants receive more moisture (Fletcher, 1940). Therefore, *P. seriatus* populations associated with horsemint plants in low rainfall areas are small and relatively isolated from cotton. Small *P. seriatus* populations resulting from low horsemint abundance in Lubbock, San Angelo, and Weslaco may lead to stronger genetic drift and could promote HAD in these locations.

Dispersal ability may play an important role in determining genetic population structure (Roderick, 1996; Peterson & Denno, 1998). Although the dispersal ability of *P. seriatus* has not been assessed with any mark–recapture study, personal observations (by AK Barman & CPC Suh) indicate that *P. seriatus* is a weak flier, which is consistent with reports from other insects in the same family (King, 1973; Stewart & Gaylor, 1994; Lu et al., 2009). Studies addressing airborne dispersal of *P. seriatus* have shown that very few individuals take high altitudinal flight and that in wind tunnels they tend to remain attached to their host plant at wind speeds as high as 48 km h^{-1} (Almand et al., 1976). Several studies have shown that habitat fragmentation may lead to increased population differentiation of insects by restricting gene flow, particularly if the insect has limited dispersal ability (Van Dongen et al., 1998; Sato et al., 2008). In the locations at which HAD of *P. seriatus* occurs, the distribution of horsemint is fragmented.

The most striking difference among the geographic locations studied was the differential presence of woolly croton. Woolly croton is absent, or present at low numbers, in the three locations at which HAD was found (Figure 1). This plant species is the primary host of *P. seriatus* in the fall and serves as a hibernating substrate for eggs at the end of the cotton season (Almand et al., 1976; Gaylor & Sterling, 1977). Thus, woolly croton may act as bridge connecting *P. seriatus* populations from cotton in the fall with horsemint in the spring. Therefore, the absence (e.g., in Lubbock and San Angelo) or low numbers (e.g., in Weslaco) of woolly croton is likely to keep *P. seriatus* populations in cotton and horsemint reproductively isolated, promoting their genetic differentiation. In addition, *P. seriatus* strongly prefers horsemint and woolly croton to cotton (Beerwinkle & Marshall, 1999; AK Barman, unpubl.) strengthening the isolation of *P. seriatus* populations associated with horsemint and cotton. Host plant preference has been invoked as a mechanism keeping herbivorous insects associated with different host plant species reproductively isolated (Hokkanen, 1991; Feder et al., 1994; Cunningham et al., 1999). If strong host plant preference exists, herbivorous insects may also track the phenologies of their host plants adding a temporal component to their reproductive isolation owing to phenological differences among their host plants.

Intimate association of insect herbivores with their host plants enables them to match their biology with their host plant phenologies. Differences in host plant phenology have been found to act as a pre-mating barrier among insect herbivore populations associated with different host plant species and thereby facilitate HAD (Feder et al., 1994; Groman & Pellmyr, 2000; Berlocher & Feder, 2002; Dres & Mallet, 2002). For example, a difference of ca. 25 days in fruiting phenology between apples and hawthorns translates into reproductive isolation and HAD of *Rhagoletis pomonella* (Walsh) (Feder et al., 1994). The three host plant species we studied (i.e., horsemint, cotton, and woolly croton) show minimum to no phenological overlap at the locations in which we found HAD (i.e., Lubbock, San Angelo, and Weslaco). *Pseudatomoscelis seriatus* life span is ca. 32 days (Gaylor & Sterling, 1975). In Lubbock and San Angelo there is a time difference of ca. 30–35 days in flowering and fruiting phenology between horsemint and cotton, which is long enough to reproductively isolate *P. seriatus* populations on these two host plant species. In contrast, at the locations that did not show HAD (i.e., College Station and Corpus Christi) the three host plant species had overlapping phenologies.

