TEXAS A&M AGRILIFE RESEARCH & EXTENSION

COTTON ENTOMOLOGY

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TEXAS A&M AGRILIFE RESEARCH, PATRICK J. STOVER, DIRECTOR
THE TEXAS A&M UNIVERSITY SYSTEM, COLLEGE STATION, TEXAS

COTTON ENTOMOLOGY PROGRAM

RESEARCH ACTIVITY ANNUAL REPORT

2018

SUBMITTED TO:

PLAINS COTTON IMPROVEMENT COMMITTEE
PLAINS COTTON GROWERS, INC.

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Introduction

Plains Cotton Growers, Inc. (PCG) has been a strong supporter of cotton insect research and extension activities in west Texas for many years. Most notably, PCG was instrumental in securing state funds for the Boll Weevil Research Facility at the Lubbock Center, and provided both financial and political support to conduct boll weevil biology and ecology research even before the boll weevil became a significant economic pest of the High Plains region. After the initial entry of the boll weevil into the eastern edge of the High Plains, PCG promoted and along with USDA-APHIS administered the boll weevil diapause suppression program involving a team effort that continued to include Texas A&M University. PCG also supported Texas Cooperative Extension (now Texas A&M AgriLife Extension Service) efforts to annually evaluate the diapause suppression program, conduct applied research trials to develop boll weevil management practices that would enhance the diapause suppression program's efforts and in the 1990s supported an annual survey of High Plains overwintering sites and grid trapping of cotton across the High Plains area. Under the strong and cooperative leadership of PCG, the boll weevil eradication program for the High Plains area progressed much more rapidly than anticipated. Now, the successful boll weevil eradication program has eliminated the boll weevil from this region for 15 years. In 2015, all 11 West Texas zones (Southern Rolling Plains, El Paso/Trans Pecos, St. Lawrence, Permian Basin, Rolling Plains Central, Western High Plains, Southern High Plains/Caprock, Northern Rolling Plains, Northern High Plains, Northwest Plains, and Panhandle) have been declared boll weevil eradicated and is managed as a single zone called West Texas Maintenance Area (WTMA). The team effort of PCG, Texas A&M AgriLife Research and AgriLife Extension Service over several decades has resulted in a comprehensive understanding of boll weevil ecology and behavior.

With a successful boll weevil eradication program and increased adoption of the transgenic *Bt* technology (now >70%), the cotton insect research and extension program focus has changed considerably during the last 18 years. Our current research/extension focus is on developing ecologically intensive strategies for cotton pest management, including crop phenology, cultivar, non-crop habitat, irrigation, and fertility management towards reducing insect pest pressure. Our research has demonstrated the need for continuing investigation of basic behavior and life patterns of insects while having a strong field-based applied research to bridge the gap between basic, problem-solving science and producer-friendly management recommendations. We have assembled a strong group of people to work as a team to examine multiple disciplines within the broad theme of Cotton IPM. We invest considerable time and manpower resources in investigating the behavior and ecology of major cotton pests of the High Plains with the goal of developing management thresholds based on cotton production technology and economics, with particular focus on limited water production system. Our Program has successfully leveraged research funds based on the funding provided by PCIC to support our research effort. We are excited about and greatly value our Cotton Entomology research and extension partnerships with multidisciplinary scientists at the Texas A&M AgriLife Research Center, together with area IPM agents in the region, to continue this partnership as we challenge ourselves to deliver the best cotton insect-pest management recommendations to our Texas High Plains producers.

Texas A&M AgriLife Research & Extension Center at Lubbock

COTTON ENTOMOLOGY PROGRAM

Megha N. Parajulee, Ph.D.
Professor, Faculty Fellow, and Texas A&M Regents Fellow

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

EFFECT OF NITROGEN FERTILITY ON COTTON CROP RESPONSE TO INSECT DAMAGE

A long-term study investigating the effects of differential nitrogen fertility on cotton aphids and cotton fleahopper population dynamics in a typical drip-irrigation Texas High Plains cotton production system has been ongoing since 2002. Differential nitrogen fertility (0, 50, 100, 150, and 200 lbs N/acre) is being examined for its effect on cotton plant physiological parameters, thereby influencing cotton insect injury potential and plant compensation. Recent focus has been to examine the effect of residual nitrogen on crop response to simulated fleahopper damage.

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

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

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

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

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

The objective of this study was to conduct a statewide monitoring of *Helicoverpa armigera* in Texas which would serve as the foundation for the development of management strategies. Traps and pheromone lures were used to collect moths; moths were either dissected to identify OWB based on genital characteristics or examined via molecular diagnostic tests. We found no evidence of OWB invasion in Texas at the present time.

ECONOMIC EVALUATION OF INSECT-PEST MANAGEMENT IN WATER-DEFICIT COTTON PRODUCTION

Reduced water availability, low rainfall, higher pumping cost of limited water, and increased input cost limit cotton productivity in the Texas High Plains and correspondingly lower profit margins, warranting for higher water use efficiency in our crop production. The impact of two key insect-pests at two distinct cotton phenological stages (thrips – seedling stage and cotton fleahopper – early squaring stage) will be evaluated with five combinations of single versus multiple-species infestations under three water-deficit (dryland, deficit irrigation, and full-irrigation) conditions (15 management scenarios). This study will enable development of research-based action thresholds considering variable yield potential under different water deficit scenarios. Data will be utilized to develop a dynamic optimization economic model that maximizes the net returns from management of single versus multiple pest infestations under water-deficit crop production conditions. This will enable real-world decision support under various production settings and empower producers to optimize input resources for profitable cotton production.

Predictable occurrence of thrips at seedling stage and cotton fleahopper during the early squaring stage in the Texas High Plains

THRIPS MANAGEMENT IN TEXAS HIGH PLAINS COTTON: INSECTICIDE PRODUCT EVALUATION

Multi-year statewide studies are being conducted at several Texas locations to represent cotton fields surrounded by variable vegetation/crop complexes and thrips population pressure in cotton. The study objective is to evaluate the efficacy, residual performance, and economic competitiveness of selected products in thrips management. Seed treatment products including Gaucho®, Cruiser®, Aeris®, and Avicta® Elite are evaluated with and without foliar application of acephate for their efficacy and cost effectiveness in managing thrips populations in cotton relative to an untreated control. Detailed plant growth parameters and yield will be measured and economics of seed treatment calculated.

Field evaluation of thrips insecticide products

EFFECT OF NITROGEN FERTILITY ON COTTON CROP RESPONSE TO SIMULATED COTTON FLEAHOPPER DAMAGE

M.N. Parajulee, A. Hakeem, Katie Lewis, S.C. Carroll, and J.P. Bordovsky

Objective: The objective was to evaluate the effect of artificial injury to cotton squares mimicking acute cotton fleahopper damage under variable nitrogen application rates on cotton fiber yield and quality.

Methodology: A high-yielding cotton cultivar, NG3406 B2XF, was planted at a targeted rate of 54,000 seeds/acre on May 25, 2018. The experiment was laid out in a split-split-plot randomized block design with five nitrogen fertility rate treatments applied for 16 years as main plots (16-row plots), split into two 8-row sub-plots: 1) nitrogen applied annually as previous years, and 2) nitrogen not applied since 2016, and two artificial cotton square injury treatments mimicking acute cotton fleahopper infestation as sub-sub-plots with four replications (total 80 experimental units). Within each of the five main-plot treatments that included pre-bloom side-dress applications of augmented N fertilizer rates of 0, 50, 100, 150, and 200 lb N/acre for 16 years, sub-plot treatments included N augmentation using a soil applicator injection rig on July 3, 2018 as in previous years versus no N augmentation. Pre-treatment soil samples (consisting of three 0 to 12 and 12 to 24-inch depth soil cores each), were collected from each of the 40 sub-plots on June 22, 2018. Within each sub-plot, two 8-ft. sections of uniform cotton were flagged in the middle two rows, each receiving hand removal of 100% cotton squares three weeks into squaring or control (no square removal). Five plants were removed to determine biomass. Treatment plots were harvested for lint yield and fiber analysis.

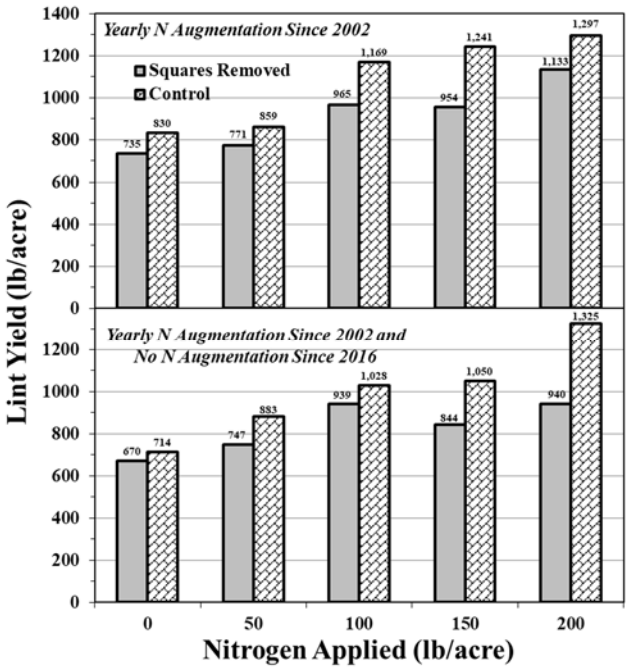

Fig. 1. Lint yield following 100% square removal 3-week into squaring as affected by continuous long-term augmentation of varying N rates versus N withdrawal for two years after 16-year augmentation.

Results: Considerably higher residual soil nitrogen was recorded from plots that received the two highest N rates in preceding 16 years. Withdrawing of N following 16 years of continuous augmentation resulted in lower leaf N and slightly lower lint in all N rate treatments (Fig. 1). However, lint yield was similar across all five N treatments in 2017 when N augmentation was ceased and the crop only experienced the long-term residual N. Removal of 100% squares 3-week into squaring did not significantly impact lint yield at lower N levels, but the yield was reduced by 20-30% at the two highest N levels; greater impact of square removal was observed on N-withdrawn plots compared to that in continuous N augmented plots. Only a slight reduction in yield without N augmentation for two years after 16 years of N augmentation indicates the presence of enough residual N in the soil.

TITLE:

Cotton yield response to manual square removal mimicking cotton fleahopper infestations as influenced by irrigation level and cultivar treatments, Lamesa, TX, 2018.

AUTHORS:

Megha Parajulee – Professor, Faculty Fellow, and Regents Fellow
Abdul Hakeem – Asst. Research Scientist
Wayne Keeling - Professor

MATERIALS AND METHODS:

Plot Size:	4 rows by 300 feet, 3 replications
Planting date:	May 21
Fertilizer:	115-35-0
Cultivars:	FiberMax 1911 GLT and Deltapine 1646 B2XF

Irrigation:

	Low	High
Preplant	3.8”	3.8”
In Season	4.2”	8.4”
Total	8.0”	12.2”

Cotton fleahopper:	Two treatments [*Control* (zero square removal), and *Manual removal* (100% squares removed manually three weeks into squaring]
Herbicides:	Prowl 3 pt/A – April 12 Gramoxone 1 qt/A + Caparol 26 oz/A – May 21 Roundup 1 qt/A – June 19 Roundup + Outlook 1 qt/A – July 23 Roundup 1 qt/A – August 22 ETX 1.25 oz/A + Ethephon 3 pt/A – October 10 ETX 1.25 oz/A – October 26
Treatment date:	July 17 at fleahopper susceptible stage
Harvest date:	October 1 (hand-harvested)

Effect of manual removal of early stage fruits versus control was evaluated on two cotton cultivars, FM 1911 GLT and DP 1646 B2XF, as influenced by irrigation water level. Two seasonal irrigation levels, *High* (12.2 inches) and *Low* (8.0 inches) were evaluated under a center pivot irrigation system. Experimental design consisted of two square abscission treatments (*manual removal of 100% squares to mimic severe cotton fleahopper infestation* versus *control*), two water levels (*high* versus *low*), and two cultivars (*FM 1911 GLT* versus *DP 1646 B2XF*), replicated three times and deployed in a randomized complete block design (total 24 plots). In order to mimic a natural early-season acute infestation of cotton fleahoppers and severe damage to the squaring cotton, a 10-ft section was flagged in each plot and treatments were applied. Square abscission treatments, 1) *control* (zero square removal) and 2) *manual removal* (removal of 100% squares from the plant), were deployed when cotton was highly vulnerable to fleahopper injury (2-3 weeks into cotton squaring). No squares were removed from control plots. The test plots were monitored for the

occurrence of any other insects, but no such occurrences were observed throughout the growing season. Test plots were harvested on October 1, 2018 and ginning was done on November 17. Fiber samples have been sent to Cotton Incorporated for HVI analysis.

RESULTS AND DISCUSSION:

As expected, 'High' water increased overall yield by about 50% (1,182 lb/acre) compared to that in 'Low' water regime (792 lb/acre). Both cotton cultivars, DP 1646 B2XF and FM 1911 GLT, showed similar response to the irrigation water treatment, partly attributed to a significant water supplement through rain events during the boll maturation stage. Removal of pre-flower squares did not render any lint yield reduction regardless of the irrigation water regime or cultivar type. Early season drought and late season frequent rain events complicated the scope of the study. It is generally expected that the 'Low' water treatment will be more significantly impacted by a severe shedding of pre-flower squares, but the yield was similar in our study in 2018. In addition, there was much greater yield variation in 'Low' water treatment plots compared to that in 'High' water treatment plots (Fig. 1). In 'High' water treatment plots, manual removal resulted in numerically higher lint yield, suggesting that the sufficient water availability increases the compensatory potential of cotton plants. Previous studies demonstrated that the square removal (>20%) by cotton fleahoppers significantly reduced the lint yield, but the impact was greater in water-stressed production situation. We will continue to investigate the intricate cultivar-water relationship on cotton's compensatory potential.

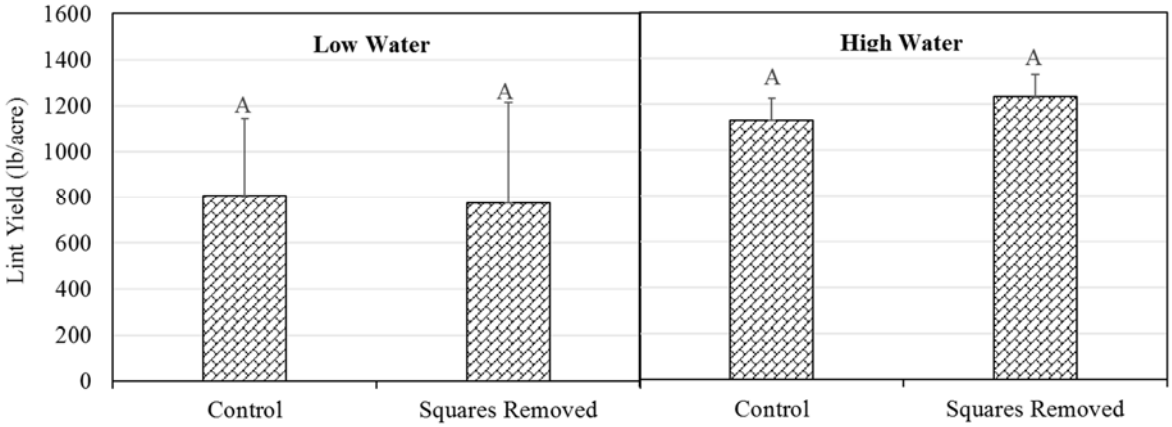

Figure 1. Average lint yield following manual removal of 100% squares prior to first flower versus control plots under high and low irrigation regimes, Lamesa, Texas, 2018.

Effect of *Lygus* on Drought-Stressed Cotton

Cotton Incorporated Core Program
Project Number: 16-354
PI: Megha N. Parajulee

PROJECT SUMMARY

Western tarnished plant bug, *Lygus hesperus,* is the primary *Lygus* species inhabiting cotton and several other hosts in the Texas High Plains. Our previous studies have documented that several non-cotton hosts including alfalfa, sunflower, corn, grain sorghum, as well as weedy habitats along roadside bar-ditches and turnrows could impact *Lygus* severity in adjacent cotton. Our previous projects, supported by the Cotton Incorporated State Support Program, have generated significant information on the damage potential of adult and immature *Lygus* on maturing cotton bolls. A three-year field study has quantified the boll age (measured in terms of heat units from flowering) that is safe from *Lygus* damage. Boll damage assessment based on heat unit-delineated maturity provided a boll-safe cutoff value of 350 heat units (~2-3 weeks from flowering), although *Lygus* adults and nymphs both cause external lesions on bolls throughout boll development and may give farmers a false impression of *Lygus* damage. A 4-year TSSC project (2012-2015) developed economic threshold-based management recommendations for *Lygus* in Texas High Plains cotton, which is expected to recommend a boll management threshold for early versus late season *Lygus* infestations.

While the Texas High Plains is fortunate to experience insignificant *Lygus* pressure in cotton during the recent years, the research on *Lygus* feeding behavior as it relates to low-input production systems in the Texas High Plains needs to continue. In particular, the characteristic low annual rainfall and decreasing irrigation water availability in the region has resulted in increased dryland cotton acreage. This project examined the feeding behavior and plant response to *Lygus* injury in relation to drought conditions. In 2016 and 2018, drought-stress treatments included two irrigation levels [full irrigation versus dryland (2016-2017) or supplemental irrigation (2018)], each nested with two cotton cultivars (early maturing DP 1518 versus full-season DP 1044). Each irrigation x cotton maturity combination received two *Lygus* infestation levels [untreated control versus 2X threshold (high infestation)], each with four replications, resulting in a total of 32 plots. In 2017, only DP 1518 was evaluated.

In 2016, effect of drought-stress on *Lygus*-induced injury was more pronounced in DP 1518 (38.8% lint loss) compared to that in DP 1044 (28.2%), suggesting that DP 1518 may be more susceptible to *Lygus* injury under dryland or water-stressed conditions. Irrigated plots had significantly lower lint loss in both cotton cultivars due to *Lygus* feeding compared with that in dryland plots (14.1% in DP 1518 and 9.3% in DP 1044). In 2017, *Lygus*-induced injury reduced lint yield by 41% in dryland versus 29.8% in irrigated cotton. In 2018, *Lygus*-induced injury reduced lint production by 42.7% in supplemental irrigation treatment verses 18% in full irrigation cotton. Our study demonstrated that the impact of *Lygus* injury was more pronounced under dryland and water-deficit growing conditions; likely because late-season lint yield compensations were limited due to reduced water availability limiting future plant growth and fruiting.

Effect of *Lygus* on Drought-Stressed Cotton

INTRODUCTION

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

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

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

METHODOLOGY

A four-year study was initiated in 2016 in a multi-factor split-plot randomized block design with four replications (blocks). Drought-stress parameters included two irrigation levels (full irrigation versus dryland) that served as main plot factors, whereas two cotton cultivars (early maturing versus full-season) were used as subplot factors to create an interaction of cultivar maturity and drought-stress situations to mimic the Texas High Plains (THP) scenario during dry summers. The full irrigation water level was created via 100% replenishment of evapotranspiration (ET) requirement for THP, whereas the dryland treatment received no supplemental irrigation. Two cotton cultivars included in the study were DP 1518 (short-season) and DP 1044 (full-season)

(2016-2017) and DP 1820 B3XF and DP 1823NR B2XF (2018), planted on May 25, 2016, May 26, 2017, and May 29, 2018. While both DP 1820 B3XF and DP 1823NR B2XF are early to mid-maturing cultivars, DP 1823NR B2XF offered significant nematode tolerance, offering cultivar differences for comparison. Each irrigation treatment (2) x cotton maturity (cultivar type) treatment (2) received two *Lygus* infestation levels [untreated control, 2X threshold (high infestation)], each with four replications, resulting in a total of 32 plots. In 2017, due to logistic limitations, the study was conducted only on DP 1518.

Lygus density treatments were applied on one 3-ft cotton row section per plot on August 11, 2016, August 18, 2017, and August 22, 2018. For insect release plots, a single release of *Lygus* adults (5 adult *Lygus* per plant, resulting in 1 bug per plant after 80% field mortality) was timed to simulate the acute infestation of *Lygus* while cotton was at boll development/maturation stage. Multi-plant (7 plants) cages were used to contain the released adults (Fig. 1). The control plots were flagged and sprayed with insecticides. Two weeks after the deployment of insect release treatments, all experimental plots were sprayed with insecticide Orthene® 97 to ensure that the released insects were removed. One to two plants from each treatment were removed and processed for *Lygus* damage assessment. Variables including number of fruits aborted and internal/external damage to developing bolls were measured. Pre-harvest plant mapping was conducted, and crop was hand-harvested on November 5 (2016), November 2 (2017), and November 5 (2018) and ginned. Lint samples were sent to Cotton Incorporated for fiber quality analysis.

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

RESULTS

2016 Study. As expected, higher numbers of internal warts were observed in bolls collected from *Lygus*-infested plants compared to that in control plots (Fig. 2). *Lygus* appeared to cause greater damage to dryland-grown plants compared to that in full irrigation plots. It is somewhat interesting to note that the dryland plots received greater boll injury while the bolls in dryland plots are expected to possess a tougher carpel wall. It is possible that the water-stressed bolls are more sensitive to *Lygus* feeding injury.

Averaged across the water level and cultivar treatments, total boll density on *Lygus*-infested plants was lower (2.27 bolls per plant) compared to that on uninfested control plants (3.2 bolls per plant) two weeks after *Lygus* infestation, suggesting possible abortion of small bolls due to *Lygus* feeding. Within varieties, DP 1518 had slightly more (2.8 bolls per plant) bolls compared to DP 1544 (2.6 bolls per plant), but this difference was not statistically significant.

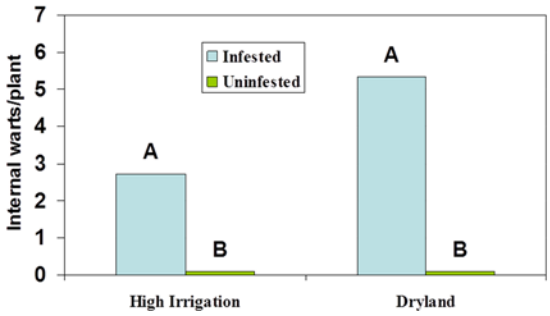

Figure 2. Internal injury warts in developing bolls caused by *Lygus* feeding on plants grown under full irrigation versus dryland, Lubbock TX, 2016.