Although both College Station and Corpus Christi *P. seriatus* populations lack HAD, the Corpus Christi population is genetically distinct from all the others (Figures 2 and 3). The genetic differentiation between the Corpus Christi population and the rest cannot be explained by isolation-by-distance (result not shown). In addition, there are no apparent geographic barriers between Corpus Christi and remaining study locations. Without any further ecological and behavioral studies, it is difficult to speculate why the Corpus Christi population would be genetically differentiated from the rest. Perhaps a non-Texas propagule of *P. seriatus* may have colonized Corpus Christi, since marine routes connect this area with parts of Mexico and other US states such as Florida. The overwintering eggs of *P. seriatus* can easily be transported along with plant material from other locations. Thus, human-mediated movement (Kuhnle & Muller, 2011) of *P. seriatus* populations cannot be ruled out. Relatively high genetic diversity and percent polymorphism of the Corpus Christi population suggest that it could be ancestral to the sampled populations in other locations or that there may have been multiple introductions of fleahopper populations at this site. Future research using different molecular markers will be needed to further examine the above possibilities.

In summary, asynchrony in growth phenology among different host plant species, lower population abundance of horsemint, strong preference for the native host, and finally the absence of woolly croton (the preferred hibernating host plant) may have collectively contributed to the observed pattern of HAD of *P. seriatus* at the geographic scope of this study. Our results indicate that *P. seriatus*, although considered a widespread generalist, could be a specialist at certain locations. Our results suggest that geographic variation in vegetation composition may influence

population structure of herbivorous insects on different host plant species. Incorporating evolutionary ecology information in pest management practices will not only enrich our understanding of population genetic processes at a local scale but will also aid in the formulation of more efficient and sustained insect management strategies than the ones currently in place.

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Host Preference of Cotton Fleahopper, *Pseudatomoscelis seriatus* (Reuter) is not Labile to Geographic Origin and Prior Experience

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ABSTRACT Several phytophagous insects exhibit distinct preference for their host plants. In widely distributed generalist insects, host preference can be influenced by geographic variation in host plant distribution and abundance as well as by prior experience. We have studied host preference of the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), a pest of cotton in Texas and other neighboring states, by measuring olfactory orientation to horsemint (*Monarda punctata* L.) and cotton (*Gossypium hirsutum* L.). Horsemint is one of the primary, native, wild hosts of cotton fleahopper during late-spring and early summer in Texas, and it is commonly believed to be the main source of this pest in cotton. Although the abundance of horsemint, and therefore the fleahopper exposure to it, varies geographically, cotton fleahopper's preference for this native host-plant is maintained across two ecoregions in Texas, TX High Plains (Lubbock area) and Brazos Valley (College Station area). Similarly, preference for horsemint was retained regardless of prior experience with cotton throughout all the life stages of the insect. This fixed preference of cotton fleahopper to horsemint could be because of their ancestral insect–plant interaction, better fitness of cotton fleahopper on horsemint, and relatively low abundance of horsemint compared with cotton. Information gained from this study could be used to implement cultural control practices such as trap cropping, to develop attractants to monitor this pest, or both.

KEY WORDS olfactometer, Hemiptera, horsemint, cotton, prior experience

Host plant selection is a fundamental behavior in herbivorous insects, which allows them to successfully feed, shelter, mate, and oviposit (Bernays and Chapman 1994, Schoonhoven et al. 2005, Bruce and Pickett 2011). The process of host plant selection is broadly subdivided into three major steps that are not always clearly delineated: searching (orientation), recognition and selection (landing and probing), and acceptance (feeding and oviposition) (Visser 1986, Schoonhoven et al. 2005). The orientation behavior is mainly dependent on olfactory, visual cues, or both from the herbivore's host plants (Patt and Setamou 2007, Wenninger et al. 2009). The ability of herbivorous insects to track specific blends of volatile chemicals allows them to find their host plants even when the insects occupy diversified, complex habitats where their host plants are surrounded by nonhost plant species (Bruce et al. 2005, Schoonhoven et al. 2005, Bruce and Pickett 2011). Assessing the olfactory orientation of herbivorous insects is the first step toward screening their host-plant preference.

Herbivore's host-preference may be subject to geographic variation (Newby and Etges 1998, Kawecki and Mery 2003, Verdon et al. 2007, Utsumi et al. 2009). For example, *Helicoverpa armigera* (Hübner) (Firempong and Zalucki 1990), *Malacosoma disstria* Hübner (Parry and Goyer 2004), *Drosophila mojavensis* (Patterson and Crow) (Newby and Etges 1998), *Uroleucon ambrosiae* (Thomas) (Funk and Bernays 2001) and *Leptopilina clavipes* (Hartig) (Pannebakker et al. 2008) show geographic variation in their host preference. Herbivorous insects seem to respond to variation in the distribution and abundance of their host plant species in regard to host preference (Kuussaari et al. 2000). Therefore, herbivore populations may specialize and prefer locally abundant host plant species (Fox and Morrow 1981, Sword and Dopman 1999). These geographic differences in host plant preference may be because of genetic variation among geographic populations (Jaenike 1990, Via 1990, Singer and Parmesan 1993), because of geographic variation in the composition of host-plant species (Bernays and Funk 1999, Funk and Bernays 2001, Davis and Stamps 2004, Troncoso et al. 2005), or both. Thus, a herbivore insect's host preference cannot be generalized from observations based on just a few, focalized geographic locations, or both, and should be investigated at multiple geographic locations across the entire distribution of the insect.

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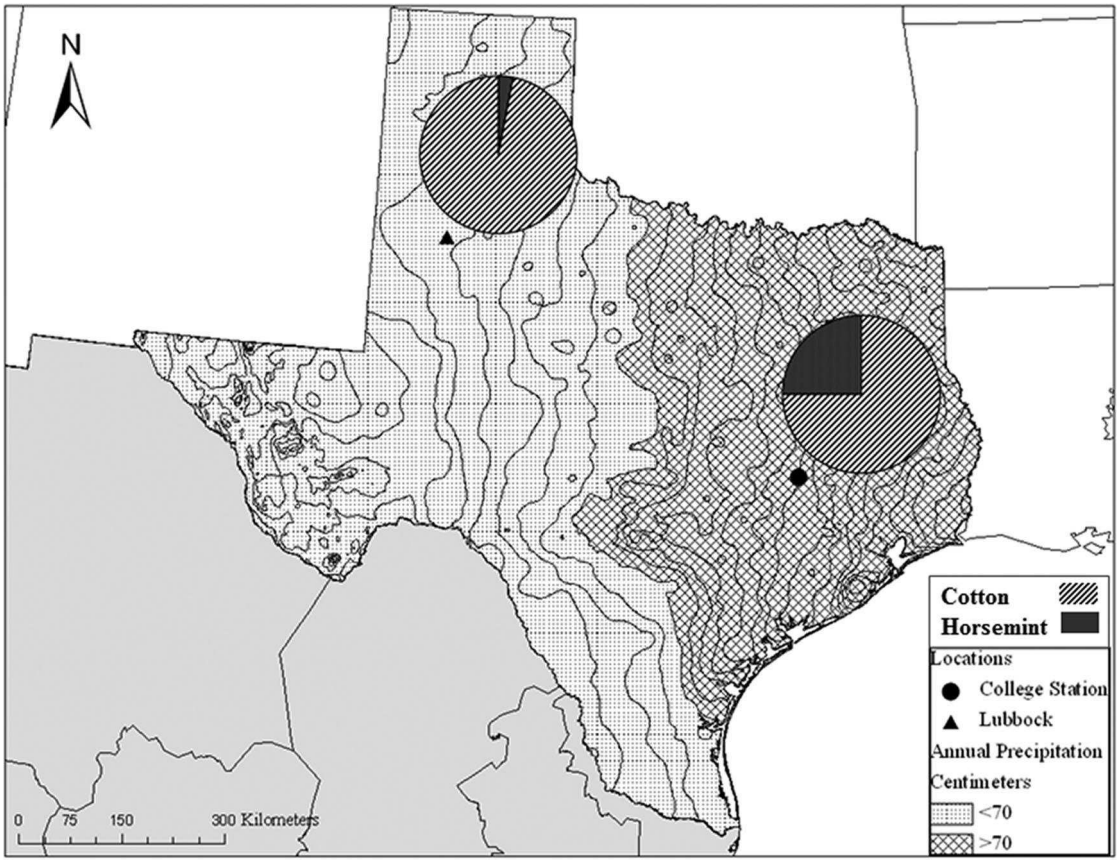

Fig. 1. Map indicating annual rainfall in the two geographic study locations.