Averaged across cultivars and irrigation treatments, no significant difference in lint yield was observed between *Lygus*-release treatments and non-release control treatments. However, drought-stress induced a significantly greater impact of *Lygus* injury on cotton lint yield. *Lygus* injury caused 34.83% lint yield loss in dryland cotton compared to only 11.3% loss in irrigated cotton (Fig. 3), suggesting a reduced *Lygus* injury sensitivity on full irrigated cotton compared to that in water-stressed production situation.

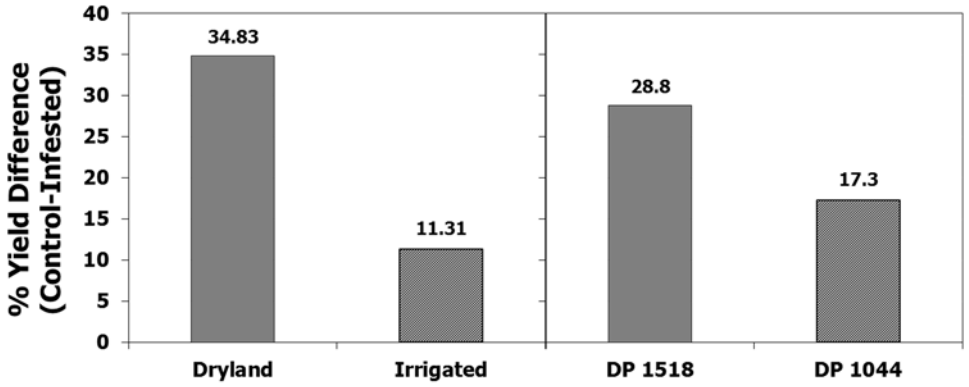

Figure 3. Effect of *Lygus* bug-induced damage on lint yield of cotton under dryland and irrigated production conditions and between the two cultivars, Lubbock, TX, 2016.

Lygus injury sensitivity varied between cultivars. While no significant difference in total lint yield was observed between the two cotton cultivars evaluated, *Lygus*-induced lint yield reduction was significantly greater (28.8%) in DP 1518 compared to 17.3% in DP 1044 (Fig. 3).

Effect of drought-stress was more pronounced in DP 1518 (38.8% lint loss) compared to that in DP 1044 (28.2%) (Fig. 4), suggesting that DP 1518 may be more susceptible to *Lygus* injury under dryland or water-stressed conditions. Irrigated plots had significantly lower lint loss in both cotton cultivars due to *Lygus* feeding compared with that in dryland plots (Fig. 4). The 2016 study indicated that DP 1044 appeared to show lower sensitivity to *Lygus* injury under both dryland and irrigated conditions, but the impact was more pronounced under dryland condition.

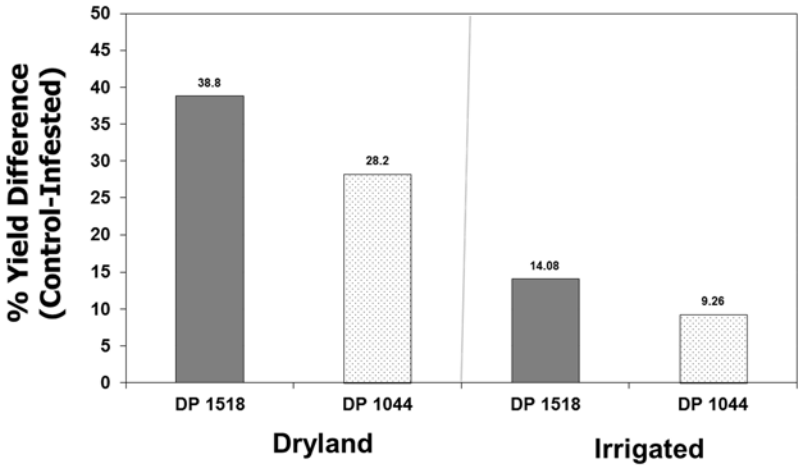

Figure 4. Percentage yield losses due to *Lygus* infestation under dryland versus irrigated production conditions, Lubbock, Texas, 2016.

2017 Study. *Lygus* augmentation exerted significant injury to the maturing bolls in both dryland and irrigated cotton (Fig. 5). There was a slight increase in the number of external lesions, internal boll injury warts, and damaged seeds in irrigated cotton compared to that in dryland cotton, but the trend was similar between the two irrigation treatments. Even though the *Lygus* injury caused a lower amount of visible damage in dryland cotton compared to that in fully irrigated cotton (Fig. 5), drought-stress appeared to render greater boll vulnerability to *Lygus* injury for continuing boll growth, lint development, and fiber quality.

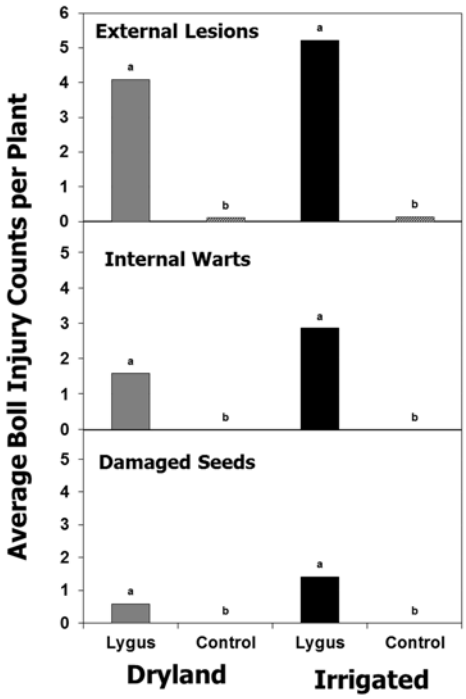

Figure 5. External lesions, internal injury warts, and damaged seeds in developing bolls caused by *Lygus* feeding on plants grown under full irrigation versus dryland, Lubbock TX, 2017.

Lygus augmentation significantly reduced lint yield in both fully irrigated and dryland conditions. As expected, dryland plots produced lower lint yield compared to that in irrigated plots (Fig. 6). Within dryland, un-augmented control plots produced 1,292 lbs of lint per acre compared to 762 lbs per acre when *Lygus* bugs were augmented and injury was inflicted to the maturing crop. Similar relationship was observed under full irrigated crop production system, with 1,974 lbs per acre lint yield in control plots and 1,386 lbs per acre in *Lygus*-augmented plots (Fig. 6). Irrigated plots had significantly lower lint loss (29.8%) due to *Lygus* feeding compared with that in dryland plots (41.0%) (Fig. 7).

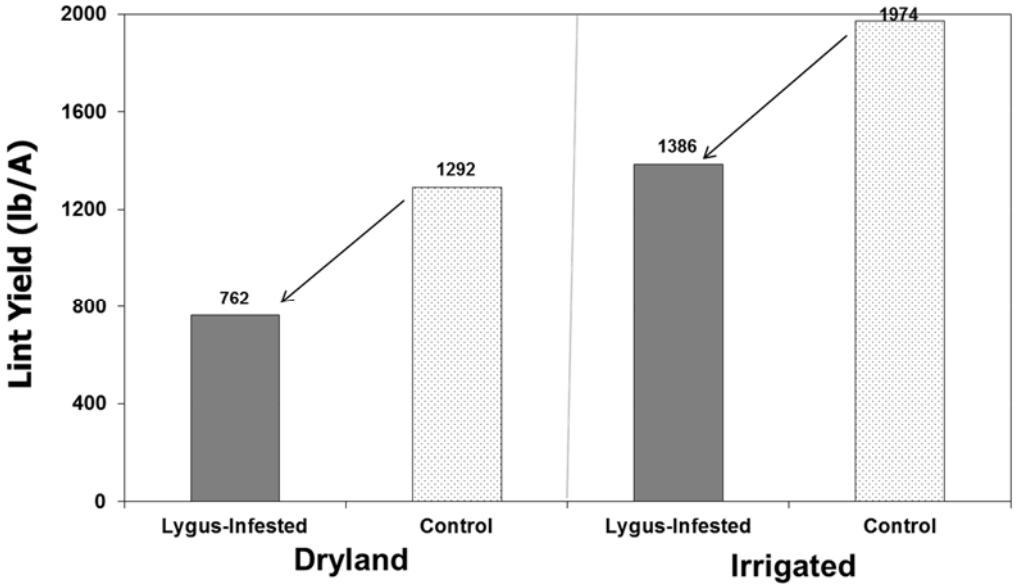

Figure 6. Cotton lint yield losses due to *Lygus* infestation under dryland versus irrigated production conditions, Lubbock, Texas, 2017.

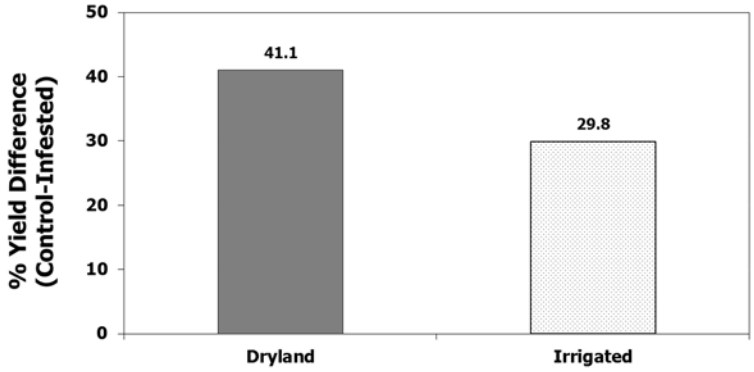

Figure 7. Percent lint yield losses due to *Lygus* infestation under dryland versus irrigated production conditions, Lubbock, Texas, 2017.

2018 Study. In 2018, *Lygus* bugs were released on two varieties (DP 1820 B3XF and DP 1823 NRB2XF) under low (supplemental) and high water (full irrigation) regimes. *Lygus* augmentation exerted significant boll injury in both supplemental and full irrigation treatments. As expected, significant external lesions, internal warts, and seed damages were observed in Lygus-infested plots compared to that in uninfested control plots (Fig. 8). Overall, *Lygus* exerted greater damage to bolls in full irrigation treatments compared to that in cotton with supplemental irrigation.

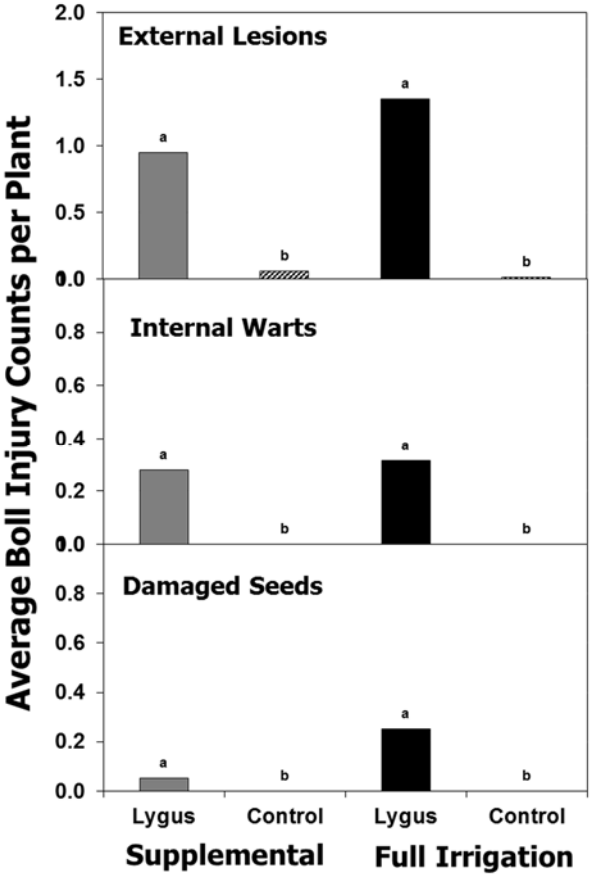

Figure 8. External lesions, internal injury warts, and damaged seeds in developing bolls caused by *Lygus* feeding on plants grown under supplemental versus full irrigation, Lubbock TX, 2018.

Lint yield significantly varied between the two cultivars, with higher lint yield in DP 1823 NR B2XF compared with that in DP 1820 B3XF regardless of the irrigation amount (Fig. 9). Also, as expected, full irrigation increased lint yield significantly in both cultivars compared with supplemental irrigation. However, full irrigation produced significantly higher lint yield compared with that in supplemental irrigation in DP 1823 NR B2XF (1,223 lb/acre versus 1,020 lb/acre) while the yield was similar between the two irrigation regimes for DP 1820 B3XF (961 lb/acre versus 950 lb/acre). *Lygus* infestation negatively impacted the lint yield in both cultivars at both irrigation regimes (Fig. 9). Combined over two cultivars, supplemental irrigation resulted in 642 and 985 lbs/acre in *Lygus*-infested and control plots, respectively, whereas the full irrigation increased lint yield to 816 lb/acre and 1,092 lb/acre for *Lygus*-infested and control plots, respectively.

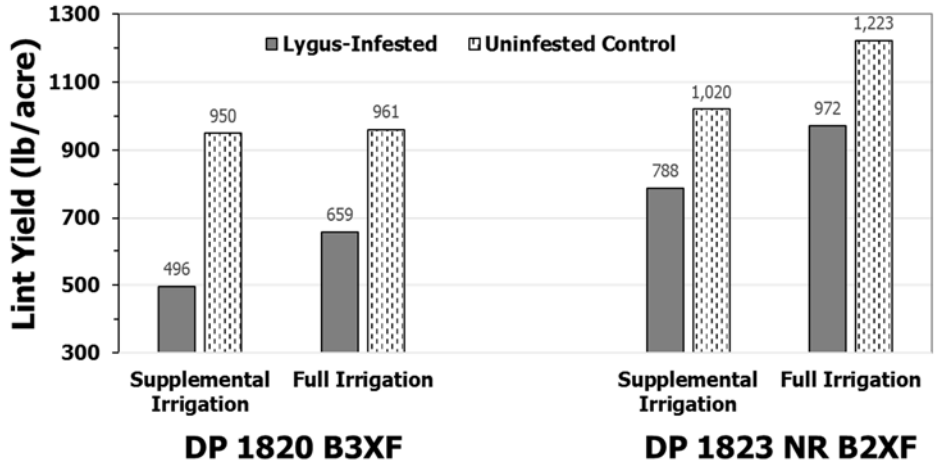

Figure 9. Cotton lint yield losses due to *Lygus* infestation under supplemental versus full irrigation production conditions, Lubbock, Texas, 2018.

The effect of *Lygus* was more pronounced on DP 1820 B3XF compared to that for DP 1823 NR B2XF (Fig. 10). The highest yield reduction (48%) was observed in DP 1820 under supplemental irrigation, followed by DP 1820 full irrigation (31%), and lowest reductions were observed in DP 1823 supplemental (23%) and full irrigation (21%) (Fig. 10). As observed in previous years, *Lygus* induced greater lint yield loss under water-deficit conditions compared to that under full irrigation conditions. However, the difference was not significant with DP 1823 NR B2XF. It is illustrated that the *Lygus* impact on lint loss that is modulated by the amount of irrigation/water availability is also influenced by the cultivar's potential to tolerate *Lygus* injury.

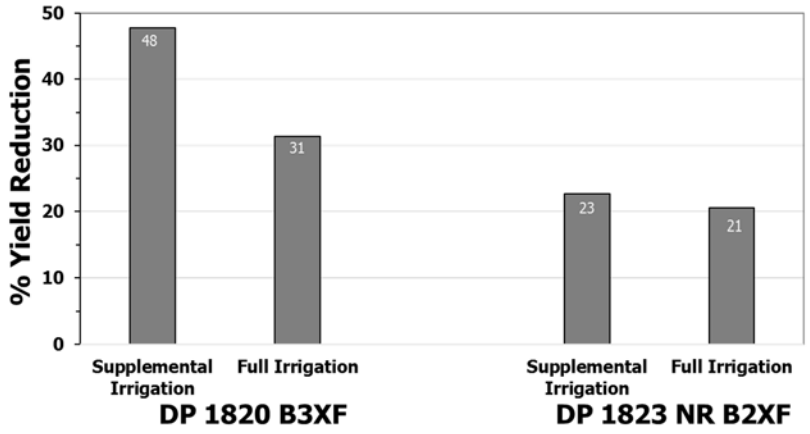

Figure 10. Percent lint yield losses in two cotton cultivars due to *Lygus* infestation under supplemental versus full irrigation production conditions, Lubbock, Texas, 2018.

Acknowledgments

Research funding which facilitated this study came from Cotton Incorporated Core program.

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

FINAL REPORT

Cotton Incorporated Texas State Support Program

Project Number: 16-272TX

PI: Megha N. Parajulee

Project Summary

A four-year study was initiated in 2015 in the Texas High Plains and South Texas to investigate the seasonal moth flight activity patterns of *Helicoverp*a spp. and to possibly detect the presence of the 'Old World' bollworm (OWB, *H. armigera*), if it has already been introduced into the Texas bollworm population. The primary objectives of the study were to: 1) Investigate the effectiveness of species-specific pheromone lures obtained from two vendors, and 2) Determine the efficiency of two different trap designs in capturing *Helicoverpa* spp. moths. Trap type x pheromone lure combination treatments were deployed in mid- to late July each year, followed by all traps being monitored and the captured moths counted approximately weekly through mid-November. All traps were re-baited with fresh lures approximately every two weeks. In 2016, sub-samples of up to 25 moths per trap per sample date (total of 1500 moths) were dissected to determine if the Texas High Plains moth populations contained any *H. armigera*. In 2017 and 2018, thirty-eight (1 - 28 moths per sample) and 52 samples (7 - 1,187 moths per sample) were sent to USDA Center for Plant Health Science and Technology, Fort Collins, CO for molecular diagnosis of OWB specimens. Our current hypothesis is that OWB invasion has not occurred in Texas. In the absence of *H. armigera*, it is therefore, impossible to determine which lure type and/or lure vendor has the best pheromone lure formulation for attracting *H. armigera*. Among the five selected experimental treatments, the Texas Traps baited with Trécé™ *H. armigera* lure captured the highest number of *Helicoverpa* spp. moths during all four years of the study. The *H. armigera* baited traps with the USDA Cooperative Agricultural Pest Survey (CAPS) lures captured significantly fewer moths compared to that in 2015, but caught an equal or greater number of tobacco budworm moths [*Heliothis virescens* (F.)]. The Trécé™ (*H. zea* and *H. armigera*) lure baited traps did not attract tobacco budworm moths, yet both Trécé™ species-specific lures captured numerous *H. zea* specimens. Improvement in the CAPS lure since 2016 has discriminated *Helicoverpa* moth species significantly and the moth capture by CAPS lure in Texas has been greatly reduced, indicating the greater sensitivity of the USDA lure in possible monitoring of OWB moths. However, the 2018 CAPS lure failed to discriminate *H. zea* moths as observed in both 2016 or 2017 CAPS lure formulations. On the other hand, Trécé™ *H. armigera* lure is totally ineffective in discriminating the *Helicoverpa* moth species. Sample dissections of 2016 resulted in no positive identification of OWB samples from Texas populations. Similarly, the molecular examination of 2017 samples resulted in no positive identification of OWB while the 2018 samples are being processed. Based on our current data, we do not believe that the *H. armigera* has been introduced to Texas at this time.

Introduction

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

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

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

Materials and Methods

Survey area for the study included four trapping sites situated in a west-to-east orientation along Texas FM1294 in northern Lubbock County, TX. Five selected experimental treatments included: 1) 'Texas Trap' baited with Trécé™ *H. zea* lure, 2) 'Texas Trap' with Trécé™ *H. armigera* lure, 3) 'Bucket Trap' (green) with Trécé™ *H. zea* lure, 4) 'Bucket Trap' (green) with Trécé™ *H. armigera* lure, and 5) 'Bucket Trap' (green) with USDA CAPS *H. armigera* pheromone lure. Each treatment was represented at each trapping site, including five treatments and four sites (replications) deployed in a randomized block design. Bucket trap with USDA CAPS *H. armigera* pheromone lure was also used to trap moths in Hidalgo Co. to represent South Texas. In 2018, an additional treatment was added to compare the formulations of 2015-2017 CAPS lure versus the new 2018 formulation.

Trapping (surveying) periods for all four study years included typically deploying the traps during mid- to late July with monitoring extending until mid-November annually. Traps were inspected weekly and re-baited at two-week intervals. All captured moths were counted, placed into Zip-Loc™ bags, and then samples were placed into a freezer for species identification dissections at a later date (2016) or sent to USDA Lab in Fort Collins, CO for molecular identification (2017-2018).

Figure 1. Two trap designs, 'Texas Trap' (A) and green 'Bucket Trap' (B), deployed at four Lubbock County sites, 2015-2018.

Results and Discussion

'Texas Trap' with Two Associated Pheromone Lure Treatments

The Trécé™ *H. armigera* and Trécé™ *H. zea* lure baited Texas traps yielded 2015 seasonal weekly mean captures of 119 and 83 bollworm moths per trap, respectively; while during 2016, similar seasonal weekly moth capture averages of 116 and 84 were observed (Figs. 2 and 3). Moth captures in 2017 were higher than that in the previous two years, with 234 and 177 moths per trap per week for *H. armigera* and *H. zea* lures, respectively. The 2018 moth captures were similar to 2015-2016, with 107 and 106 moths per trap per week for *H. armigera* and *H. zea* lures, respectively. Overall, it should be noted that among the five study treatments, the Texas Traps baited with Trécé™ *H. armigera* lure captured the highest number of *Helicoverpa* spp. moths during three of the four years of the study (Figs. 2, 3 and 4). Because *H. zea* cross-responds to *H. armigera* lure and the Trécé™ *H. armigera* lure is not sufficiently sensitive to species specificity, it appears that the Trécé™ lure that is designed for *H. armigera* is as much or more attractive to *H. zea* (Figs. 2, 3, and 4). Therefore, the Trécé™ *H. armigera* lure is not a viable monitoring tool in OWB survey.

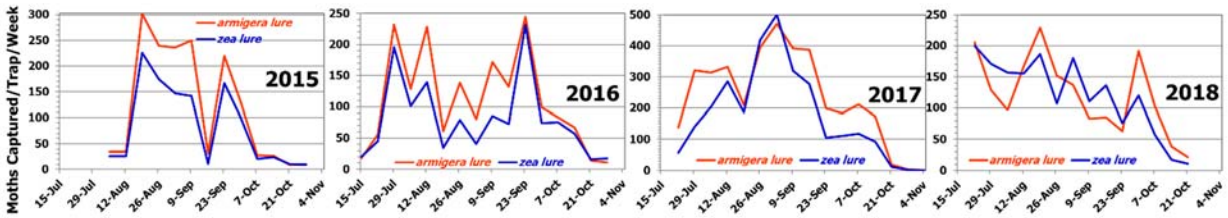

Figure 2. Texas Traps [also referred to as Texas Pheromone Trap, TP Trap or Hartstack Trap (Hartstack et al. 1979)]: Weekly *Helicoverpa* spp. male moth captures during 2015-2018 on 'Texas Traps' baited with *H. zea* or *H. armigera* Trécé™ pheromone lures.