Host preference in polyphagous herbivorous insects may vary not only because of geographic differences in host plant composition, genetic variation among insect populations, or both, but also insect's behavioral modification as a result of prior experience. Prior experience on a particular host plant may result into induced preference of an insect for the host plant over another host plant they have never encountered (Jermy et al. 1968, Dethier 1982). Induced preference for experienced host plants has been shown in several lepidopteran larvae (Jermy et al. 1968, Zhang et al. 2007) and in coleopterans (Messina et al. 2009, Coyle et al. 2011). Polyphagous insects exhibiting induced preference and distributed across wide geographic areas are likely to show greater variation in host preference than insect with localized distributions, because of heterogeneity in host plant composition. However, variation in host preference is not ubiquitously present in insects. There are insect species in which host preference is unchanged (conserved) regardless of geographic differences in host composition and prior host exposure (Wehling and Thompson 1997, Davis and Stamps 2004, do Valle et al. 2011, Wellenreuther et al. 2011). Thus, host selection behavior in phytophagous insects could range from highly variable to strictly conserved.

Several integrated pest management (IPM) strategies such as the use of trap crops, resistant varieties, and botanicals (attractants and deterrents) are based on pest's host preference behaviors. Thus, investigation into insect's host preference will increase successes of these IPM strategies. For example, if an insect species is subjected to variation in host preference behavior because of induced preference, a trap crop would not be as useful to attract insect populations as it would be to attract pests with fixed host preferences (Hokkanen 1991, Thaler et al. 2008, Guillemaud et al. 2011, Midega et al. 2011).

The cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), is an insect pest of cotton that has a wide distribution in the United States (Henry 1991), with >160 host plant species belonging to 35 different families (Esquivel and Esquivel 2009). Extensive sampling and survey for both host plant and cotton fleahopper abundance in Texas, indicates that the host plant complex of this pest varies in time and space. That is, at any given time there are only 3–4 dominant host plant species at a given local landscape. In this study, we have selected two locations in Texas: Texas High Plains and Brazos Valley, hereinafter referred to as Lubbock and College Station, respectively (Fig. 1). These two locations are 620 km apart and

differ considerably in average annual rainfall and both availability and abundance of host plants for *P. seriatus.* Each of these locations represents the eco-region to which they belong in terms of host plant composition and cotton fleahopper abundance. Two cotton fleahopper host plant species were selected for this host preference study: cotton and horsemint. Cotton is an important crop in the region we studied and the cotton fleahopper is considered a pest species on this crop in central and eastern Texas. Horsemint is a wild, native host plant of cotton fleahopper and has overlapping growth phenology with cotton in central and eastern Texas. Previous studies reported that among the wild host plants, cotton fleahoppers prefer horsemint (Holtzer and Sterling 1980, Beerwinkle and Marshall 1999). Also, horsemint supports more cotton fleahopper than cotton and other wild host plants, indicating that horsemint is one of the most suitable host plants for this insect. Horsemint (*Monarda punctata* L.) is limited in northwest Texas (e.g., Lubbock), although highly abundant in central and eastern Texas (e.g., College Station). In addition, the cotton fleahopper's primary overwintering host plant, woolly croton (*Croton capitatus* Michx.), is absent in northwest Texas. In contrast, cotton is common at both locations (i.e., Lubbock and College Station), although it is more concentrated in Lubbock than in College Station.

The current study explored whether *P. seriatus* shows variation in host preference. We specifically asked: 1) does host plant preference of *P. seriatus* vary geographically, and 2) do prior experiences result in induced preference to host plants in *P. seriatus*?

Materials and Methods

Insect. The cotton fleahopper is a small (3–4 mm long) sucking insect. It completes its lifecycle (from egg to adult mortality) in 32–40 d. Adults are weak fliers and live up to 10–15 d. In this study we used adult insects of mixed sex. Insects were collected either using a sweep net or with an aspirator directly from the host plant. Host preference bioassays were conducted within 2–3 h after insect collection to avoid mortality. Only active, healthy adults were used in bioassays.

Host Plant. We selected two host plant species, horsemint (*Monarda punctata*) and cotton (*Gossypium hirsutum*), for evaluating olfactory preference of cotton fleahopper. Horsemint is a native wild host plant of cotton fleahopper in the study area. Although horsemint is a perennial species, fresh aboveground biomass can only be seen in the study areas from early April to late June. Horsemint plants are 0.3–1.5 m tall depending on the habitat. Each stem bears an apical dense whorl of flowers. We used a bouquet of 3–4 stems, wrapped with cotton wool at the bottom and soaked in water in a glass beaker as one of the odor sources for olfactometer bioassays. Cotton is a cultivated crop in the study areas and preflowering (squaring) stage of the plant is susceptible to cotton fleahopper attack (Reinhard 1926, Ring et al. 1993). We used 3–4 cotton branches, bearing flower buds as another odor source in olfactometer bioassays. Approximately equal amount of plant material from both plant species were placed within the bioassay arena. The control treatment consisted of an empty glass beaker of the same size and with same amount of water used in the other treatments.

Olfactometer Setup. A glass Y-tube olfactometer was used (12-cm common tube, 5-cm arms, and 1.3-cm internal diameter; Analytical Research System, Gainesville, FL) to conduct the bioassays. The odor sources, control treatment, or both were placed in a glass chamber (15 cm internal diameter, 32 cm tall), with two openings. The glass chamber was airtight to prevent any exchange of volatiles. One opening of the glass chamber was connected to the air-delivery system (Analytical Research System, Gainesville, FL), which allowed air flow through the glass chamber. The other opening of the glass chamber was connected to an arm of the Y-tube. Thus, the two arms of the Y-tube olfactometer received odors coming out of the two glass chambers. Charcoal-filtered air was flowing through the two arms of the Y-tube at constant rate (1 l/min., 18.5 psi) throughout the experiment.

Bioassay. Air was released for 10–15 min before the bioassay so that plant odors traveled through the arms of the Y tube. A paper straw (equal in length to the longer arm of the Y-tube) was placed on the floor of the long arm of the Y-tube to facilitate movement of cotton fleahoppers to the point of rendezvous (where long and two short arms meet). In earlier trials without the paper straw, we found that fleahopper adults were incapable of walking normally on the glass surface of the Y-tube and could not make a choice. Adult cotton fleahoppers were placed individually on the rear tip of the paper straw inside the long arm of the Y-tube. Cotton fleahopper individuals took from 30 s to 5 min to travel between the points of release and choice. Fleahoppers that took >7 min to arrive to the rendezvous point were considered as nonrespondents and were not included in the analysis. We allowed three additional minutes of response time for fleahoppers to make a choice (i.e., landing into either one of the two arms of Y-tube). When an individual stayed for >1 min in any of the arms, we considered it a 'choice'. Orientation behavior was observed on each individual fleahopper adult only once. Thirty to fifty individuals were tested for each set of experiments. For every experiment, the orientation (left to right) of the Y-tube was changed by flipping it after testing a series of five consecutive individuals, to avoid any orientation biased behaviors. Before each bioassay, we also tested if fleahoppers showed any particular orientation to either of the two arms in the absence of odor sources, and found no significant deviation from the expected ratio (0.50). After each experiment, the Y-tube, glass chambers, and connecting tubes were washed with ethanol and rinsed with water, and air-dried to remove any residual odors from previous assays. Two sets of bioassays were conducted during 2009 and 2010.

Effect of Geography. In this study we evaluated preference of cotton fleahopper to horsemint and cot-

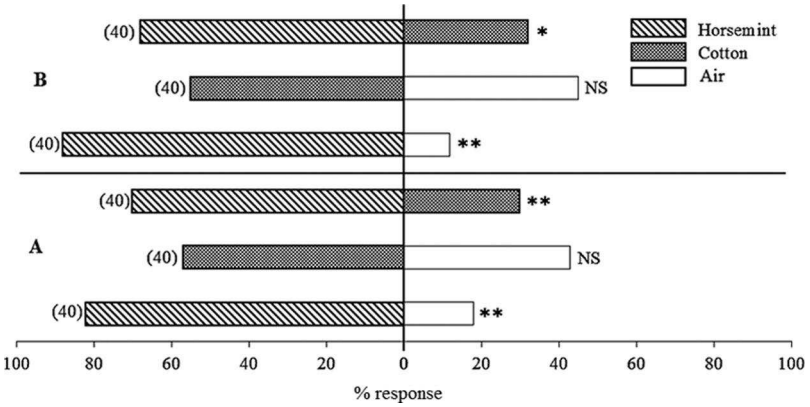

Fig. 2. Olfactory preference of two geographic populations of cotton fleahopper to cotton and horsemint. A. Lubbock population. B. College Station population. Statistically significant χ^2 test is indicated as * = $P < 0.05$ or ** = $P < 0.0001$. Number within parentheses on the left side of each test indicates number of individuals tested.