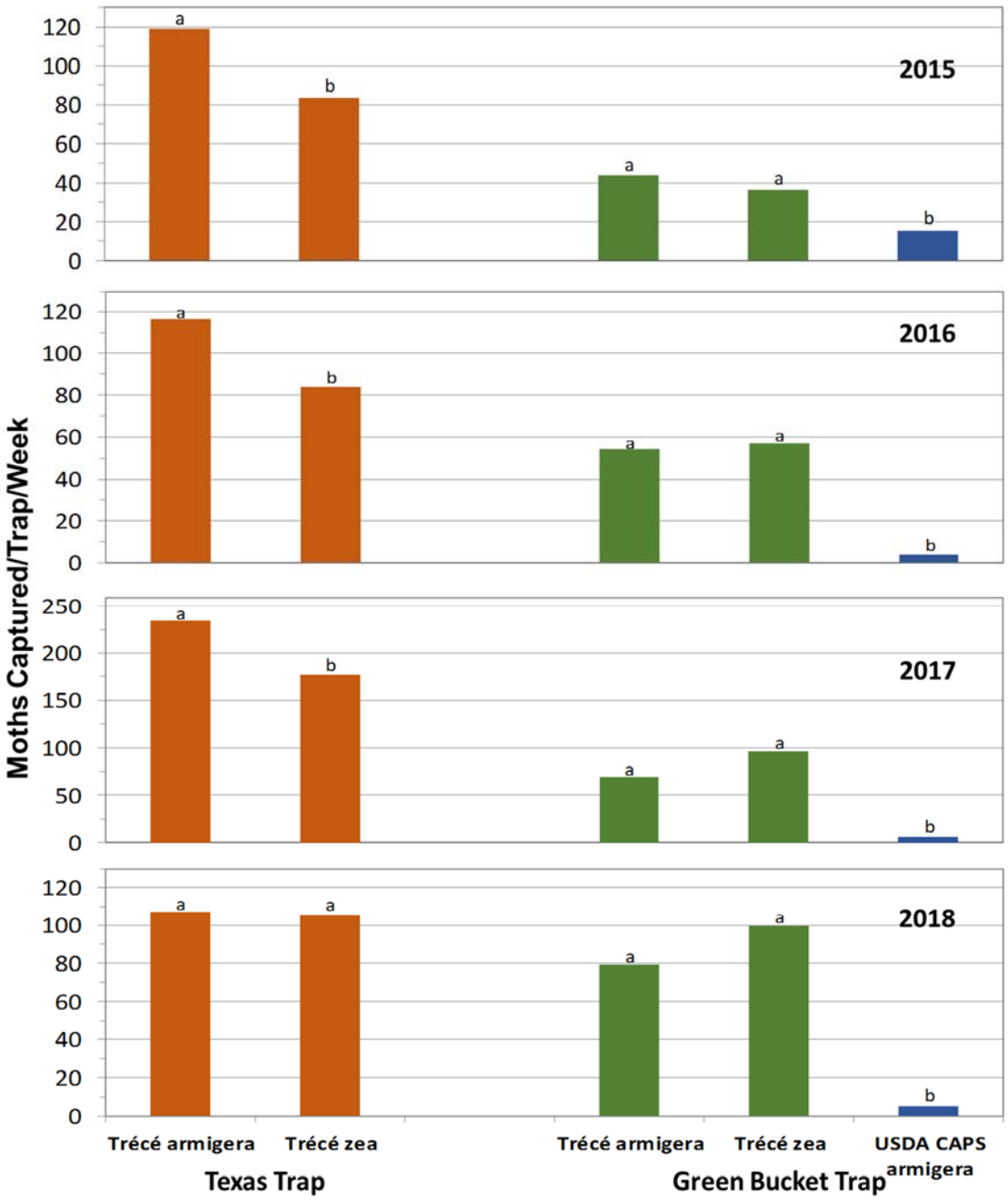

Figure 3. Seasonal mean number of *Helicoverpa* spp. male moths captured per week per trap on Texas Traps (orange bars) baited with Trécé™ *H. armigera* and *H. zea* pheromone lures. Likewise, the two green bars indicate weekly means for green Bucket Traps baited with Trécé™ *H. armigera* and *H. zea* lures. The blue bar illustrates the data for green Bucket Traps baited with the USDA CAPS *H. armigera* lures (lures formulated in 2017 were used for the 2018 survey as well). Seasonal means within each trap type indicated by different lowercase letters indicate statistical difference between these means.

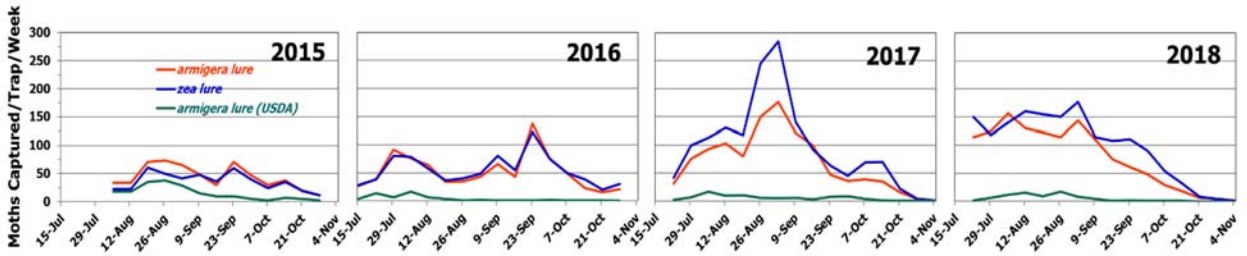

Figure 4. Green 'Bucket Traps': Weekly *Helicoverpa* spp. male moth captures during 2015-2018. Traps were baited with *H. zea* or *H. armigera* Trécé™ pheromone lures, and *H. armigera* USDA CAPS lure.

Green 'Bucket Traps' with Three Associated Pheromone Lure Treatments

Overall, green bucket traps baited with the Trécé™ *H. armigera* and *H. zea* lures yielded lower numbers of bollworm moths than the Texas Traps, yet overall peak trap response periods were observed on both trap designs similarly (Figs. 2, 3, and 4). The Trécé™ *H. armigera* and Trécé™ *H. zea* lure baited green bucket traps yielded 2015 seasonal weekly moth captures of 44 and 36 bollworm moths per trap, respectively, reflecting the same general moth activity trend as observed from the Texas traps (Figs. 2 and 3).

During 2016, a slightly different numerical trend was observed in which the Trécé™ *H. zea* lure baited traps captured a seasonal mean of 57 moths per trap, whereas the Trécé™ *H. armigera* lure captured slightly lower moth numbers (although not statistically different) at 54 moths per trap (Fig. 3). Trap captures in 2017 were significantly higher than that in 2016, with 96 and 69 moths captured in Trécé™ *H. zea* lure and *H. armigera* lure baited traps, respectively. In 2018, Trécé™ *H. zea* lure and *H. armigera* lure baited traps captured 99 and 79 moths, respectively.

What should be noted is that the moth captures on the USDA CAPS baited green bucket traps did not reflect the same moth trap response activity patterns of the other four treatments which utilized lures obtained from Trécé™, Inc. Figures 3 and 4 clearly illustrate that the moth numbers were much lower and only the early season peak trap responses were slightly reflected by USDA CAPS lure as compared to the other pheromone lure treatments. While *H. armigera* lure is expected to cross-capture *H. zea*, USDA CAPS lures were designed to be more sensitive toward *H. armigera* compared to commercially available *H. armigera* lure. At the present time, *H. armigera* does not appear to be in the Texas High Plains bollworm population (see below in *Identification* section); therefore, it is difficult to determine which lure type and/or lure vendor has the best pheromone lure formulation for attracting *H. armigera*. Nevertheless, USDA CAPS lure of 2015-2017 seems to discriminate *H. zea* moths significantly as shown by drastically lower moth captures in CAPS lure baited traps versus Trécé™ OWB lure.

During 2016, the traps baited with the USDA CAPS lures were observed to also capture tobacco budworm [*Heliothis virescens* (F.)] moths, while the Trécé™ (*H. zea* and *H. armigera*) lure baited traps did not attract tobacco budworm moths. During the 11-week trapping period of 18 August to 4 November 2016, the four USDA CAPS lure baited traps captured a total of 172 tobacco budworm moths, while during the same time period these traps captured a slighter higher total of only 245 *Helicoverpa* spp. moths. In 2017, CAPS lure showed similar trend as in 2016, with 347 *Helicoverpa* spp. moths and 99 tobacco budworm moths in 38 samples. The 2017 CAPS lure that was used in 2018 survey captured 289 *Helicoverpa* spp. moths and 162 tobacco budworm moths.

Interestingly, the 2018 formulation of CAPS lure attracted considerably higher abundance of moths compared to the formulations for 2015-2017; the 2018 CAPS lure captures were only slightly lower than (TP traps) or similar (Bucket trap) to the Trécé™ *H. armigera* lure (Fig. 5). The 2018 formulation of CAPS lure did not capture any tobacco budworm moth specimens.

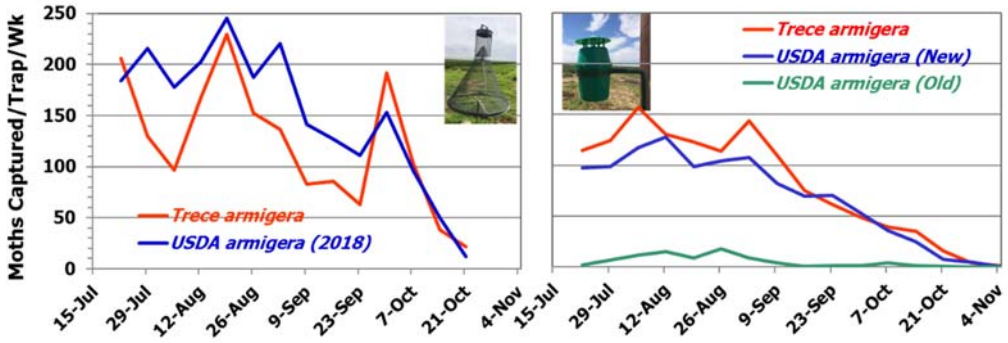

Figure 5. Comparing lure performance between Texas Pheromone traps and Bucket traps: Weekly *Helicoverpa* spp. male moth captures during 2018. Traps were baited with *H. armigera* Trécé™ pheromone lures versus the new USDA CAPS lure provided for 2018 survey (left) and *H. armigera* Trécé™ pheromone lures versus 2018 USDA CAPS lure versus 2017 CAPS lure.

Dissections to Determine *Helicoverpa* spp. Identifications

A total of 1,500 moths from Trécé™ and USDA CAPS *H. armigera* lure baited traps in the Texas High Plains were dissected in 2016. These dissections resulted in no positive identification of OWB in the Texas High Plains moth populations. All dissected male moths appeared to be *H. zea* specimens. Approximately 500 moths per year were dissected from Hidalgo Co. in 2016 and 2017 and no positive OWB samples were detected in that region either. Thirty-eight samples including 347 specimens from 2017 survey and 52 samples (7 – 1,187 moths per sample) were sent to USDA Center for Plant Health Science and Technology, Fort Collins, CO for molecular diagnosis of OWB specimens and their molecular examination also resulted in no positive identification for OWB in 2017 while the 2018 samples are currently being processed.

Acknowledgments

Research funding which facilitated this ongoing study came from Cotton Incorporated State Support Committee and Plains Cotton Growers, Inc. USDA CAPS lures were provided by USDA APHIS PPQ. Stanley Carroll performed the entire four-year trapping survey. Molecular identification of collected specimens was conducted by Dr. Todd Gilligan at USDA Center for Plant Health Science and Technology, Fort Collins, CO.

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Economic Evaluation of Insect-Pest Management in Water-Deficit Cotton Production

Cotton Incorporated - Texas State Support Committee

Project Number: 18-099TX
PI: Megha N. Parajulee
CO-PIs: Abdul Hakeem, Suhas Vyavhare, Katie Lewis, Wayne Keeling, and Donna McCallister

PROJECT SUMMARY

The Texas High Plains (THP) is a semi-arid region with characteristic low rainfall, with production agriculture supported by limited irrigation or rain-fed. As a result, the cropping system in this region is largely low-input and the producer decision-making in economically profitable input use is a challenge. THP has been facing some significant drought conditions in recent years, including the drought of 2011 that claimed much of the Texas production agriculture, reducing total cotton yield that year by 55%. Drought conditions ensued the next 3 years that disproportionately depleted the underground water, significantly shifting the cotton production outlook in THP to even more low-input with dryland acreage reaching to >65%. The shift in cotton production system due to devastating droughts in an already semi-arid region has altered our input resources, cultivars, and management practices. Low cotton market price, increased nitrogen fertilizer price, and reduced water availability have forced farmers to move toward reorganizing available input resources to sustain their production enterprise. Thus, transitioning to the new crop production reality via developing economic data-based input management practices has become our priority to sustain producer profitability.

The objectives of this project were to: 1) quantify the impact of single (thrips or cotton fleahoppers) versus multiple (thrips and cotton fleahoppers sequentially) pest infestations on cotton lint yield and fiber quality under two irrigation water regimes (water-deficit treatments), and 2) develop a dynamic optimization economic model that maximizes the net returns from management of single versus multiple pest infestations under water-deficit crop production conditions. Thus, the scope of this proposed work entails integrating production practices and pest management options under numerous cotton management scenarios (10 total scenarios) and the management options would be developed based on breakeven value and net return of each option for farmers to choose depending on the availability of water resources on their farms.

In 2018, thrips and fleahoppers impacting cotton production risks were evaluated with five combinations of single versus sequential infestations under two water-deficit (near-zero deficit or full irrigation and high deficit or dryland) regimes, replicated four times (total 40 plots). Water deficit conditions and insect infestations impacted crop growth profile as well as lint yield. For example, fleahopper infestation resulted in increased apical growth of the plants in water-deficit conditions, whereas sequential infestation of two insect pests increased the plant apical growth in irrigated plots. Lint yield was similar across all five treatment combinations under dryland condition while the sequential infestation of two pests significantly reduced the lint yield compared to that under irrigated condition, indicating the impact of drought conditions on modulating the effect of insect pests as well as the plant's compensatory ability. Once multi-year data are available, a robust economic analysis will be presented.

Economic Evaluation of Insect-Pest Management in Water-Deficit Cotton Production

INTRODUCTION

The Texas High Plains (THP) is a semi-arid region with characteristic low rainfall (average annual rainfall of 15-18 in.), with production agriculture supported by limited irrigation or rain-fed. As a result, the cropping system in this region is largely low-input and the producer decision-making in economically profitable input use is a challenge. THP has been facing some significant drought conditions in recent years, including the drought of 2011 that claimed much of the Texas production agriculture, reducing total cotton yield that year by 55%. Drought conditions ensued the next 3 years that disproportionately depleted the underground water, significantly shifting the cotton production outlook in THP to even more low-input with dryland acreage reaching to about 70%. The shift in cotton production system due to devastating droughts in an already semi-arid region has altered our input resources, cultivars, and management practices. Low cotton market price, increased nitrogen fertilizer price, and reduced water availability have forced farmers to move toward reorganizing available input resources to sustain their production enterprise. While the drought and heat conditions are unpredictable, the anticipated changes in global climate patterns may exacerbate the water-deficit conditions further in the THP. Thus, transitioning to the new crop production reality via developing economic data-based input management practices has become our priority to sustain producer profitability and for future success of the U.S. cotton industry.

Much has been reported on direct and indirect effects of drought stress on cotton, but the effect of drought stress on cotton insect pest dynamics, feeding potential, and plant's response to insect injury under drought-stressed conditions are limited. In addition, the paucity of information on integration of pest management decisions and crop production decisions has hindered producers' ability to predict economic risks of optimizing limiting input resources. Predicting pest populations under different water-deficit crop production scenarios and understanding how these conditions influence those populations to impact crop production risks, are critically important components for implementing pest management strategies as crop cultivars and other input variables continue to change. Reduced water availability, low rainfall, higher pumping cost of limited water, and increased input cost may result in lower yields and correspondingly lower profit margins, warranting for higher water use efficiency in our crop production. Therefore, cotton producers must carefully consider costs of pest management options against potential benefits to overall net profit margin of the crop production enterprise. The objectives of this project are to: 1) Quantify the impact of five combinations of single versus sequential infestations of two major insects (thrips and cotton fleahoppers) on cotton lint yield and fiber quality under two irrigation water regimes (water-deficit treatments – near dryland versus full irrigation), and 2) Develop a dynamic optimization economic model that maximizes the net returns from management of single versus sequential pest infestations under water-deficit crop production conditions. Thus, the goal of this project aims to integrate production practices and pest management options under numerous cotton management scenarios (10 total scenarios) and the management options will be developed based on breakeven value and net return of each option for farmers to choose depending on the availability of water resource on their farms.

METHODOLOGY

A multi-year study was initiated in 2018 on a five-acre subsurface drip irrigation cotton field located at the Lubbock Texas A&M AgriLife Research farm (Lubbock County, TX).

Irrigation water level treatments. Two irrigation water levels (dryland and full irrigation) simulated two water-deficit production conditions, including high water-deficit (dryland condition) and no water deficit. A high-water treatment maintained >90% evapotranspiration replenishment through subsurface drip irrigation throughout the crop growing season, whereas the dryland treatment received pre-planting irrigation to facilitate proper seed germination and no additional irrigation. Cotton cultivar DP 1646 B2XF (seed with no insecticide or fungicide seed treatment) was planted on 31 May 2018.

Insect infestation treatments. Two key early-season insect-pest species (thrips and cotton fleahoppers) impacting cotton production risks were evaluated with five combinations of single versus sequential infestations (Table 1) under two water-deficit (zero versus high) regimes, replicated four times (total of 40 experimental plots). Targeted insect management options were achieved via artificial infestation of insect pests. Because Texas High Plains cropping conditions rarely warrant more than a single insecticide application to suppress either of the two major insect pest groups (thrips at seedling stage and cotton fleahopper at early squaring stage), our experiment was designed to infest our treatments at the most vulnerable stage of crop for the species infested.

Table 1. Five insect management scenarios evaluated in dryland versus full irrigation cotton, Lubbock, Texas, 2018

Treatment #	Insect Infestation Treatment Simulated via Artificial Infestation
1	All insects suppressed (No insect infestation) (sprayed control)
2	Thrips occurring at 1-2 true leaf stage
3	Cotton fleahoppers occurring during the first week of squaring
4	Thrips and cotton fleahoppers infested sequentially
5	No insect management (untreated control)

Insect augmentation

Thrips. Thrips were released to seedling cotton on June 19, 2018 when the crop was at 1-2 true leaf stage. Thrips infested alfalfa terminals were excised from a healthy alfalfa patch and these terminals were laid at the base of young cotton seedlings. Thrips were expected to move onto the cotton seedlings as excised alfalfa sections began to dry. Approximately 6 thrips per seedling were released to two 5 row-ft sections (approximately 12 plants per section) per plot (approximately 140 thrips per thrips-augmented plot). Thrips were released on all 16 thrips-augmentation plots (treatments #2 and #4 x 2 water levels x 4 replications) on the same day. Thrips were released on four additional plots to estimate thrips movement onto the cotton seedling via absolute sampling of seedlings and washing of thrips 3 days post-release. Data showed that the seedlings received an average of 1.2 live thrips per seedling which is the threshold density for 1-2 leaf stage seedling cotton.

Uncharacteristic high daytime temperatures for the next 7 days following the thrips release (103-107 ^{o}F) contributed to low thrips feeding performance and perhaps high thrips mortality after the thrips moved to the seedlings. Consequently, no visible signs of thrips-feeding effect were observed in thrips-augmented plots.

Cotton fleahoppers. Woolly croton, with embedded overwintering fleahopper eggs, was harvested from rangeland sites near College Station, Texas, in early February 2018 and then placed into cold storage. Eighty 1-gallon sheet metal cans, each containing 4 ounces of dry croton twigs per can, were initiated to generate the required number of cotton fleahopper nymphs for the experiment. Conditions conducive to cotton fleahopper emergence were simulated in a laboratory environment in order to induce hatching of overwintered eggs embedded in the croton stems, and emerged cotton fleahoppers were subsequently reared on fresh green beans. The single release of nymphal cotton fleahoppers (2nd instars) was timed to simulate the acute heavy infestation of cotton fleahoppers (4-5 days of feeding) while cotton was highly vulnerable to the fleahopper injury (1st week of squaring). The release was accomplished on 10 July by transferring second-instar fleahopper nymphs from the laboratory colony into 15 cm X 10 cm plastic containers, then cautiously depositing them onto the terminals of plants in each treatment plot at the rate of 5 nymphs per plant. Immediately after cotton fleahoppers were released onto the fleahopper-augmentation plots (treatments #3 and #4; total 16 plots), control plots were sprayed with Orthene® 97. All treatment plots, except treatment #1, were sprayed with Orthene® 97 on 17 July and kept insect-free for the remainder of the study to isolate the effect of various treatments.

Parameters measured. The flowering profile was monitored from all 40 experimental plots for five sample dates (31 July, 6 August, 9 August, 15 August and 28 August 2018) to determine the effect of insect infestation and water deficit condition on fruiting delays and/or flowering patterns. Plant height was also recorded from all plots at the time of harvest. Hand harvesting was done on 16 November from flagged area and cotton was ginned on 17 December 2018. Lint will be sent to Cotton Incorporated for fiber analysis.

RESULTS

Extremely high temperatures during the seedling stage complicated the study in 2018, especially the released thrips failed to exert the desired significant infestation on the young cotton seedlings. As a result, thrips damage to seedlings was not apparent on visual observation. Cotton fleahoppers caused about 20% square loss overall across all experimental plots. Because cotton fleahoppers were released when plants had 2-3 total squares (all were fleahopper susceptible squares), the effect was not apparent immediately and plants outgrew the effect of early season fleahopper-induced square loss. Nevertheless, insect injury manifested some noticeable effect on flowering patterns, plant height, and lint yield.