ton in two geographic locations, i.e., Lubbock and College Station. At each location, cotton fleahopper adults were collected from horsemint during May (College Station) and June (Lubbock). Insect collections were made from relatively pure patches (ranging from 0.3 to 30 m^2) of horsemint at both locations. Because we observed both nymph and adults in the plants, we assumed that the collected individuals were completing their life cycle in horsemint. To test if host-preference varied at each location, three pairs of treatments were offered to the collected adult fleahoppers: 1) horsemint versus air, 2) cotton versus air, and 3) horsemint versus cotton.

Effect of Prior Experience. This study was conducted during the summer of 2010 in College Station, where both cotton and horsemint are abundant. In College Station, horsemint grows naturally during April–May and cotton is planted during mid-April. Cotton fleahoppers (nymphs and adults) were collected from horsemint patches close to cotton fields. We called this the 'source population' because we used individuals from this population and reared them on cotton and horsemint in confinement. We tested the host-preference of the source population as described in the previous experiment (See Effect of Geography section). Two similar source populations were collected from the same location and cotton fleahopper nymphs and adults were released into two field cages where cotton or horsemint plants were growing free of cotton fleahoppers. We allowed ≈50 d to elapse to ensure that any cotton fleahopper introduced in the cage as an adult was dead and that the offspring of the released adults completed at least one generation on their respective host inside the cage. After 50 d, newly emerged adult fleahoppers were collected using an aspirator from their respective host plant inside the cage. Thus, adult cotton fleahoppers use in these bioassays experienced either cotton or horsemint during the entire duration of their immature stages before conducting the experiments. We refer this fleahoppers as "host-associated populations" (i.e., horsemint-associated population or cotton-associated population). An average of 50 adults from each host-associated population were tested in the Y-tube olfactometer using the same treatment combinations used for testing for geographic differences (see previous). This design allowed us to test if host-preference of cotton fleahopper was influenced by prior host plant experience.

Statistical Analysis. Host-preference data were analyzed as percent response using χ^2 analysis ($\alpha = 0.05$) under the null hypothesis that cotton fleahopper oriented with an expected probability of 0.5 for each treatment (Proc FREQ, SAS 2006).

Results

Test for Effect of Geography. Cotton fleahoppers from both Lubbock area and College Station area preferred horsemint to cotton. In Lubbock (Fig. 2A), 70% of tested individuals preferred horsemint to cotton ($\chi^2 = 16.0$; df = 1; $P < 0.001$). Similarly, in College Station (Fig. 2B), 68% of tested individuals preferred horsemint to cotton ($\chi^2 = 12.96$; df = 1; $P = 0.0003$). A significantly larger proportion of individuals preferred horsemint over the control (air) in both Lubbock and College Station. In contrast, preference of cotton fleahopper to cotton was nonsignificant when tested against the control.

Test for Effect of Prior Experience

Source Population. Eighty per cent of the individuals from the source population (Fig. 3A) preferred horsemint to cotton ($\chi^2 = 30.5$; df = 1; $P < 0.0001$). When individuals were allowed to choose between horsemint and the control, significant preference for horsemint was observed, whereas there was no preference to cotton when tested against the control.

Horsemint-associated Population. Individuals of this population spent all their life stages on horsemint. Eighty-four percent of the individuals from the horsemint-associated populations (Fig. 3B) preferred horsemint to cotton ($\chi^2 = 22.2$; df = 1; $P < 0.0001$). When individuals were allowed to choose between horsemint and the control, significant preference for