Untreated control plots showed slightly higher flower densities in irrigated versus dryland cotton effect all throughout the month-long monitoring period, with significantly higher flower densities in late August. Contrasting to this phenomenon, the flowering patterns were near identical between irrigated and dryland plots when cotton fleahoppers were infested singly or sequentially with thrips infestation (Fig. 1). When thrips were infested alone, flowering patterns between dryland and irrigated main-plot treatments were generally similar to what was observed in untreated or sprayed control plots. Overall, average flower abundance was similar across five insect augmentation

treatments within each irrigation treatment (Fig. 2). While cotton flowering occurs daily during the active flowering period and the average of flower monitoring only five times may not reflect the production potential of cotton, these patterns clearly indicate that insect infestation, particularly cotton fleahoppers, rendered overall flowering patterns between irrigated and dryland similarly (Figs. 1-2). The average flower abundance was significantly lower in dryland compared to that in irrigated cotton only at untreated control plots while all other treatments were not significantly different between the two irrigation regimes (Fig. 2). These data suggest that the insect infestation during pre-flower stage exerts some significant physiological response to cotton during the flowering stage. Multi-year data will hopefully add more insights into this phenomenon.

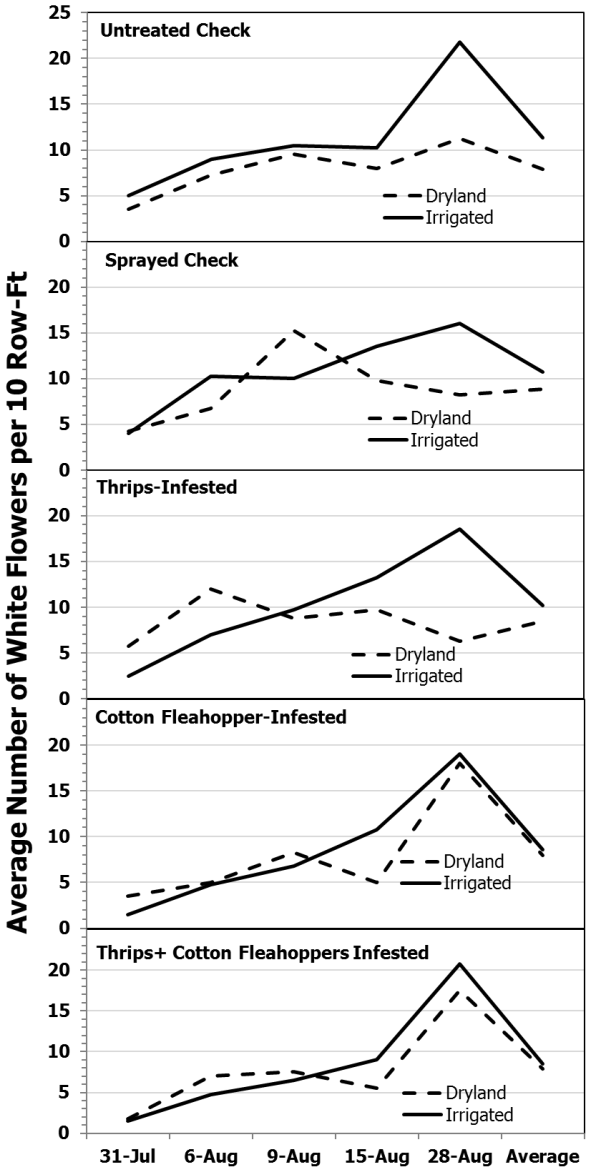

Figure 2. Temporal abundance of white flowers (number of white flowers per 10 row-ft per sample date) recorded from thrips and fleahopper infested plots under dryland versus irrigated production conditions, Lubbock, Texas, 2018.

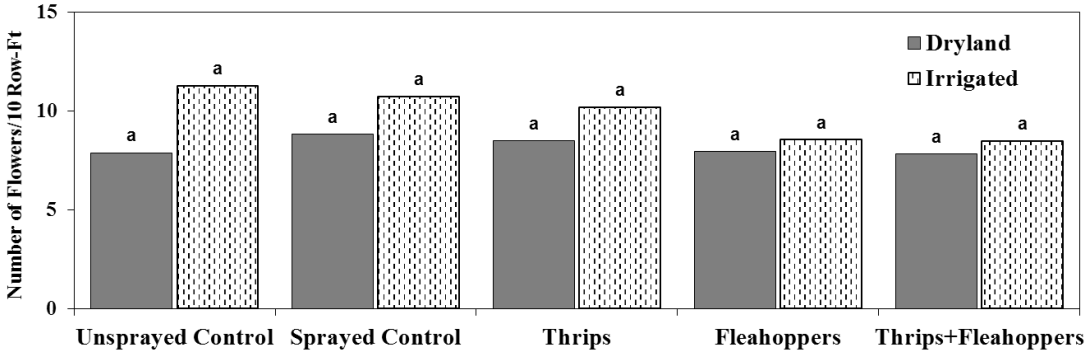

Figure 2. Average abundance of white flowers (number of white flowers per 10 row-ft; n=5 sample dates) recorded from thrips and fleahopper infested plots under dryland versus irrigated production conditions, Lubbock, Texas, 2018. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

Pre-harvest plant measurement showed that insect-augmented plots in irrigated cotton had significantly taller plants compared to that in untreated control plots, but the effect was considerably diminished under dryland conditions (Fig. 3). There was significant "noise" on plant height data under dryland condition in which fleahopper-infested plants resulted in the tallest plants while thrips followed by fleahoppers resulted in the shortest plant heights. We find no reasonable explanation for why cotton fleahopper-infested plots resulted in both tallest and shortest plants.

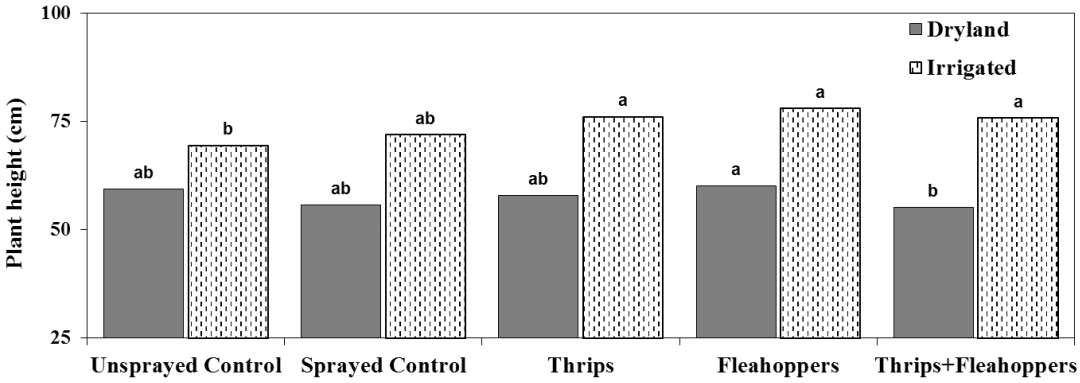

Figure 3. Plant height impacted by thrips and fleahopper infestations under dryland versus irrigated production conditions, Lubbock, Texas, 2018. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

Lint yield was significantly higher in irrigated cotton compared to that in dryland cotton across all five treatment combinations (Fig. 4). This suggests that the dryland plots were sufficiently water-stressed during the growing season, despite several rainfall events during the crop maturation phase in late September - early October. The highest lint yield under irrigation treatment was observed in the untreated control treatment (1,607 lb/acre), while the lowest (1,253 lb/acre) was recorded in the thrips+fleahopper sequential infestation treatment (Fig. 4). Lint yield in other

treatments (spray control, thrips only, and fleahoppers only) did significantly differ from the untreated control or thrips+fleahopper sequential treatments (Fig. 4). Lint yield did not significantly vary across five insecticide augmentation treatments. As expected, the yield threshold in dryland cotton is much lower than that for irrigated cotton and thus the lower yield across all treatments can be partially attributed for lack of insecticide treatment effect on lint yield. Fiber parameters will be included in the 2019 report.

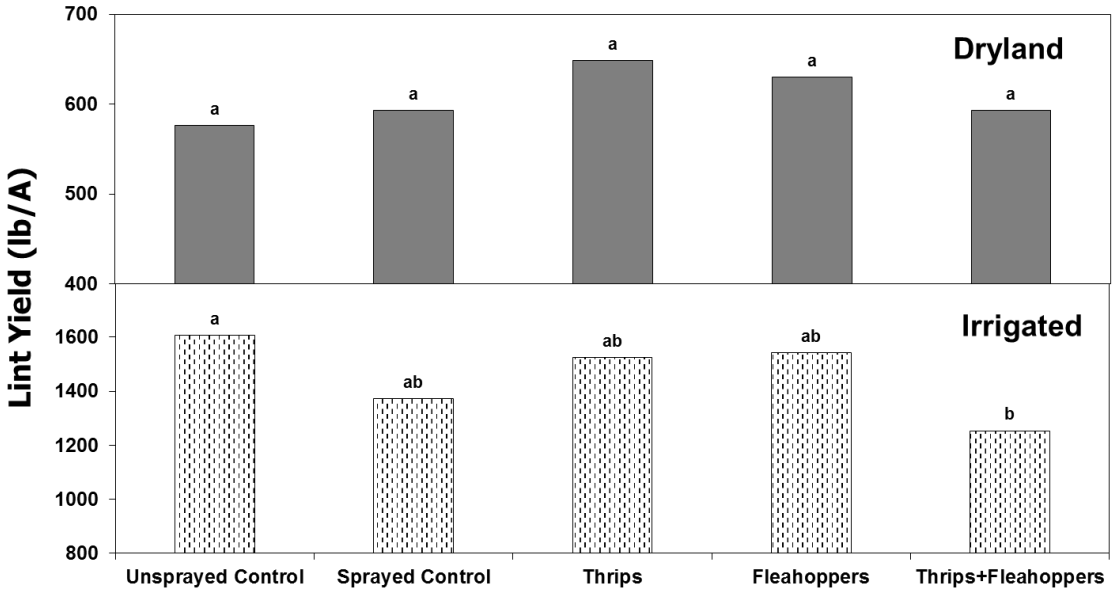

Figure 4. Cotton lint yield losses due to thrips and fleahopper infestation under dryland versus irrigated production conditions, Lubbock, Texas, 2018. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

Profitability model will be developed by using at least two years of data. This data will be used to analyze and compare the economics of management of thrips and cotton fleahoppers singly or in sequential combinations under two water-deficit production regimes. A set of economic profitability models will empower cotton producers in production decision-making in their specific production scenarios (insect pest management options in relation to water availability in their production enterprise). Economic decision-making models will be developed based on crop yield response and crop budget analyses. Crop yield response functions will be generated for each of the 5 insect management treatments within each water-deficit production systems, with 10 separate production scenarios. Cotton yield response to each insect treatment under two water levels will be fitted to calculate the slope (coefficient) of each treatment. Functional form will consider cotton yield and insect exposure (treatment) as fixed effect, and year as random. Insect management treatments within each water level will be ranked based on likelihood ratio test.

Acknowledgments

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

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INTRODUCTION

A long-term study (2002 to 2018) was conducted in the southern Texas High Plains (THP) region to investigate the year-around weekly moth flight activity patterns of cotton bollworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), and beet armyworm, *Spodoptera exigua* (Hübner).

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

The regional adoption of cotton and corn crop cultivars incorporating *Bt* technology has been instrumental in reducing the current threat of these lepidopteran pests yet diminishing underground water availability for irrigation is necessitating vigilant economic cost/benefit evaluations of high-cost crop inputs, such as genetically modified seeds, for our increasing dryland/limited irrigation crop production acreage.

MATERIALS & METHODS

Study Duration: March 2002 to December 2018 **Study Sites:** Lubbock County, Texas

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

Survey Protocol: Nine pheromone traps [3 lepidopteran species monitored X 3 study sites] were placed in Lubbock County representing the approximate center of the southern Texas High Plains. The three sites were selected and one trap for each pest species was placed, then baited and monitored weekly (growing season) to twice monthly (non-crop months) throughout the year. Trap types included: 1) Texas pheromone trap (Hartstack et al. 1979) for bollworms and tobacco budworms, and 2) Bucket traps (green) for beet armyworms. The three species-specific pheromone lure types were secured from a single source (Trece®, Inc., Adair, OK). Bollworm and tobacco budworm pheromone lures were replaced approximately every two weeks, while *beet armyworm* pheromones were replaced monthly. GPS coordinates for each of the three selected Lubbock County trapping sites were recorded along with the weekly/bimonthly trap captures.

RESULTS & DISCUSSION

Seasonal abundance and flight patterns of cotton bollworm, tobacco budworm, and beet armyworm moths were determined based upon captures in pheromone traps monitored all months of the year (Parajulee et al. 1998, 2004). For each species, this 17-year trapping study has been sub-divided into four successive periods, including: 1) 2002-2006, 2007-2011, 2012-2016, and 2017-2018, roughly representing boll weevil eradicated and beginning of *Bt* cotton adoption in THP, low *Bt* cotton acreage (<50%), majority *Bt* cotton (70%), and the most recent 2-year period, respectively.

Cotton Bollworm. The cumulative annual number of bollworm moths captured per trap averaged 10,188, 6,389, 3,858 and 2,781 for 2002-2006, 2007-2011, 2012-2016, and 2017-2018, respectively. The observed trend suggests a decreasing, yet high bollworm numbers during years 2002 to 2011, followed by a leveling-off of numbers beginning in 2012 to 2018. Fig. 1 (top-left panel) and Fig. 2 (left-panel) clearly illustrates this trend of decreasing trap captures during the first 10 years, followed by lower, yet relatively level, overall annual bollworm total captures (per trap) from 2012 to 2018. Interestingly, although bollworm numbers decreased over time, the seasonal flight profiles remained quite similar over the four periods (Fig. 1 top-left panel).

Tobacco Budworm. The cumulative annual number of tobacco budworm moths captured per trap averaged 767, 148, 255 and 87 for 2002-2006, 2007-2011, 2012-2016, and 2017-2018, respectively. Higher numbers of tobacco budworm moths were trapped during the early 2002-2006 period and then numbers decreased and have remained low in the past 12 years with the exception periods for peak flight from late August through September (Fig. 1 top-right; Fig. 2 center-panel). Although the number of trapped budworm moths varied between the four defined periods, the overall flight activity patterns had somewhat similar profiles with activity starting in late April, peak activity during early August to early October and most trap response ending by late October.

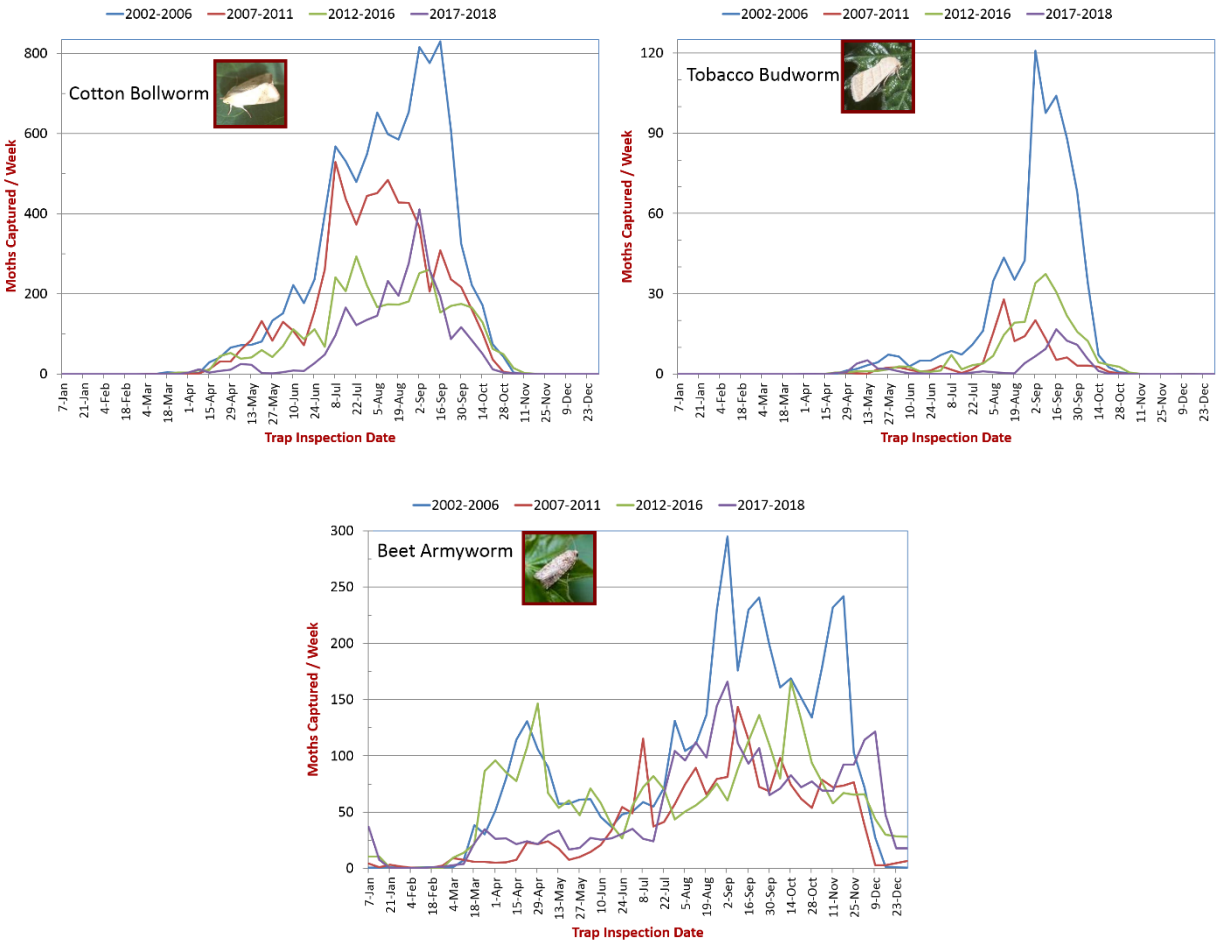

Figure 1. Number of bollworm (top-left panel), tobacco budworm (top-right panel), and beet armyworm (bottom-center panel) moths captured per week, averaged across three selected consecutive 5-year groupings (2002-2006, 2007-2011, 2012-2016), plus the most recent two years (2017-2018), Lubbock County, TX, 2002-2018.

Beet Armyworm. The cumulative annual beet armyworm moths captured per trap averaged 4,397, 1,979, 3,104, and 2,635 for 2002-2006, 2007-2011, 2012-2016, and 2017-2018, respectively. Although beet armyworm moths were often captured during all months of the year, they were primarily active during the period of mid-March to early December (Fig. 1, bottom-center panel). Unlike decreasing bollworm and tobacco budworm numbers since the beginning of the study, no obvious population trends are evident as seen in Fig. 1 (lower-center panel) and Fig. 2 (right panel).

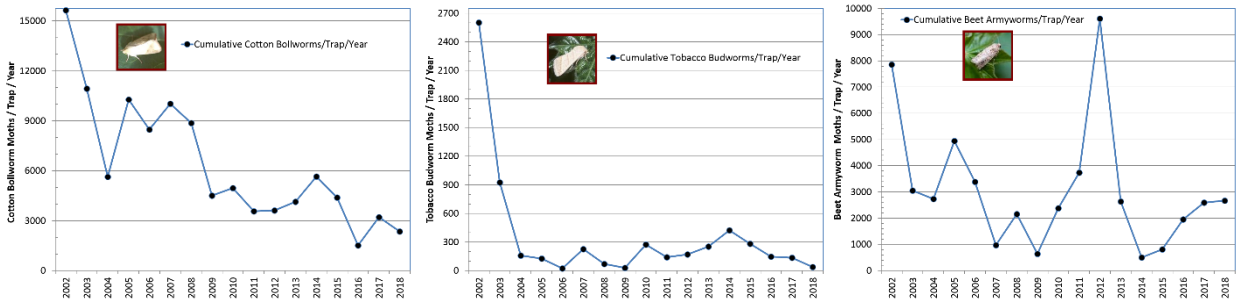

Figure 2. Average annual cumulative moths per trap: 1) Bollworm (left panel), 2) Tobacco budworm (middle panel), and 3) Beet armyworm (right panel), Lubbock County, TX, 2002-2018.

Influence of annual rainfall on moth abundance and flight profiles. Within the 17-yr study period, cumulative annual rainfall ranged from a low of 5.7-in. to a high of 33.3-in. The two years of lowest rainfall were 2003 (8.8-in.) and 2011 (5.7-in.), while the two highest rainfall years were 2004 (33.3-in.) and 2015 (29.5-in.). For each of the three selected cotton pest species, the seasonal abundance and flight profiles are plotted for the two highest and two lowest rainfall years (Fig. 3).

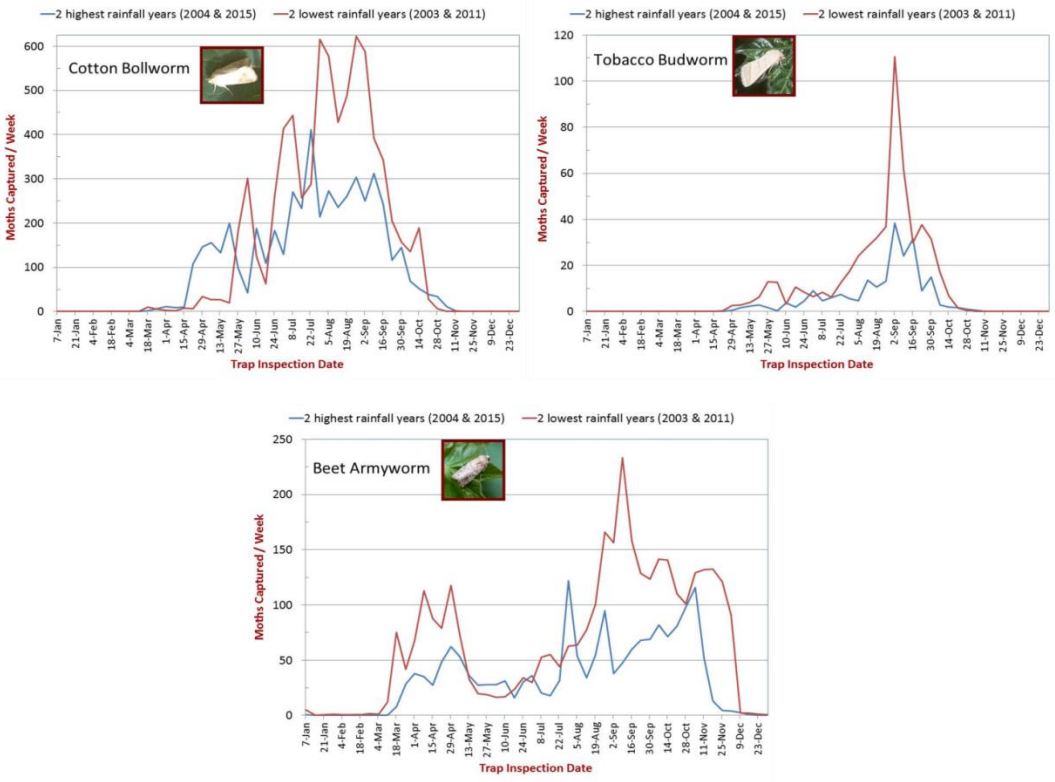

Figure 3. Cotton bollworm (top-left), tobacco budworm (top-right), and beet armyworm (bottom center) moth seasonal flight profiles averaged for: 1) Two study years with the highest rainfall (2004 & 2015), and 2) Two lowest rainfall years (2003 & 2011). Lubbock County, 2002-2017.