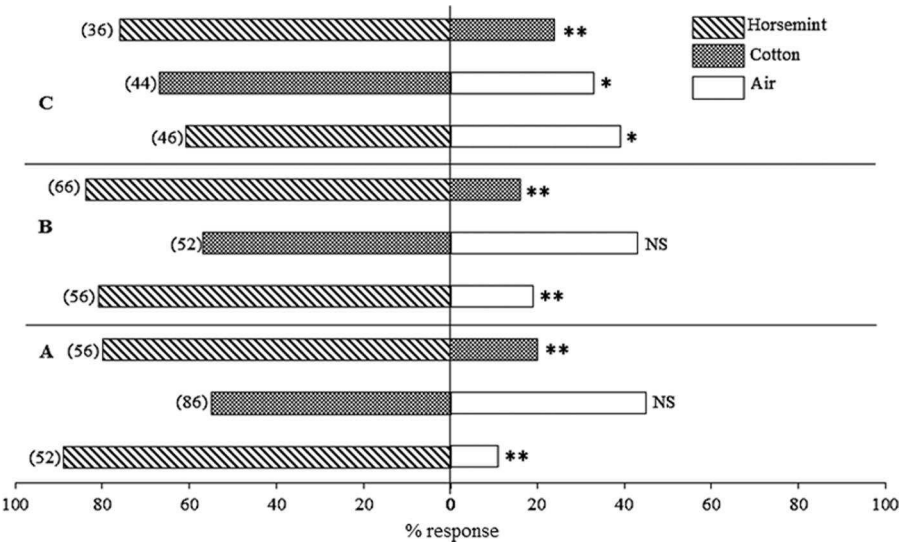

Fig. 3. Olfactory preference of cotton fleahopper population with and without prior experience to cotton. A. Source population B. horsemint-associated population, and C. cotton-associated population. Statistically significant χ^2 test is indicated as $*P < 0.05$ or $**P < 0.0001$. Number within parentheses on the left side of each test indicates number of individuals tested.

horsemint was observed, whereas there was no preference to cotton when tested against the control.

Cotton-associated Populations. Individuals of this population spent all their life stages on cotton. Seventy-six percent of the individuals from the cotton-associated populations (Fig. 3C) preferred horsemint to cotton ($\chi^2 = 28.4$; df = 1; $P < 0.0001$). When individuals were allowed to choose between horsemint and the control, significant preference for horsemint was observed. There was a statistically significant proportion of individual that preferred cotton over air.

Discussion

Our results indicate that the cotton fleahopper prefers horsemint to cotton regardless of its geographic origin and prior experience. In five independent experiments, an average of 76% of tested individuals preferred horsemint to cotton. The previous study by Beerwinkle et al. (1999) also found that cotton fleahopper strongly preferred horsemint over cotton and suggested its potential use as a trap crop. Unlike our study, Beerwinkle et al. and others (Van Tol et al. 2002, Midega et al. 2011), focus their studies on insects collected from a single geographic location and recommended pest management options based on their host-preference results. Since, host-preference may vary among geographic populations of herbivore insect pests, development of trap crop strategies should consider testing multiple geographic populations.

The influence of locally abundant host plants in insect host preference has been documented in several instances where insect populations are adapted to the locally most abundant host plant and prefer it over other potential hosts (Harrison 1987, Newby and Etges 1998, Funk and Bernays 2001, Kawecki and Mery 2003, Gotthard et al. 2004, Ferrari et al. 2006, Logarzo et al. 2011). Because the cotton fleahopper is a widely distributed, highly polyphagous insect and the abundance of its host-plant species varies geographically, this insect herbivore is bound to use different host-plant species at different geographic locations because of geographic variation. If preference for a particular host is not a fixed trait, then we expect to find differences in host preference among geographic populations of cotton fleahopper. However, contrary to this expectation, our results indicate that there was no difference in host-preference between geographic populations (Figs. 2A and 2B). The two locations we selected (i.e., Lubbock and College Station) belong to two distinct eco-regions and both differ in terms of annual rainfall (Fig. 1) and abundance of cotton and horsemint. Horsemint is limited in Lubbock, whereas it is abundant in College Station. Similarly, the area under cotton cultivation in Lubbock is much larger than in College Station (USDA-NASS 2010). In spite of the differences between the two locations we studied, cotton fleahoppers preferred horsemint to cotton in both Lubbock and College Station locations.