*Cotton Bollworm***.** The overall timing of the flight profiles was similar between high and low rainfall years, except in regard to the magnitude of the peak numbers of moths captured (Fig. 3, top-left panel). The highest cumulative number captured per trap per year was 7,254 for the low rainfall years, while the numbers in highest rainfall years declined by 31.0% to 5,005 moths.

*Tobacco Budworm***.** Again, the overall timing of the flight profiles was similar between high and low rainfall years, but more tobacco budworm moths were captured during the low rainfall years (Fig. 3, top-right panel). The average annual cumulative number captured per trap per year was 533 for the low rainfall years, while the average annual cumulative number in the highest rainfall years declined by 58.5% to 221 moths per trap.

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

ACKNOWLEDGMENTS

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CHARACTERIZATION OF COTTON CROP RESPONSE TO WESTERN FLOWER THRIPS INJURY AND ITS MANAGEMENT IN TEXAS HIGH PLAINS COTTON

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Abstract

The western flower thrips, *Frankliniella occidentalis* Pergande, is a serious pest on seedling cotton in the Texas High Plains and other regions of the U.S. cottonbelt. Thrips are an early season pest which can cause severe damage to seedling cotton. First three weeks of seedling stage is important because thrips can cause significant damage during this period when plants are in the 1-3 true-leaf stage. Heavy infestations can cause leaves to shrivel, reduction in leaf chlorophyll content and leaf area, and ultimately significant yield reduction. The manipulation of thrips populations in a cotton field setting is very challenging and maintaining selected thrips densities on cotton seedlings in an open field condition are unmanageable. Nevertheless, it is essential to use field cages and confine known number of thrips per caged plants to obtain a desired thrips density. The ultimate goals of the research project were to develop new economic thresholds for thrips based upon plant response characteristics, validating or revising the current Texas High Plains thrips treatment threshold recommendations, and precisely characterizing the cotton crop response to various levels of thrips injury at different cotton seedling ages. Results reported herein consisted of a series of two greenhouse experiments conducted at the Texas A&M AgriLife Research and Extension Center, Lubbock. In the greenhouse study, 0, 0.5, 1 and 2 thrips per plant were released at 1- to 2- true-leaf stage. Twenty-two days following the release, the seedlings were harvested, washed and thrips were counted. Significantly higher thrips densities were observed from treatments where 1 or 2 thrips were released per seedling compared to 0.5 and control seedlings. Visual plant damage ranking values of plants from thrips densities 0 and 0.5 were significantly superior (i.e., less visual damage) compared to that from thrips densities 1 and 2. Similar densities were achieved in field cages via thrips release in No-Thrips® screen cages to compensate for 80% field mortality. Significant numbers of thrips were recovered from all thrips-augmented treatments, with lowest numbers recovered from control plants. Leaf area was significantly higher in uninfested control compared to those in thrips augmented treatments. Seedling health, measured by visual ranking, declined progressively with increased thrips densities. Thrips densities @ 0.5 released thrips per plant or greater significantly reduced plant vigor. Thrips densities of 0.5, 1, and 2 per plant at early seedling stage all reduced lint yield significantly compared to that in uninfested control plots. Similar densities were achieved in field cages via thrips release in No-Thrips® cages to compensate for 80% field mortality. Thrips densities of 0.5, 1, and 2 per plant at early seedling stage all reduced lint yield significantly compared to that in uninfested control plots.

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) is a serious pest on seedling cotton in Texas and other regions of the U.S. cottonbelt. Thrips are an early season pest which can cause severe damage to seedling cotton. Heavy infestations can cause leaves to shrivel and cause the loss of leaf chlorophyll, reduced leaf area and cause delayed maturity, thereby causing significant yield reduction, if not controlled. Several insecticides, including Orthene®, are commonly used to reduce thrips infestations during the early cotton growth stages. The overall objective of this study was to estimate thrips damage potential and management tactics against thrips in the Texas High Plains. The specific objectives were to determine the effect of thrips densities on seedling cotton in the greenhouse, and to quantify thrips-induced losses to cotton lint yield.

Materials and Methods

Greenhouse Study:

Two experiments were conducted at the Texas A&M AgriLife Research Center-Lubbock, Texas. Six cotton cultivars were planted in 16-oz Styrofoam® cups (1 plant per cup). Thrips were reared on green beans in the laboratory. Immature thrips were released onto cotton at the 1- to 2-true leaf stage. Greenhouse was disinfected by spraying three days before the start of the experiment. Four thrips density treatments (No thrips, one thrips per two plants, one thrips per plant and two thrips per plant) were applied and thrips were allowed to feed for three weeks. Visual leaf tissue damage rankings were recorded (scale of 1-10), 1 = healthy plants; 10 = plants killed by thrips. Chlorophyll meter was

used to record chlorophyll readings. Seedlings were clipped, washed and thrips counted. Leaf area was also recorded using a LI-COR® leaf area meter.

No-Thrips® Field Cage Study:

Wooden cages were covered with No-Thrips® screen and placed in the field. Each cage contained 8-13 seedlings. Thrips were reared on green beans in the laboratory. At the 1- to 2-true leaf stage, immature thrips were released onto the cotton seedlings. Cages were removed five days after releases and Orthene®97 was sprayed. Treatments included no thrips (control), two thrips per plant, five thrips per plant, and ten thrips per plant to achieve 0.0, 0.5, 1, and 2 thrips per plant, after accounting for 20% field survivorship. Five days after thrips release, two plants from each cage were clipped, washed and counted immature and adult thrips. Remaining plants were harvested for lint yield.

Results and Discussion

Greenhouse Study:

Significantly higher number of thrips were recovered from thrips densities released treatment plants than control plants (Fig. 1). Leaf area was significantly higher in uninfested control compared to that in thrips-augmented treatments (Fig. 2). Seedling vigor was significantly reduced with increased thrips densities (Fig. 3).

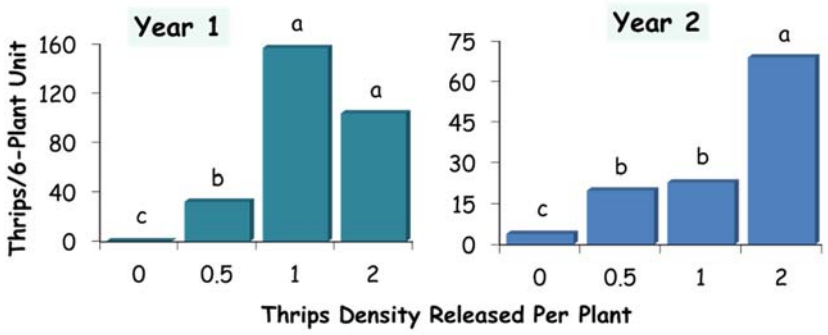

Figure 1. Thrips recovery from seedling cotton using whole-plant washing technique.

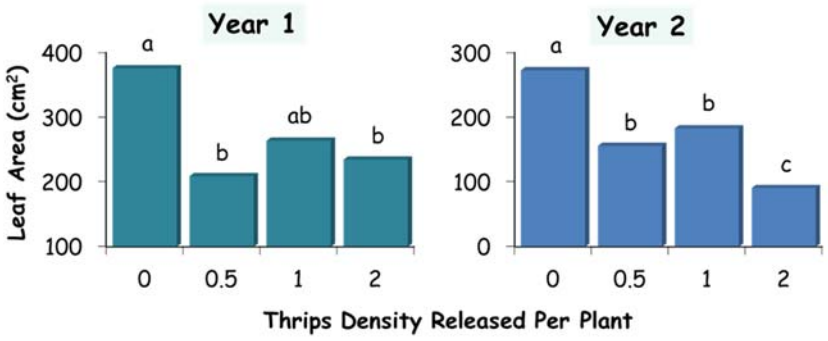

Figure 2. Effect of thrips densities on seedling growth, represented by seedling leaf surface area.

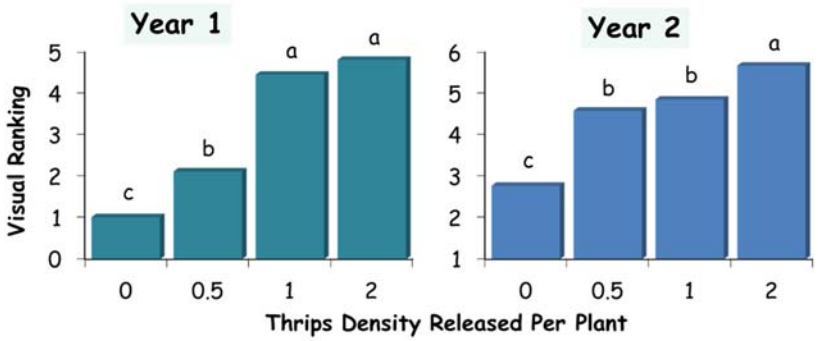

Figure 3. Effect of thrips densities on seedling health, as measured by visual ranking (1=no thrips damage; 10=plants killed by thrips injury).

No-Thrips Field Cage Study:
No-Thrips® cages allowed to maintain the desired thrips densities of 0, 0.5, 1, and 2 thrips/plant relatively well. Thrips augmentation of 0.5 thrips per plant and higher densities reduced the lint significantly compared with that in uninfested control plots in both years (Fig. 4).

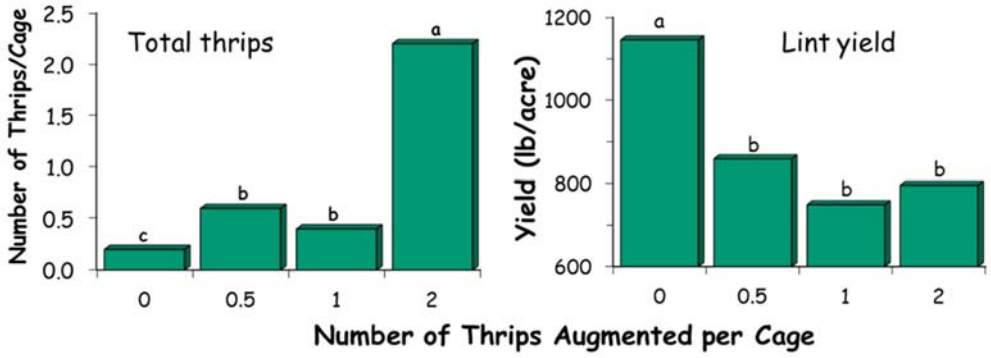

Figure 4. Thrips recovered from different treatments using whole-plant washing (left) and lint yield (right).

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EARLY-SEASON INSECT MANAGEMENT IN DRYLAND COTTON IN THE TEXAS HIGH PLAINS

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Abstract

The Texas High Plains (THP) is a semi-arid region with characteristic low rainfall, with production agriculture supported by limited irrigation or rain-fed. As a result, the cropping system in this region is largely low-input and the producer decision-making in economically profitable input use is a challenge. The shift in cotton production system due to devastating droughts in an already semi-arid region has altered our input resources, cultivars, and management practices. Low cotton market price, increased nitrogen fertilizer prices, and reduced water availability have forced farmers to move toward reorganizing available input resources to sustain their production enterprise. Thus, transitioning to the new crop production reality via developing economic data-based input management practices has become our priority to sustain producer profitability.

The objectives of this project were to quantify the impact of single versus multiple pest infestations on cotton lint yield and fiber quality under two irrigation water regimes. Thus, the scope of this proposed work entails integrating production practices and pest management options under numerous cotton management scenarios and the management options would be developed based on breakeven value and net return of each option for farmers to choose depending on the availability of water resources on their farms.

In 2018, thrips and fleahoppers impacting cotton production risks were evaluated with five combinations of single versus sequential infestations under two water-deficit regimes, and replicated four times. Water deficit conditions and insect infestations impacted crop growth profile as well as lint yield. For example, fleahopper infestation resulted in increased apical growth of the plants in water-deficit conditions, whereas sequential infestation of two insect pests increased the plant apical growth in irrigated plots. Lint yield was similar across all five treatment combinations under dryland conditions, while the sequential infestation of two pests significantly reduced the lint yield under irrigated condition, indicating the impact of drought conditions on modulating the effect of insect pests as well as the plant's compensatory ability.

Introduction

The Texas High Plains (THP) is a semi-arid region with characteristic low rainfall (average annual rainfall of 15-18 in.), with production agriculture supported by limited irrigation or rain-fed. As a result, the cropping system in this region is largely low-input and the producer decision-making in economically profitable input use is a challenge. THP has been facing some significant drought conditions in recent years, including the drought of 2011 that claimed much of the Texas production agriculture, reducing total cotton yield that year by 55%. Drought conditions ensued the next 3 years that disproportionately depleted the underground water, significantly shifting the cotton production outlook in THP to even more low-input with dryland acreage reaching to about 70%. The shift in cotton production system due to devastating droughts in an already semi-arid region has altered our input resources, cultivars, and management practices. Low cotton market price, increased nitrogen fertilizer price, and reduced water availability have forced farmers to move toward reorganizing available input resources to sustain their production enterprise. While the drought and heat conditions are unpredictable, the anticipated changes in global climate patterns may exacerbate the water-deficit conditions further in the THP. Thus, transitioning to the new crop production reality via developing economic data-based input management practices has become our priority to sustain producer profitability and for future success of the U.S. cotton industry.

Much has been reported on direct and indirect effects of drought stress on cotton, but the effect of drought stress on cotton insect pest dynamics, feeding potential, and plant's response to insect injury under drought-stressed conditions are limited. In addition, the paucity of information on integration of pest management decisions and crop production decisions has hindered producers' ability to predict economic risks of optimizing limiting input resources. Predicting pest populations under different water-deficit crop production scenarios and understanding how these conditions

influence those populations to impact crop production risks, are critically important components for implementing pest management strategies as crop cultivars and other input variables continue to change. Reduced water availability, low rainfall, higher pumping cost of limited water, and increased input cost may result in lower yields and correspondingly lower profit margins, warranting for higher water use efficiency in our crop production. Therefore, cotton producers must carefully consider costs of pest management options against potential benefits to overall net profit margin of the crop production enterprise. The objectives of this project are to: 1) Quantify the impact of five combinations of single versus sequential infestations of two major insects (thrips and cotton fleahoppers) on cotton lint yield and fiber quality under two irrigation water regimes (water-deficit treatments – near dryland versus full irrigation), and 2) Develop a dynamic optimization economic model that maximizes the net returns from management of single versus sequential pest infestations under water-deficit crop production conditions. Thus, the goal of this project aims to integrate production practices and pest management options under numerous cotton management scenarios (10 total scenarios) and the management options will be developed based on breakeven value and net return of each option for farmers to choose depending on the availability of water resource on their farms.

Materials and Methods

A multi-year study was initiated in 2018 on a five-acre subsurface drip irrigation cotton field located at Texas A&M AgriLife Research farm, Lubbock, TX.

Irrigation water level treatments. Two irrigation water levels (dryland and full irrigation) simulated two water-deficit production conditions, including high water-deficit (dryland condition) and no water deficit. A high-water treatment maintained >90% evapotranspiration replenishment through subsurface drip irrigation throughout the crop growing season, whereas the dryland treatment received pre-planting irrigation to facilitate proper seed germination and no additional irrigation. Cotton cultivar DP 1646 B2XF (seed with no insecticide or fungicide seed treatment) was planted on 31 May 2018.

Insect infestation treatments. Two key early-season insect-pest species (thrips and cotton fleahoppers) impacting cotton production risks were evaluated with five combinations of single versus sequential infestations under two water-deficit (zero versus high) regimes, replicated four times (total of 40 experimental plots). Targeted insect management options were achieved via artificial infestation of insect pests. Because Texas High Plains cropping conditions rarely warrant more than a single insecticide application to suppress either of the two major insect pest groups (thrips at seedling stage and cotton fleahopper at early squaring stage), our experiment was designed to infest our treatments at the most vulnerable stage of crop for the species infested.

Insect augmentation.

Thrips. Thrips were released to seedling cotton on 19 June 2018 when the crop was at 1-2 true leaf stage. Thrips infested alfalfa terminals were excised from a healthy alfalfa patch and these terminals were laid at the base of young cotton seedlings. Thrips were expected to move onto the cotton seedlings as excised alfalfa sections began to dry. Approximately 6 thrips per seedling were released to two 5 row-ft sections (approximately 12 plants per section) per plot (approximately 140 thrips per thrips-augmented plot). Thrips were released on all 16 thrips-augmentation plots (treatments #2 and #4 x 2 water levels x 4 replications) on the same day. Thrips were released on four additional plots to estimate thrips movement onto the cotton seedling via absolute sampling of seedlings and washing of thrips 3 days post-release. Data showed that the seedlings received an average of 1.2 live thrips per seedling which is the threshold density for 1-2 leaf stage seedling cotton.

Uncharacteristic high daytime temperatures for the next 7 days following the thrips release (103-107 ^{o}F) contributed to low thrips feeding performance and perhaps high thrips mortality after the thrips moved to the seedlings. Consequently, no visible signs of thrips-feeding effect were observed in thrips-augmented plots.

Cotton fleahoppers. Woolly croton, with embedded overwintering fleahopper eggs, was harvested from rangeland sites near College Station, Texas, in early February 2018 and then placed into cold storage. Eighty 1-gallon sheet metal cans, each containing 4 ounces of dry croton twigs per can, were initiated to generate the required number of cotton fleahopper nymphs for the experiment. Conditions conducive to cotton fleahopper emergence were simulated in a laboratory environment in order to induce hatching of overwintered eggs embedded in the croton stems, and emerged cotton fleahoppers were subsequently reared on fresh green beans. The single release of nymphal cotton fleahoppers (2nd instars) was timed to simulate the acute heavy infestation of cotton fleahoppers (4-5 days of feeding) while cotton was highly vulnerable to the fleahopper injury (1st week of squaring). The release was accomplished on 10 July by transferring second-instar fleahopper nymphs from the laboratory colony into 15 cm X 10 cm plastic

containers, then cautiously depositing them onto the terminals of plants in each treatment plot at the rate of 5 nymphs per plant. Immediately after cotton fleahoppers were released onto the fleahopper-augmentation plots (treatments #3 and #4; total 16 plots), control plots were sprayed with Orthene® 97. All treatment plots, except treatment #1, were sprayed with Orthene® 97 on 17 July and kept insect-free for the remainder of the study to isolate the effect of various treatments.

Parameters measured. The flowering profile was monitored from all 40 experimental plots for five sample dates (31 July, 6 August, 9 August, 15 August and 28 August 2018) to determine the effect of insect infestation and water deficit condition on fruiting delays and/or flowering patterns. Plant height was also recorded from all plots at the time of harvest. Hand harvesting was done on 16 November from flagged area and cotton was ginned on 17 December 2018. Lint will be sent to Cotton Incorporated for fiber analysis.

Results and Discussion

Extremely high temperatures during the seedling stage complicated the study in 2018, especially the released thrips failed to exert the desired significant infestation on the young cotton seedlings. As a result, thrips damage to seedlings was not apparent on visual observation. Cotton fleahoppers caused about 20% square loss overall across all experimental plots. Because cotton fleahoppers were released when plants had 2-3 total squares (all were fleahopper susceptible squares), the effect was not apparent immediately and plants outgrew the effect of early season fleahopper-induced square loss. Nevertheless, insect injury manifested some noticeable effect on flowering patterns, plant height, and lint yield.

Untreated control plots showed slightly higher flower densities in irrigated versus dryland cotton effect all throughout the month-long monitoring period, with significantly higher flower densities in late August. Contrasting to this phenomenon, the flowering patterns were near identical between irrigated and dryland plots when cotton fleahoppers were infested singly or sequentially with thrips infestation. The average flower abundance was significantly lower in dryland compared to that in irrigated cotton only at untreated control plots while all other treatments were not significantly different between the two irrigation regimes. These data suggest that the insect infestation during pre-flower stage exerts some significant physiological response to cotton during the flowering stage. Multi-year data will hopefully add more insights into this phenomenon.

Lint yield was significantly higher in irrigated cotton compared to that in dryland cotton across all five treatment combinations (Fig. 1). This suggests that the dryland plots were sufficiently water-stressed during the growing season, despite several rainfall events during the crop maturation phase in late September - early October. The highest lint yield under irrigation treatment was observed in the untreated control treatment (1,607 lb/acre), while the lowest (1,253 lb/acre) was recorded in the thrips+fleahopper sequential infestation treatment (Fig. 1). Lint yield in other treatments (spray control, thrips only, and fleahoppers only) did significantly differ from the untreated control or thrips+fleahopper sequential treatments (Fig. 1). Lint yield did not significantly vary across five insecticide augmentation treatments. As expected, the yield threshold in dryland cotton is much lower than that for irrigated cotton and thus the lower yield across all treatments can be partially attributed for lack of insecticide treatment effect on lint yield.

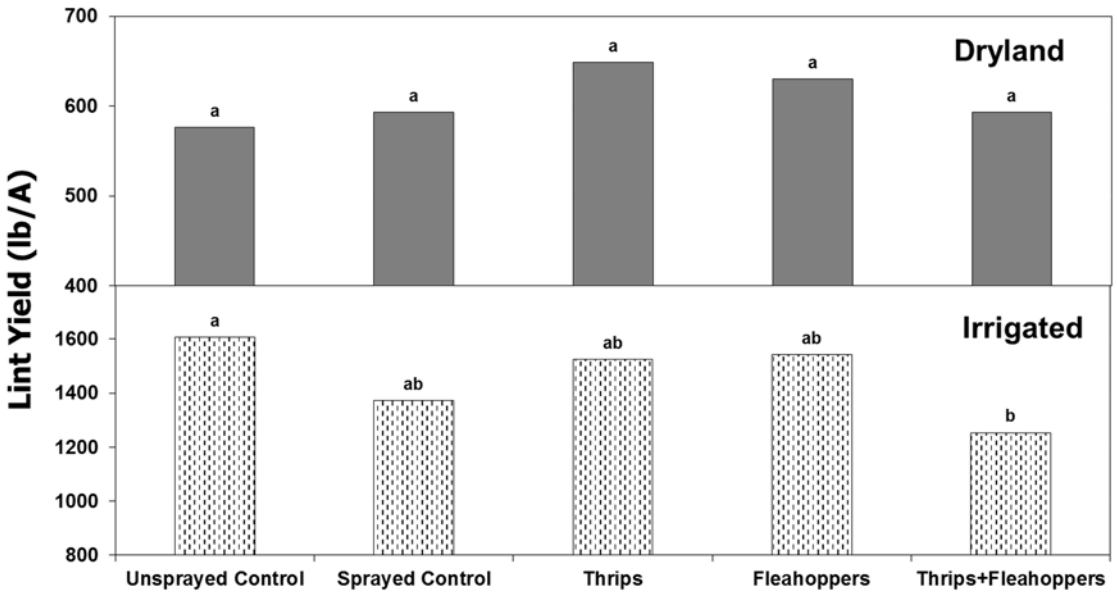

Figure 1. Cotton lint yield losses due to thrips and fleahopper infestations under dryland versus irrigated production conditions, Lubbock, Texas, 2018. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

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RESEARCH ARTICLE

Effects of transgenic *Bacillus thuringiensis* cotton on insecticide use, heliothine counts, plant damage, and cotton yield: A meta-analysis, 1996-2015

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Abstract

The primary management tactic for lepidopteran pests of cotton in the United States of America (USA) is the use of transgenic cotton that produces *Bacillus thuringiensis* Berliner (*Bt*) toxins. The primary target pests of this technology are *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.) in the eastern and central Cotton Belt of the USA. Concerns over the evolution of resistance in *H. zea* to *Bt* toxins and scrutiny of the necessity of *Bt* crops has escalated. We reviewed published and unpublished data from field trials of *Bt* cotton in the eastern and central Cotton Belt of the USA through 2015 to evaluate the effectiveness of *Bt* cotton (Bollgard, Bollgard II, WideStrike, WideStrike 3, and TwinLink). *Bt* cotton reduced insecticide usage, reduced heliothine pest numbers and damage, and provided a yield benefit, but Bollgard II and WideStrike efficacy declined in the Midsouth over the period evaluated. In the Southeastern region, heliothine damage remained constant through 2015, but yield benefits declined from 2010 until 2015. Resistance of *H. zea* to several *Bt* toxins is the most plausible explanation for the observed changes in *Bt* cotton efficacy. The introduction of new *Bt* toxins such as found in Widestrike 3 and Twinlink may preserve the benefits of *Bt* crops. However, while both Widestrike 3 and Twinlink had less damage than Widestrike, damage levels of both were similar to Bollgard II.

in the form of salary for one author (DF). NL was the USDA representative overseeing the agreement who participated as a co-author from idea development through paper review. Additional support came from Mississippi State University Hatch Project MIS-311280 for the support of FM.

Competing interests: While the agriculture industry did not fund the analysis presented in this manuscript, all the authors affiliated with a university routinely conduct research for Monsanto, Bayer CropSciences and Dow Agrosciences as well as other agrichemical companies. Most of the trial data reported in this manuscript was gathered as part of trials that were funded by these biotechnology companies. DF was a post-doc at Mississippi State University when most of the work was done and is currently employed by Provivi, Inc. Beyond providing his salary, Provivi, Inc did not have any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. These competing interests do not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Introduction

Bt crops

Lepidopteran insect control in transgenic crops is accomplished through the insertion of genes from the bacterium *Bacillus thuringiensis* Berliner (*Bt*). These genes encode for proteins with insecticidal activity in the midgut of targeted insect species. Five types of transgenic *Bt* cotton (*Gossypium hirsutum* L.) were commercialized between 1996 and 2015 in the United States (Table 1). In 2015, there were approximately 3.1 million hectares of cotton grown in Texas, the Midsouth and the Southeast combined (Fig 1), with transgenic *Bt* cotton planted on approximately 2.2 million hectares [1].

The primary pests targeted for control with *Bt* cotton in these regions are the heliothine species *Helicoverpa zea* (Boddie) (bollworm, corn earworm) and *Heliothis virescens* (F.) (tobacco budworm). These pests damage cotton by feeding primarily on and within the fruiting structures. Newly hatched *H. zea* and *H. virescens* larvae feed on plant terminals, then move to small squares, then larger squares, then bolls [2]. Estimates of insecticide usage and damage losses associated with these species following the introduction of *Bt* cotton (data from 1986–1995 compared to 1996–2015) were reduced by 61% and 47%, 79% and 60%, and 81% and 63%, respectively, in the Midsouth, Southeast, and Texas, respectively. [1] (Fig 2).

Many of the same *Bt* genes have been introduced into corn to control various lepidopteran pests, including *H. zea*. This technology has been widely accepted by corn growers, grown on 81% of the area planted to corn in the U.S. in 2015 [3]. *Bt* corn was also commercially introduced in 1996, so exposure to the *Bt* toxins in both crops has occurred simultaneously.

Helicoverpa zea is a pest of both cotton and corn, and populations of *H. zea* may spend as many as four generations per year in these crops [4–6]. Populations occurring in areas where *Bt* corn and cotton are both grown are potentially exposed to the Cry1A, Cry1F, Cry2A, and Vip3A toxins in both crops. Corn is grown on approximately 3.4 million hectares in the eastern and central Cotton Belt, and the Environmental Protection Agency (EPA) mandates a planted refuge of non-*Bt* corn consisting of 50% or 20% of corn acres in cotton growing regions for single and multi-gene *Bt* corn varieties, respectively [7]. These refuge requirements are in place to slow resistance of pests to the *Bt* toxins; however, as few as 40 percent of growers adhere to the refuge requirements [8, 9], potentially resulting in the production of fewer susceptible individuals than desired for resistance management.

Concerns over resistance to *Bt* technology

Simulation models indicated that *H. zea* resistance to single-gene *Bt* crops could occur within 7 to 30 years [5, 10–13], while dual-gene crops would be expected to last longer [13]. The pyramiding of multiple toxins and a refuge strategy were implemented to slow the development of resistance of the major target pests to *Bt* crops [14–18]. Thus far, field-evolved *Bt* resistance has not been documented for *H. virescens*; however, laboratory selection of a Cry1Ac resistant

Table 1. Cotton technologies with transgenes from *Bacillus thuringiensis* Berliner (*Bt*) commercialized in the United States, 1996–2015.

Technology	Year of commercial availability	*Bt* transgene(s)	Event
Bollgard	1996	Cry1Ac	Mon531
Bollgard II	2003	Cry1Ac, Cry2Ab	Mon15985
WideStrike	2005	Cry1Ac, Cry1F	3006-210-23 + 281-24-236
TwinLink	2014	Cry1Ab, Cry2Ae	T304-40 + GHB119
WideStrike 3	2015	Cry1Ac, Cry1F, Vip3A	3006-210-23 + 281-24-236 + Cot102

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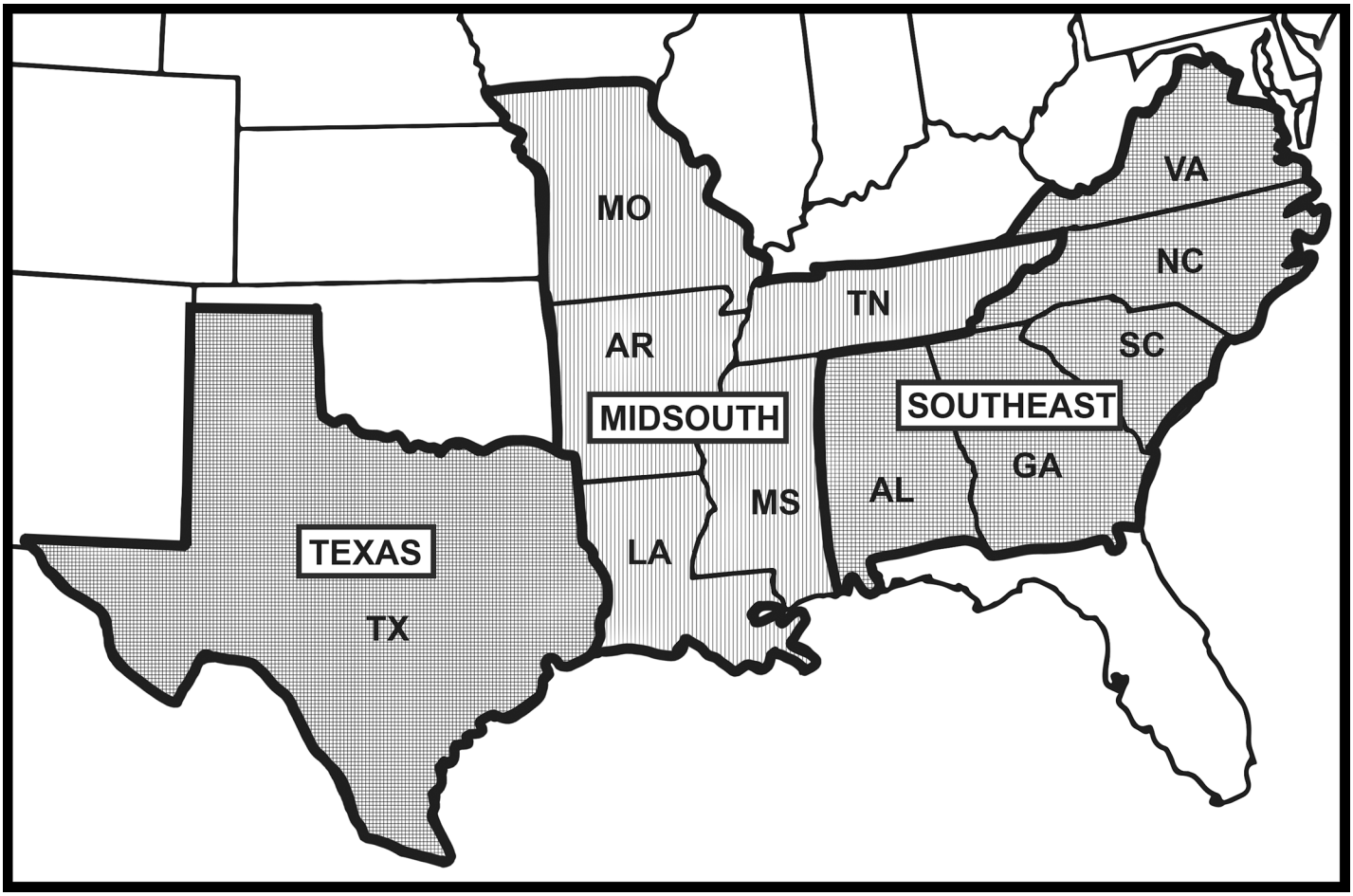

Fig 1. Map of the eastern and central Cotton Belt of the United States indicating the regions and states of trial locations used for analyses in this paper.

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colony has occurred [19]. Field-evolved resistance in populations of *H. zea* has been documented for Cry1Ab, Cry1Ac, and Cry1A.105+ Cry2Ab toxins in several locations [20–24].

Several factors may be solely or cumulatively responsible for *H. zea* resistance, including exposure of multiple generations of *H. zea* per year to *Bt* toxins in corn and cotton, lack of compliance with EPA mandated refuge requirements, exposure to the same *Bt* genes for many years, cross resistance to multiple *Bt* toxins, and the failure to express *Bt* at a high-dose from the outset [18]. Cry1Ab and Cry1Ac genes were the first *Bt* toxins commercially available and they are still found in most varieties of *Bt* corn and cotton after 20 years. The second *Bt* gene introduced for lepidopteran control in corn during 2001 and cotton during 2003 was Cry1F, and this gene also remains in many commercially available cotton and corn varieties. None of these toxins were ever considered to express a high-dose against *H. zea* [18, 25, 26]. Further increasing the likelihood of resistance development, various levels of cross-resistance to numerous Cry toxins has been documented in *H. zea* [11, 27, 28] as well as other Lepidoptera [26, 29, 30]. However, cross resistance to *Bt* toxins is not found in all studies [31]. Caprio [32] showed cross resistance has a negative impact on all resistance management strategies, but Caprio et al. [33] found that partial cross-resistance was of minor importance compared to refuge size in the evolution of resistance. The implications of continued exposure of *H. zea* to

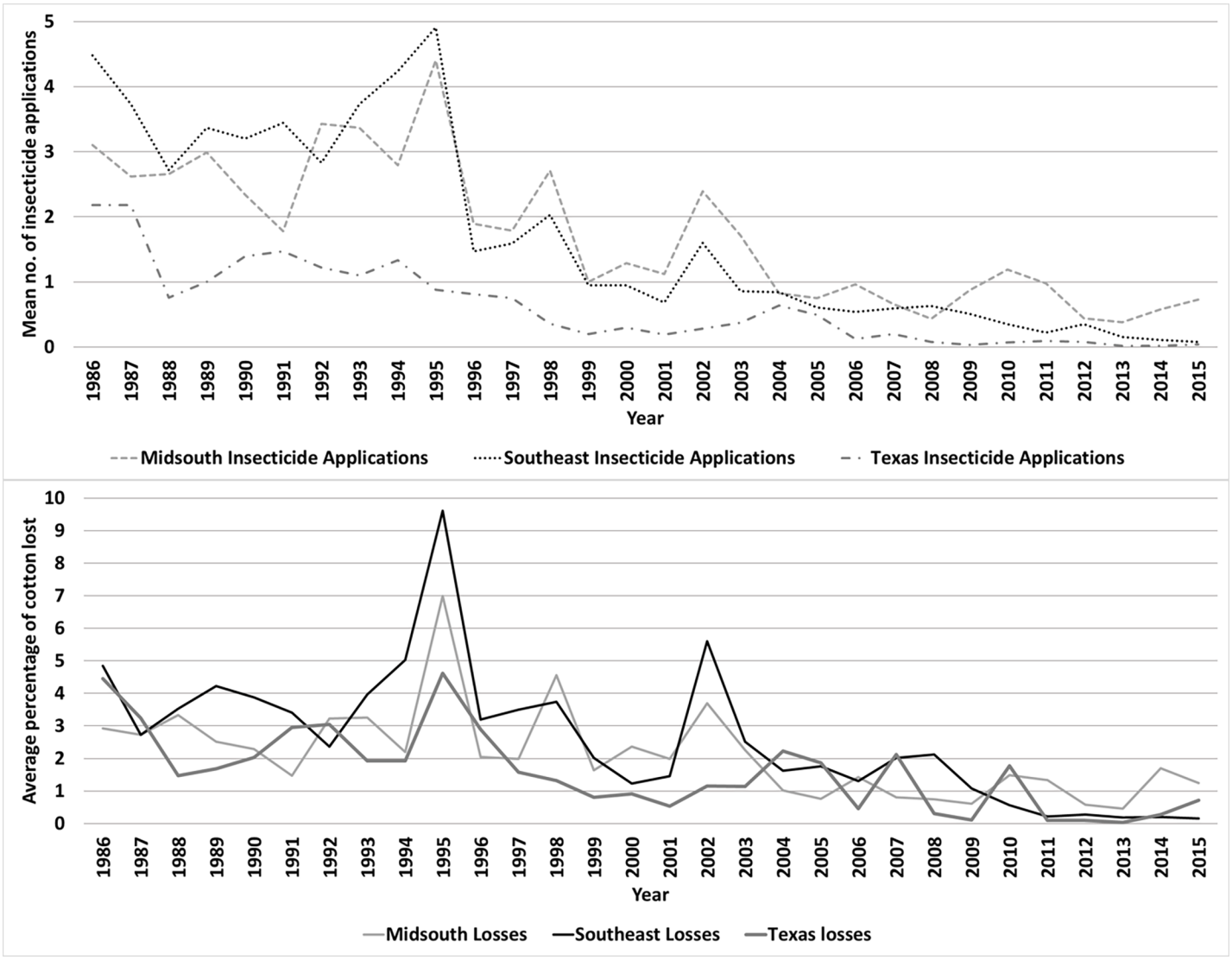

Fig 2. Changes in insecticide applications and yield losses in cotton due to heliothine infestations in the eastern Cotton Belt of the United States, 1986–2015. Compiled from Williams [1].

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similar *Bt* toxins in multiple crops is not fully known, but all these studies suggest that declining efficacy of these toxins against *H. zea* should be expected.

Need for a meta-analysis

Evaluations of *Bt* cotton efficacy on lepidopteran pests has typically involved laboratory experiments with meridic diet or plant expressed protein and insect colonies from rearing facilities. Only six refereed articles [34–39] involving replicated field experiments and natural heliothine populations in the USA have been published. These experiments are important because they validate laboratory research in biologically relevant situations, revealing the strengths and weaknesses of *Bt* cotton in a range of environments and pest densities. The use and benefits of *Bt* cotton is complex when considering the differences in environment, pest populations, and IPM strategies across the country, and as a result, data from field experiments are highly

variable or "noisy" on an individual basis [40]. Compiling large numbers of experiments together in a meta-analysis increases the precision of estimation, allowing researchers to detect small changes in susceptibility or other variables that are not possible with individual experiments [41, 42].

Five of the published field studies evaluated Cry1Ac (Bollgard), three evaluated Cry1Ac + Cry2Ab2 (Bollgard II), and one evaluated Cry1F + Cry1Ac (WideStrike). All experiments occurred between 1998 and 2003. The findings of these papers showed that *Bt* cotton reduced lepidopteran populations and the damage they cause and that this reduction further improved with the introduction of dual-gene technology. It has been nine years since the last refereed paper was published, and over fourteen years since the experiment was conducted. Since then, two *Bt* cotton technologies with three *Bt* genes new to cotton have been made commercially available (Table 1). Reduced efficacy of the older, single-gene technology has not been empirically demonstrated in field trials, nor has the efficacy of the older dual-gene technologies (Bollgard II and WideStrike), and the new dual- (TwinLink) and triple-gene (WideStrike 3) technologies been compared across multiple cotton growing regions. The results of this study will be important in predicting the longevity and benefits of the recently commercialized TwinLink Plus and Bollgard 3 technologies.

Objectives

Our primary objective is to summarize transgenic *Bt* cotton efficacy and yield data produced from 1996 to 2015 in field experiments that used natural heliothine populations in the USA. Trial locations ranged from Virginia to Texas, as these are the cotton production regions that frequently experience *H. zea* feeding. We used data from trials making threshold-based insecticide applications to assess the impacts of *Bt* technology on insecticide usage. Additionally, we used trials where insecticides targeting heliothines were not applied, to determine if changes in efficacy or yield have occurred over time and to compare efficacy and yield of various *Bt* and non-*Bt* varieties.

Methods

Compiling the dataset

Articles containing information on *Bt* cotton used in field experiments were identified using a combination of the terms *Bacillus thuringiensis*, *Gossypium hirsutum*, and one of the following: *Helicoverpa zea* or *Heliothis virescens*. Searches were conducted in Google Scholar, EBSCO through the Mississippi State University Library Discovery Service, Oxford University Press, Science Direct, Scopus, PubMed, BioOne, ISI Web of Knowledge, and the Proceedings of the Beltwide Cotton Conferences. Searches were limited to articles published no earlier than 1996. Article citations were imported into EndNote (v. X5.0.1, Thomson Reuters, www.endnote.com) and titles and abstracts were read to determine if the article contained data relevant to our objectives. Data were used if the trials included a non-*Bt* and a commercialized *Bt* variety, were conducted in a field setting, relied upon natural heliothine populations, provided a measure of variance, and if the number of observations could be determined from the information provided. Additional information was requested from authors if information in the article was insufficient or needed further clarification. In addition to these published articles, current university research and Extension Service entomologists working with cotton in the target regions were asked to provide unpublished data that met the same requirements. Researchers supplying unpublished data were asked for clarification of data they provided if information was lacking. Data that were still in doubt regarding their use in this study was ignored. All appropriate data were placed into a database for statistical analysis. While not a requirement, all but

three sources of data used in the analysis were from university and private company sponsored research plots. Fig 3 shows the PRISMA Flow Diagram. The data used for meta-analysis can be found in the Mississippi State University Institutional Repository (http://hdl.handle.net/11668/14199). A Prisma checklist was included as supplemental information to the journal (S1 Fig) [43].

Data collected included the state, city and year of the research, the type and frequency of insecticide usage for heliothine pests, the plant part(s) evaluated, yield, type of evaluation (heliothine counts, plant damage, and cotton yield), mean values, number of observations, and a measure of variance. Insecticide application types were separated as blanket sprays (same insecticide, rate, and number of applications were used over both *Bt* and non-*Bt* varieties), threshold sprays (*Bt* and non-*Bt* varieties were treated independently as pests reached the threshold for each technology), or none (no insecticide was used to manage heliothines). The threshold used was based on larval density or fruit damage as recommended by the extension service where the trial was conducted. The *Bt* and non-*Bt* varieties were not necessarily genetically related but were varieties that had similar maturities and growth habits. The specific varieties compared are listed in the repository. The larvae of *H. zea* and *H. virescens* are difficult to distinguish in field settings [44, 45], therefore, very little species-specific information was available to allow our study to evaluate the effects of *Bt* technologies separately for these two species.

Statistical analyses

The sources of reported data and numbers of observations for each technology were calculated in SAS Proc Tabulate (SAS Institute, Cary, NC, USA). Data from trials conducting threshold insecticide applications were used to evaluate the extent of insecticide reduction between *Bt* technologies (Bollgard, Bollgard II, and WideStrike; data for TwinLink and WideStrike 3 were insufficient for analysis) and non-*Bt* varieties. Differences in insecticide usage were calculated using the formula:

Number of applications for technology1—Number of applications for technology$_2$ Differences were analyzed as paired t-tests (SAS Institute, Cary, NC, USA). Pairs were made whenever both technologies were tested within the same trial. For the remaining analyses, only data from trials not using foliar insecticide to manage heliothine pests were used.