Our study also showed that preference of cotton fleahopper to horsemint is unaffected by insect's prior experience with cotton. Although both adults and nymphs spent their life on cotton (i.e., cotton host-associated), cotton fleahoppers exhibited preference to horsemint over cotton when given a choice (Fig. 3C). This indicates that host preference in cotton fleahopper is not inducible by learning; rather it seems to be a conserved behavior in this insect species. Similar results have been documented in several studies where host preference of polyphagous or oligophagous herbivorous insects does not change because of prior host experience (Janz et al. 2009, Kuhnle and Muller 2011, Midega et al. 2011). Thus, cotton flea-

hopper's host preference could be an evolutionarily conserved feature that is not affected by geographic differences in available host plants (Wehling and Thompson 1997) and prior experience (Kawecki and Mery 2003). This fixed host preference could be because of the evolutionary history of the cotton fleahopper and horsemint. The consistent preference for horsemint in cotton fleahopper could be the result of a longer association of this pest with horsemint than with cotton. The cotton fleahopper and horsemint both are native to the southern United States (Henry 1991, Turner 1994, Knutson et al. 2002). In contrast, large-scale cultivation of introduced cotton in the southern United States dates back only to late 1600 (Lewis and Richmond 1966). Thus, the cotton fleahopper has been interacting with horsemint for longer time than with cotton. This long association with horsemint might have resulted in higher fitness of this insect in horsemint than in cotton. For example, cotton fleahopper took shorter to complete its life cycle and laid significantly more eggs in horsemint than in cotton (Gaylor and Sterling 1976, Holtzer and Sterling 1980). Thus, it is not unexpected that the cotton fleahopper displays fixed preference for horsemint as revealed in our study. This as well as previous studies have shown that native host plants are preferred over cotton by the cotton fleahopper (Holtzer and Sterling 1980, Beerwinkle and Marshall 1999). One could ask then why the cotton fleahopper is found in cotton. The relatively high abundance of cotton compared with its native wild hosts may explain in part the presence of this insect in cotton. Cotton is a suboptimal host plant for the cotton fleahopper in terms of its development time and fecundity (Gaylor and Sterling 1976). Thus, although cotton may not be the host on which cotton fleahopper has the highest fitness, abundance, and predictability of cotton as a food resource may compensate for its relatively low resource quality (González-Megías and Gómez 2001). In this scenario, the evolution of preference for cotton in cotton fleahopper might be unnecessary because of this crop's extensive cultivation while maintaining its preference for a less abundant but better host-plant. In addition, there are several examples in which herbivorous insects choose plants on which their fitness is relatively low, but on which they get ecological advantages such as protection from natural enemies (Gratton and Welter 1999, Singer et al. 2004, Diamond and Kingsolver 2010). Unfortunately, no study has compared the abundance of natural enemies on cotton and horsemint. However, few studies have reported that generalist predators such as spiders and red imported fire ants (*Solenopsis invicta* Buren), are more common in cotton and woolly croton in regulating cotton fleahopper population (Breene et al. 1988, Breene et al. 1990). Agricultural practices such as pesticide use and harvesting may reduce the number of generalist predators in cotton as compared with native wild plants, making cotton a relatively enemy free space. Future studies should address these hypotheses.

Our data suggest that wild hosts of the cotton fleahopper could be used as a pest management tool. For example, horsemint could be used as a trap crop, or to develop kairomone baits. These types of cultural management options hold promises in the current cotton pest management scenario in Texas, where insecticide applications are minimized because of the use of transgenic Bt-cotton and the successful boll weevil eradication program. The conserved host preference of the cotton fleahopper suggests that trap crops could be a management option for this native pest, unlike an introduced polyphagous pest such as *Helicoverpa armigera*, which may not be feasible to control through trap crops because of their induced host preferences (Cunningham et al. 1998, Cunningham et al. 1999). Our study compared cotton fleahopper's preference to cotton and horsemint in two Texas locations, which represents two distinct ecoregions, in which cotton is grown extensively. The abundance of naturally occurring host plant species varies among different ecoregions. Because cotton fleahopper is distributed across a significant portion of North America, significant variation in spatial heterogeneity of host plant species is expected. Our study captured only a small portion of that variation. Therefore, future studies should focus on evaluating cotton fleahopper's preference for locally abundant host-plants to find if those host plants can be used in cotton fleahopper management.

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