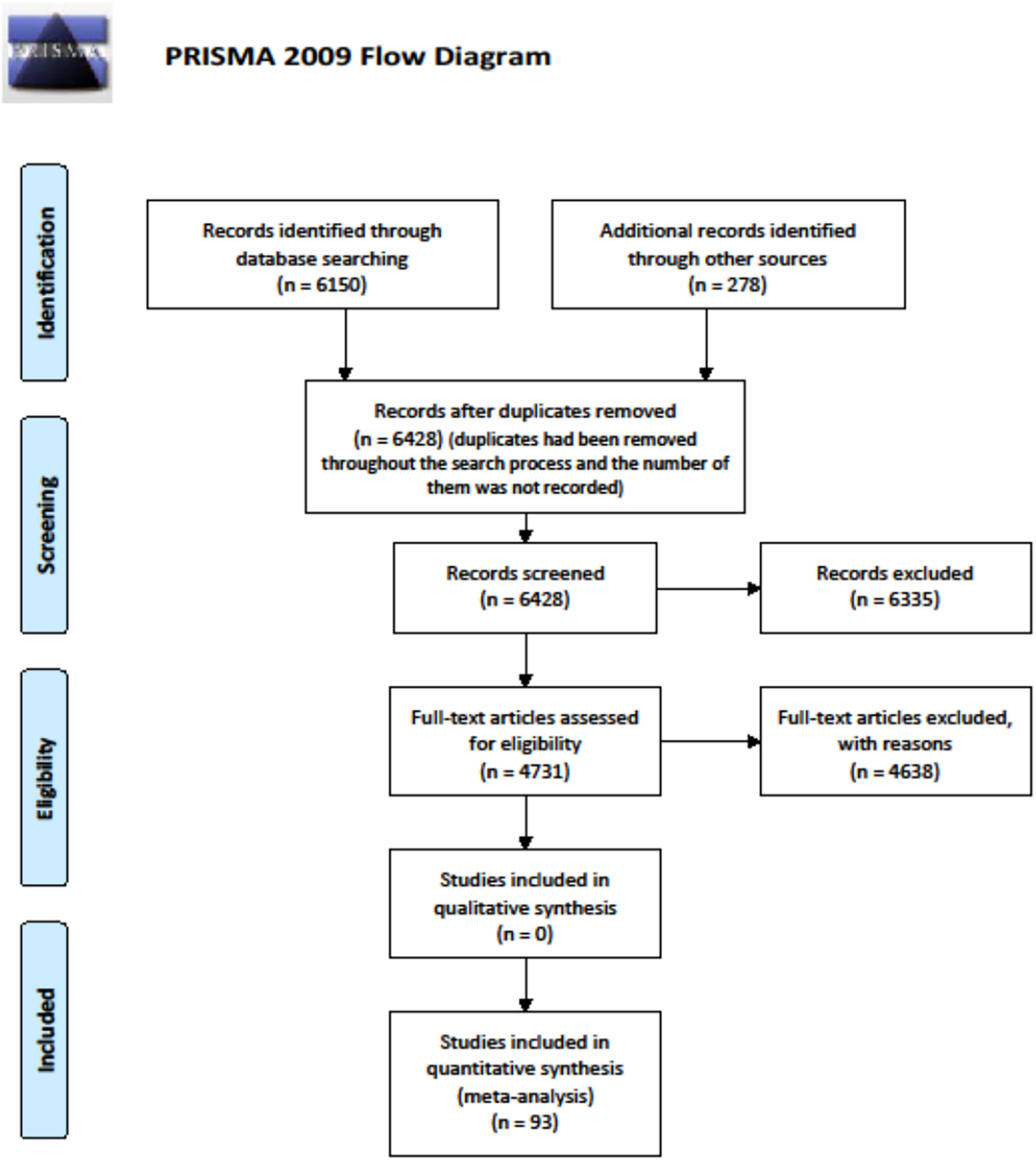
Data evaluating heliothine counts, plant damage, and yield comparisons of *Bt* to non-*Bt* cotton included results from separate studies that varied over a wide range in values. Various metrics of effect size are used in meta-analysis in order to convert these measurements to a common scale. The log response ratio [46, 47] is recommended where the outcome expresses the magnitude of the response to an experimental treatment by comparing to an experimental control group. The log response ratio (RR) and the scaled sampling variance of this metric (V_{RR}) are defined as follows:

$$RR = \ln\left([\text{Mean}_{Bt\ \text{value}} + 1]/[\text{Mean}_{\text{non}-Bt\ \text{value}} + 1]\right)$$

$$V_{RR} = (\text{Standard Error}_{Bt\ \text{value}})^2/(\text{Mean}_{Bt\ \text{value}} + 1)^2 + (\text{Standard Error}_{non-Bt\ \text{value}})^2/(\text{Mean}_{non-Bt\ \text{value}} + 1)^2$$

We modified the original formulas to use Mean + 1 in place of a mean. In some cases, the mean was zero or close to zero which caused problems when dividing by zero or a very small number.

To estimate overall means for the log response ratio and detect what factors might affect this ratio, analysis of variance was performed using a general linear mixed model (PROC GLIMMIX, SAS Institute, Cary, NC USA). Data were initially analyzed without using any weighting method but this was rejected because the quality of the V_{RR} data available from



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement.* PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

Fig 3. The PRISMA flow diagram[43].

https://doi.org/10.1371/journal.pone.0200131.g003

some studies was much better than from other studies. This was generally not a reflection of sample size, but of the statistics available to estimate V_{RR}. Secondly, the inverse V_{RR} weighting method [48] was tested. This weighting method was also rejected because weights varied by more than 1000 times in some comparisons, giving an excessive amount of weight to a small number of studies.

As a compromise between no weighting and the inverse V_{RR} weighting method, the V_{RR} were sorted from low to high and assigned a weight from 1 to 5 based on their rank. Those trials having the smallest 20% of V_{RR} were assigned a weight of 5. Those trials in the second lowest 20% were given a weight of 4 and so on, so that the 20% of observations with the largest V_{RR} were given a weight of 1. While we are not aware of this weighting system being used previously, it is basically a scaled version of the commonly used inverse V_{RR} weighting system so that no individual trial counts more than five times more than the poorest trial in the analysis.

As mentioned above, there were limited data available to estimate the V_{RR} of some trials. An estimate of variance was needed to calculate V_{RR} and these estimates were difficult to obtain from some studies. Variances were estimated for each *Bt*: non-*Bt* and *Bt*: *Bt* comparison by determining a standard error of the difference (SE diff) for each comparison. The SE diff for data using the least significant difference (LSD) values to estimate variance was calculated as LSD/t-value. The SE diff for data using standard deviation (SD) to estimate variance was calculated as SE diff = $(([\text{technology}_1\ SD^2] / [\text{technology}_1\ n]) + ([\text{technology}_2\ SD^2] / [\text{technology}_2\ n]))^{0.5}$. The SE diff for data using standard error (SE) to estimate variance was calculated as SE diff = $([\text{technology}_1\ SE^2] + [\text{technology}_2\ SE^2])^{0.5}$.

Data from trials pre-dating commercial availability of each technology (Table 1) were excluded from analyses as any changes prior to commercialization would be due to agronomic factors, and not *Bt* toxin effectiveness. Overall differences in response to the technologies were evaluated (reported as overall intercept in S6 Table). In addition, the main effects evaluated for heliothine counts and damage were plant part, region, and year, and the main effects evaluated for yield were region and year. The interaction of year and plant part was evaluated for heliothine counts and plant damage, and the interaction of year and region was evaluated for heliothine counts, damage and yield. The three-way interaction of year, plant part, and region and the two-way interaction of plant part and region were not analyzed because of a lack of data. Analyses for all main effects were done independently (i.e. the impact of plant part was not tested in the same analysis as region) since the data that met the requirement for each analysis differed. For analyses, regions and plant parts not having at least five observations were excluded from analyses involving their respective effects. Year was analyzed as a continuous variable with linear and quadratic terms. The value of year was set as year of study—1995. To analyze year as a factor, there needed to be at least 3 observations for each of 5 years (but not necessarily consecutive years). This requirement meant that year could not be analyzed for WideStrike 3 and TwinLink as they had not yet been commercialized for 5 years by 2015. Years occurring at either end of the tested time scale with less than 3 observations were deleted. To test the interaction of region or plant part with year required a region or plant part to have at least five years of data with at least three observations per year. As a result, many of the year interactions included only two regions or plant parts due to insufficient data for one or more regions or plant parts. Least square means for technology comparisons were separated using Fisher's Protected Least Significant Difference test (LSD) ($\alpha = 0.05$). Significant regressions over time were simplified by removing the non-significant terms from the final equation. Data were tested for normality of distribution and examined for outliers more than three standard

deviations from the predicted value. Nine comparisons were identified as outliers for one or more models. All outliers were for Bollgard to non-*Bt* or Bollgard 2 to non-*Bt* comparisons. All outliers occurred prior to 2009 and the *Bt* technology was always more effective than predicted by the model. Five of these outliers were from a single trial in Texas in 2004 when insect damage was high in the non-*Bt* plots, but no damage was observed in the Bollgard and Bollgard II plots. These outliers were deleted from the data set so that the analysis would not be skewed by these rare circumstances. The number of data points omitted was never more than 5% of the total number of data points analyzed for any comparison. Multiple regression was used initially for analysis, but due to a paucity of data in numerous areas, was not used because results of several factors were frequently driven by one or two trials.

Results

Literature review

Over 6,000 articles were examined for inclusion in this study. The articles (refereed or otherwise) used are listed in S1 Table. There were 910 comparisons of *Bt*: non-*Bt* cotton and 523 comparisons of *Bt* technologies to one another (S2 and S3 Tables). Additionally, 1,293 *Bt*: non-*Bt* comparisons and 915 comparisons of *Bt* technologies were collected from unpublished sources (S1–S3 Tables). Overall, 63%, 32% and 5% of the data were from the Midsouth, Southeast and Texas, respectively. No data for TwinLink or WideStrike 3 were available from Texas. The number of comparisons of *Bt*: non-*Bt* and *Bt*: *Bt* for heliothine counts, damage and cotton yield are given in S4 and S5 Tables.

Threshold-based insecticide usage

Data from comparisons with insecticide targeting heliothines on a threshold basis were used to determine the extent of the reduction of insecticide usage resulting from using *Bt* cotton. Data from Bollgard, Bollgard II, and WideStrike were available. The use of these technologies reduced insecticide usage by 1.3 to 2.6 applications (Table 2) relative to non-*Bt* cotton. Bollgard II reduced insecticide usage by approximately 1.1 applications when compared to Bollgard and 0.8 applications when compared to WideStrike (Table 2).

Efficacy comparisons

Comparisons of *Bt* cotton to non-*Bt* and other *Bt* cotton types were conducted to determine the extent of reduction of heliothine counts and damage, how efficacy of *Bt* technologies compared to each other, and how yield was affected (S6 Table). Bollgard, Bollgard II, WideStrike, and TwinLink reduced heliothine infestations relative to non-*Bt* by 49% ($p<0.0001$), 61.8%

Table 2. Paired t-test comparisons of insecticide applications based on larval thresholds for heliothine pests in trials in the eastern and central Cotton Belt of the United States for *Bt* and non-*Bt* cotton.

			Mean ± SE of the number of insecticide applications		Mean ± SE of the number of insecticide applications reduced			
						t-test results		
Technology 1	Technology 2	Study Years	Technology 1	Technology 2		df	t	p
Non-*Bt*	Bollgard	96–09	3.5 ± 0.2	2.1 ± 0.2	1.3 ± 0.1	61	9.7	<0.01
Non-*Bt*	Bollgard II	04–10	3.8 ± 0.5	1.3± 0.4	2.6 ± 0.4	17	7.3	<0.01
Non-*Bt*	WideStrike	06–11	3.5 ± 0.5	1.5 ± 0.4	2.0 ± 0.3	21	6.3	<0.01
Bollgard	Bollgard II	04–09	1.7 ± 0.7	0.6 ± 0.2	1.1 ± 0.4	8	2.5	0.04
WideStrike	Bollgard II	06–10	2.5 ± 0.5	1.6 ± 0.6	0.8 ± 0.3	12	3.2	<0.01

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(p<0.0001), 47.4% (p<0.0001), and 69.3% (p<0.0001), respectively. Bollgard II reduced heliothine infestations 17.9% more than WideStrike (p<0.0001) and 38.2% more than TwinLink (p = 0.004) (Fig 4). Bollgard, Bollgard II, WideStrike, WideStrike 3, and TwinLink reduced damage relative to non-*Bt* by 70%, 81%, 68%, 80%, and 72%, respectively (p<0.0001 for all technologies). Bollgard II reduced damage 47% more than Bollgard (p<0.0001), 33% more than WideStrike (p<0.0001), and 23% more than TwinLink (p = 0.010); TwinLink reduced damage 35% more than WideStrike (p<0.0001); WideStrike reduced damage 21% more than Bollgard (p = 0.015); and WideStrike 3 reduced damage 39% more than WideStrike (p<0.0001) (Fig 5). Bollgard, Bollgard 2, WideStrike, WideStrike 3, and TwinLink all improved yield relative to non-*Bt* by 44% (p<0.0001), 60% (p<0.0001), 54% (p<0.0001), 23% (p = 0.004), and 65% (p = 0.0002), respectively. Bollgard II and TwinLink had a higher yield than WideStrike of 7% (p = 0.0002) and 12% (p = 0.0003), respectively, and WideStrike 3 had a 13% higher yield than Bollgard II (p = 0.034) and 8% higher yield than TwinLink (p = 0.005) (Fig 6).

Bt to non-*Bt* comparison: Effects of year, region, and plant part on heliothine counts and damage

The main effects of year, region, and plant part and interactions of year with plant part and year with region were evaluated to determine if changes in *Bt* efficacy have occurred over time or if

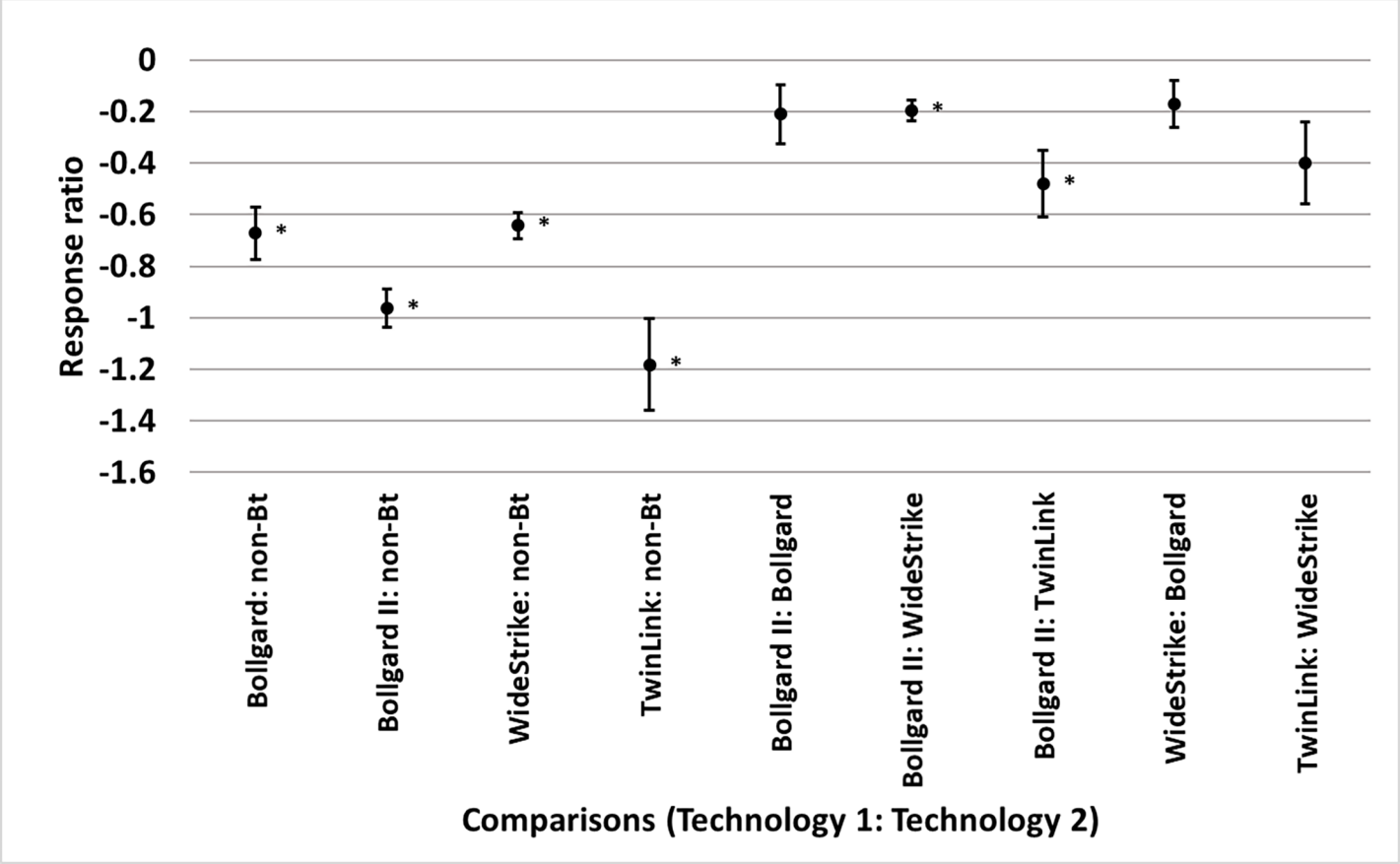

Fig 4. Least square mean ± SE of the response ratio of heliothine counts among comparisons of transgenic *Bacillus thuringiensis* (*Bt*) and non-*Bt* cotton in trials from the eastern and central Cotton Belt of the United States. Response ratio = ln ([Technology 1 $mean_x$ + 1] / [Technology 2 $mean_x$ + 1]). Comparisons marked by * indicate the technologies differed (t-test, p<0.05).

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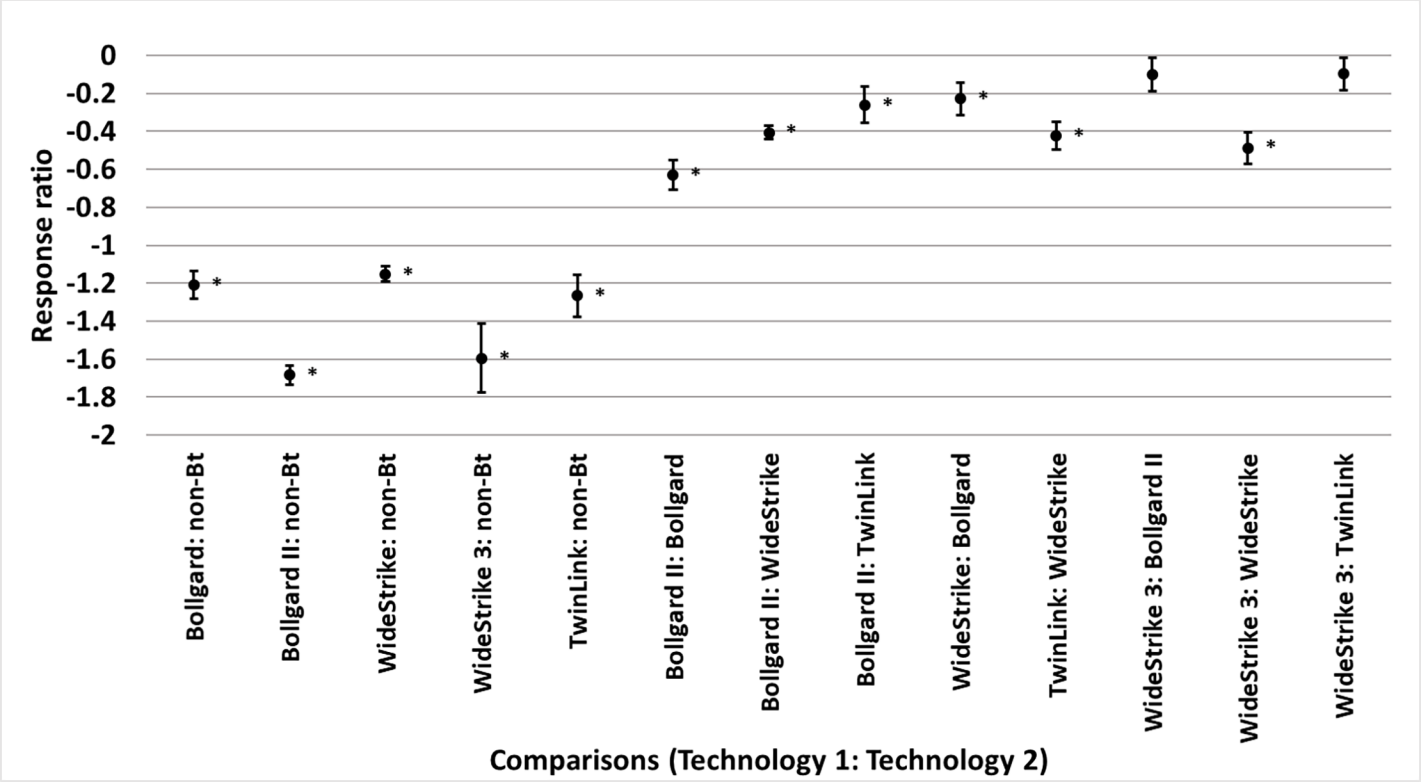

Fig 5. Least square mean ± SE of the response ratio of damage among comparisons of transgenic *Bacillus thuringiensis* (*Bt*) and non-*Bt* cotton in trials from the eastern and central Cotton Belt of the United States. Response ratio = ln ([Technology 1 $mean_x$ + 1] / [Technology 2 $mean_x$ + 1]). Comparisons marked by * indicate the technologies differed (t-test, $p<0.05$).

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efficacy is different for plant parts or regions (S6 Table). There was an interaction of year and region for Bollgard II ($p<0.01$) and WideStrike ($p<0.01$) heliothine counts. The Midsouth had an increase in heliothine numbers collected from both Bollgard II and WideStrike relative to non-*Bt* as time progressed (Fig 7). Heliothine counts in the Southeast increased over time in Bollgard II and WideStrike relative to non-*Bt*; however, after 2010 counts began decreasing (Fig 7).

There was an interaction of year and region for Bollgard II ($p<0.01$) and WideStrike ($p<0.01$) damage. As time progressed, damage increased for both technologies in the Midsouth compared to non-*Bt*, but there was not a change in damage for either technology in the Southeast (Fig 8). Region influenced Bollgard ($p = 0.040$) and WideStrike 3 ($p = 0.007$) damage. Damage in Bollgard relative to non-*Bt* was reduced by 65% in the Midsouth compared to 74% and 77% in the Southeast and Texas, respectively (Fig 9). Damage in WideStrike 3 relative to non-*Bt* was reduced by 89% in the Southeast compared to 71% in the Midsouth. Plant part influenced the amount of damage reduction provided by Bollgard ($p = 0.045$) and Bollgard II ($p = 0.022$) technologies relative to non-*Bt*. Damage in Bollgard was reduced less on flowers (48%) than on bolls (72%) and squares (75%) (Fig 9). Damage in Bollgard II was reduced less on flowers (74%) than on bolls (83%) and squares (83%) and damage on terminals (77%) was reduced less than damage on bolls (83%) (Fig 10).

Bt to non-*Bt* comparisons: Effects of year and region on yield

The main effects and interaction of year and region were evaluated to determine if changes in yield of *Bt* technologies occurred over time or if yield was affected by region (S6 Table). There

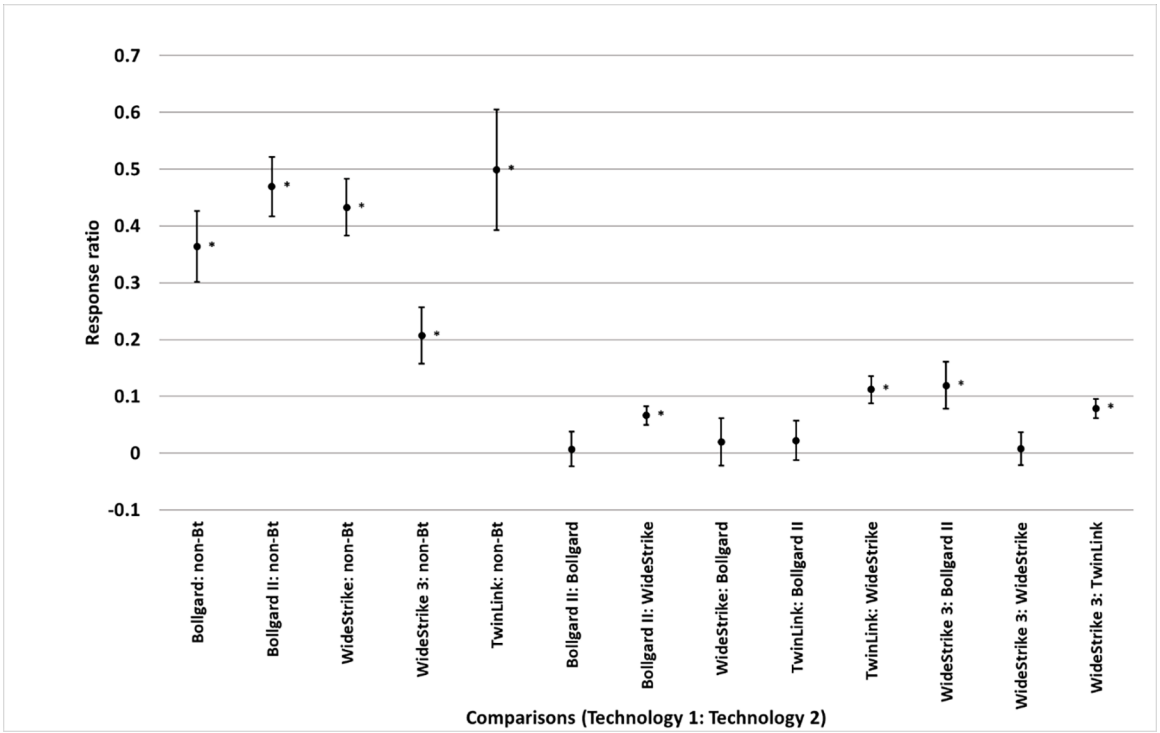

Fig 6. Least square mean ± SE of the response ratio of yield among comparisons of transgenic *Bacillus thuringiensis* (*Bt*) and non-*Bt* cotton in trials from the eastern and central Cotton Belt of the United States. Response ratio = ln ([Technology 1 $mean_x$ +1] / [Technology 2 $mean_x$ + 1]). Comparisons marked by * indicate the technologies differed (t-test, $p<0.05$).

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was an interaction of year and region for Bollgard II (Fig 11). The yield benefit over non-*Bt* cotton initially increased in both regions, but then began to decline beginning around 2010. This is consistent with the increased heliothine counts and damage observed in the Midsouth. WideStrike yields followed a similar trend (Fig 12). Region influenced Bollgard yield relative to non-*Bt* cotton ($p = 0.0415$). Yield increase of Bollgard was greater in the Southeast (73%) than in the Midsouth (25%) (Fig 13).

Effects of plant part and region on *Bt* technologies

The main effects of year, plant part, and region were evaluated to compare heliothine counts, damage and yield between *Bt* technologies (S6 Table). Year influenced heliothine counts ($p<0.01$) and damage ($p = 0.03$) in the Bollgard II: WideStrike comparison. Over time, the difference between Bollgard II and WideStrike increased for both heliothine counts and damage (Fig 14) as efficacy declined more rapidly in Widestrike than in Bollgard II. Plant part influenced the damage difference observed between Bollgard II and Bollgard. Damage reduction by Bollgard II compared to Bollgard was 54% on bolls and 31% on squares (Fig 15). Relative performance of comparisons between different *Bt* technologies varied by region for damage. Damage was reduced by 41% in the Southeast and 30% in the Midsouth in Bollgard II compared to WideStrike ($p = 0.039$), by 41% in the Southeast and 11% in the Midsouth in Bollgard II compared to TwinLink ($p = 0.036$), by 49% in the Southeast and 28% in the Midsouth in TwinLink compared to WideStrike ($p = 0.034$), and 55% in the Southeast and 28% in the Midsouth in Widestrike 3 compared to WideStrike ($p = 0.006$) (Fig 16). Region influenced the Bollgard II: WideStrike comparison of yield with Bollgard II having a greater yield benefit (13%) in the Midsouth than in the Southeast (3%) relative to WideStrike ($p = 0.006$) (Fig 17).

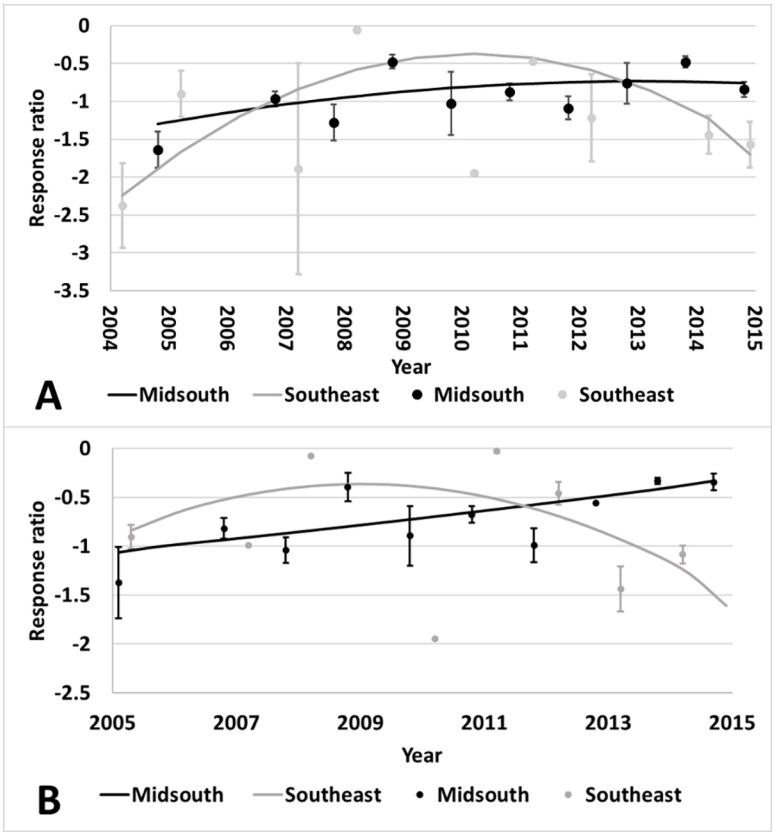

Fig 7. Change over time of heliothine counts in Bollgard II (A) and WideStrike (B) cotton by region of the eastern and central Cotton Belt of the United States. Bollgard II Midsouth equation: 0.0429x - 1.5111, Southeast equation: 1.569x - 0.05243x2–12.1179; WideStrike Midsouth equation: 0.0750x - 1.8438, Southeast equation: 0.9008x - 0.03258x2–6.5835. Response ratio (A) = ln ([Bollgard II $mean_x$ + 1] / [non-*Bt* $mean_x$ + 1]); Response ratio (B) = ln ([WideStrike $mean_x$ + 1] / [non-*Bt* $mean_x$ + 1]).

https://doi.org/10.1371/journal.pone.0200131.g007

Discussion

Literature review

This paper reviewed published literature from 20 years of commercialized use of *Bt* cotton technologies; however, only six refereed articles fit the criteria for use in this paper and these data all occurred within the first 7 years of *Bt* cotton commercialization in the USA. The remainder of the data were from non-refereed sources or were unpublished data from university entomologists. The review revealed that although a large body of field-based *Bt* research exists, most of the information has not been subjected to peer-review. The primary reason for this is that many *Bt* field trials are stand-alone experiments and would not be appropriate for peer-review publications but fit well into report style publications such as the Proceedings of the Beltwide Cotton Conferences, Arthropod Management Tests, or Extension Service bulletins. However, the scrutiny of genetically modified crops, including *Bt* technologies, is increasing, and having more refereed, field-validated data will become increasingly important.

Texas accounted for only 5 percent of the data in this analysis; however, approximately 50 percent of the United States cotton acreage is in Texas [1]. Heliothine severity in Texas is lower than in the Midsouth and Southeast [1] and *Bt* technologies have provided exceptional suppression of heliothines. Therefore, less research on *Bt* cotton efficacy has been conducted in this region.

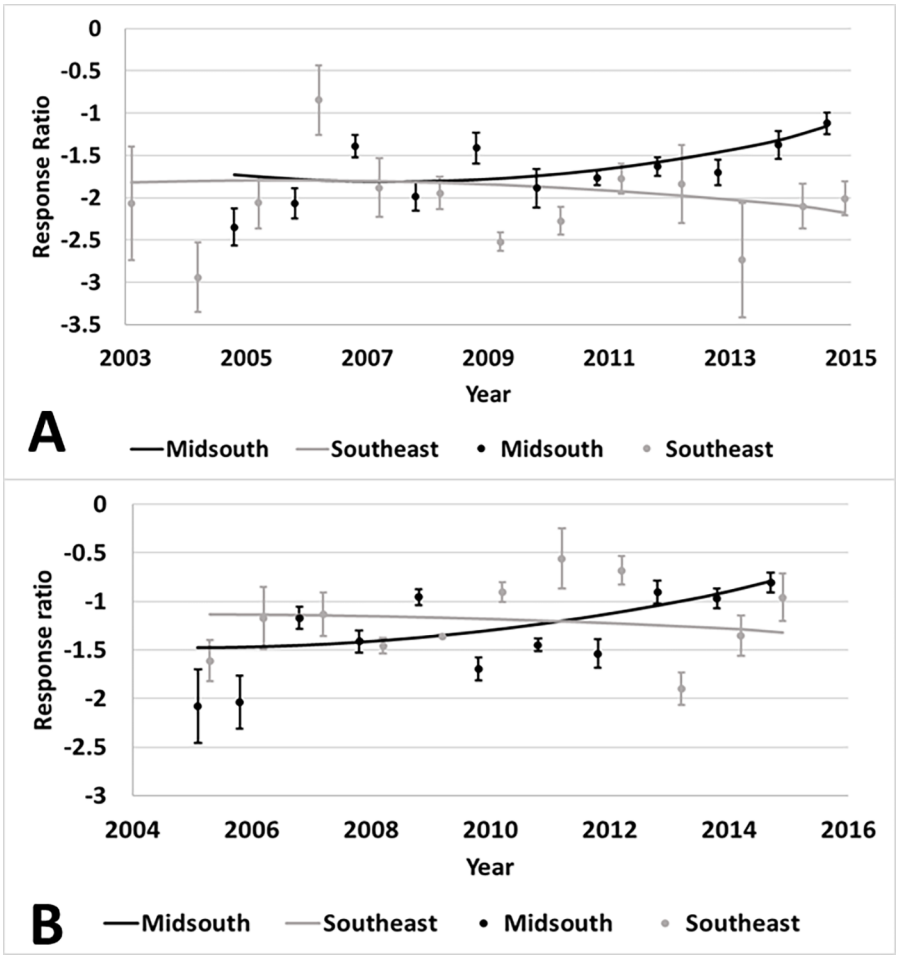

Fig 8. Change over time of damage in Bollgard II (A) and WideStrike (B) cotton by region of the eastern and central Cotton Belt of the United States. Bollgard II Midsouth equation: 0.0759x - 2.7923; Southeast equation: -1.9273; WideStrike Midsouth equation: 0.0776x – 2.4088; Southeast equation: -1.214. Response ratio (A) = ln ([Bollgard II mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]); Response ratio (B) = (ln([WideStrike mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]).

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Bias

Analyses to evaluate bias were not conducted as part of this study. Two main sources of bias were considered; however, these two sources, publication bias and selective reporting due to industry sponsorship, could not be effectively evaluated because the vast majority of the data used were from non-refereed sources and were conducted by entomologists in industry or receiving industry funds in their public university positions. This was unavoidable due to the nature of this type of research being conducted almost exclusively by entomologists who receive funding through industry to conduct applied research trials with commercial products to develop grower recommendations. Based on our knowledge, only three papers [36, 49, 50] may have been conducted without any possibility of industry influence or bias. These papers contributed 8 of 246 (3%), 5 of 585 (0.9%), and 3 of 580 (0.5%) data points for Bollgard, Bollgard II and Widestrike, respectively. This study had the advantage of having a large body of data from many sources across a wide breadth of locations and years, so the impact of any individual's bias is minimal.

Impacts of *Bt* technology on insecticide usage, heliothine counts, cotton damage, and yield

Cotton production practices in the USA have been impacted by *Bt* technology (Fig 2). The number of foliar insecticide applications in all *Bt* cotton technologies relative to non-*Bt* varieties were lowered, reducing environmental impacts from insecticides. Foliar insecticides are still often necessary in *Bt* cotton production and may become more important if resistance to *Bt* toxins becomes frequent and widespread. Newer *Bt* cotton technologies (TwinLink and WideStrike 3) were as good as or better than earlier *Bt* technologies for control of lepidopteran pests, but their impact on insecticide use could not be evaluated in this study. In the absence of foliar insecticide applications, differences between *Bt* and non-*Bt* cotton for heliothine counts, damage and cotton yield were documented for all technologies. Heliothine densities and damage were reduced, and yields of all technologies except WideStrike 3 increased. The combination of decreased insecticide use, decreased heliothine damage, and increased yields has been a substantial benefit of *Bt* technology for growers and the environment.

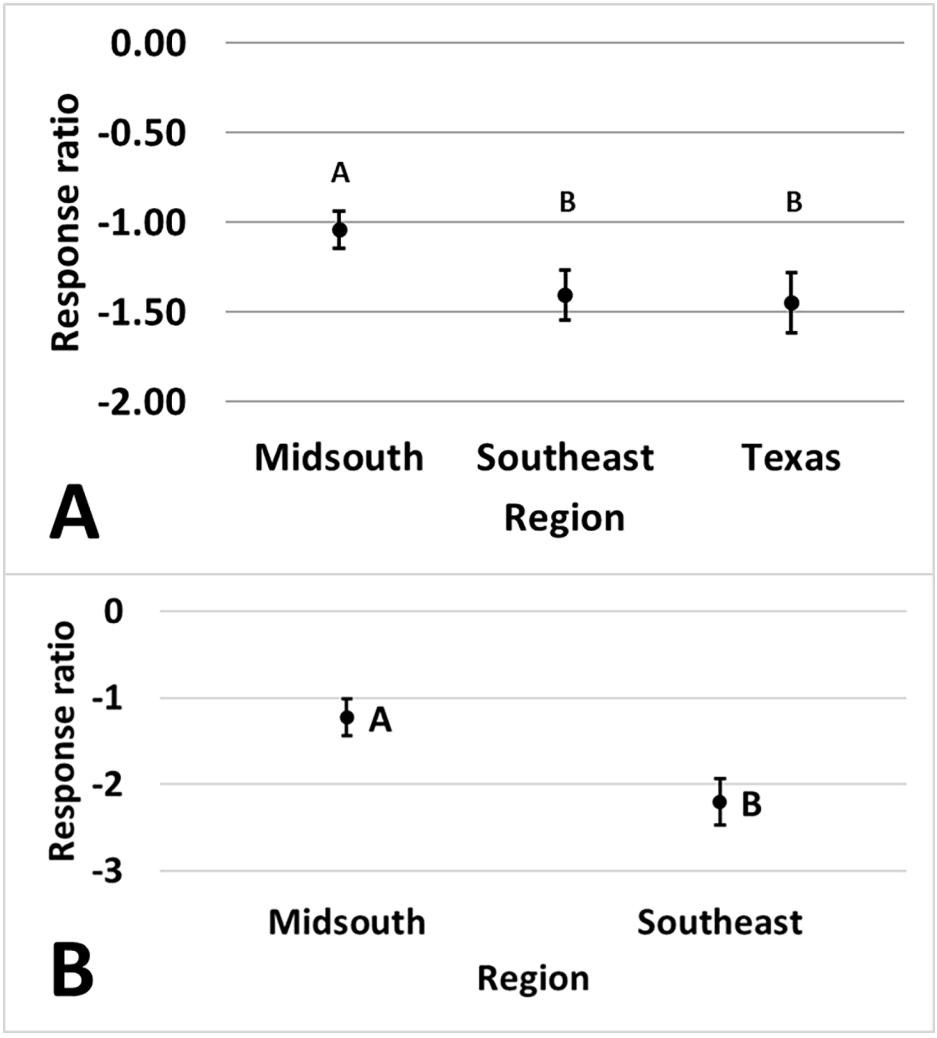

Fig 9. Least square mean ± SE of the response ratio of region of Bollgard (A) and WideStrike 3 (B) damage data from trials in the eastern and central Cotton Belt of the United States. Regions not sharing the same uppercase letter are different (Least square means $\alpha = 0.05$). Response ratio (A) = ln ([Bollgard mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]); Response ratio (B) = ln ([WideStrike 3 mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]).

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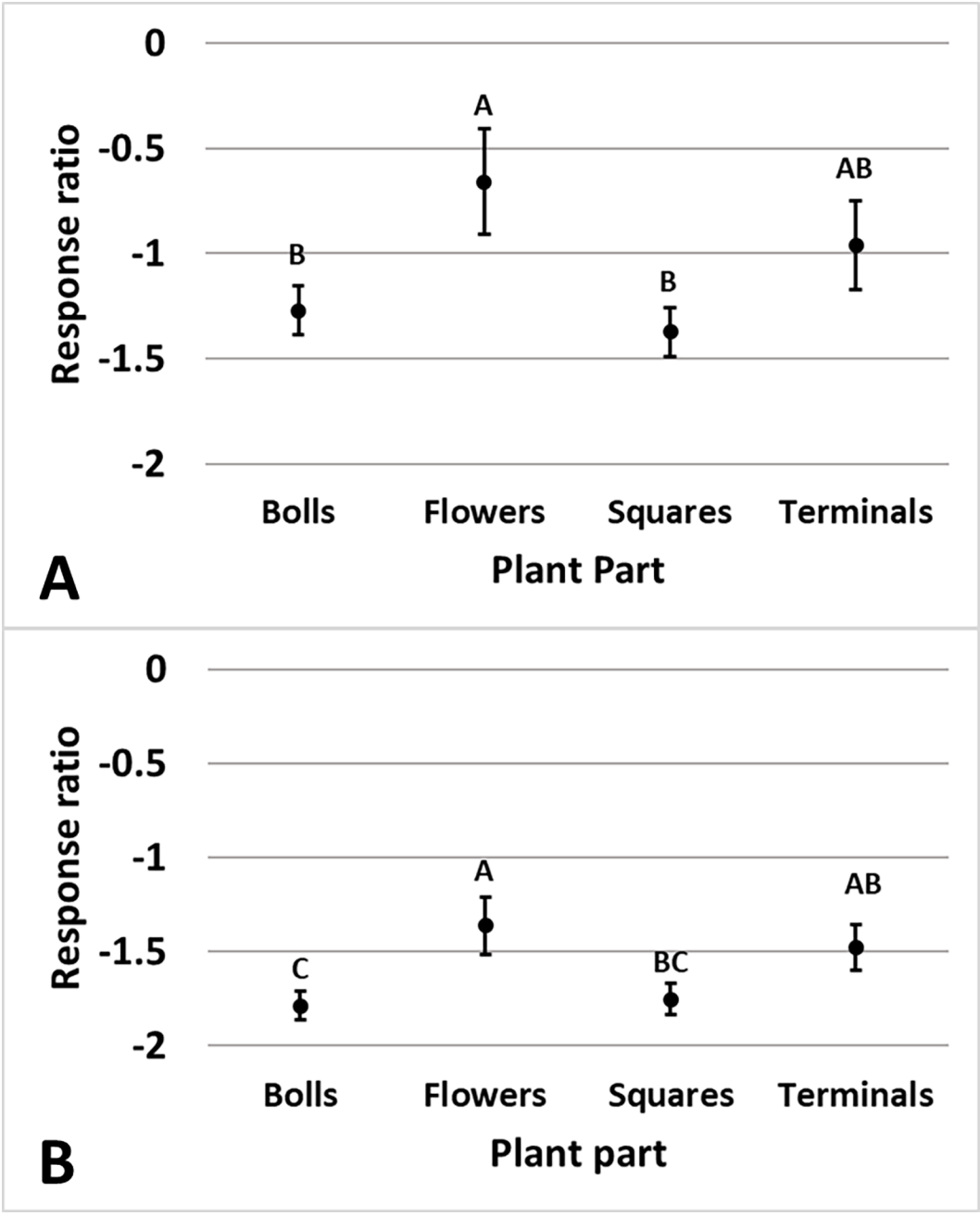

Fig 10. Least square mean ± SE of the response ratio of plant part of Bollgard (A) and Bollgard II (B) damage data from trials in the eastern and central Cotton Belt of the United States. Plant parts not sharing the same uppercase letter are different (Least square means $\alpha = 0.05$). Response ratio (A) = ln ([Bollgard mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]); Response ratio (B) = ln ([Bollgard II mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]).

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Efficacy comparisons between *Bt* technologies and non-*Bt* varieties

Regional differences were found for *Bt* efficacy as measured by heliothine counts, damage, and yield for all technologies except TwinLink. Generally, the impact of technologies was greater in the Southeast than in the Midsouth. Bollgard and Bollgard II were the only technologies that

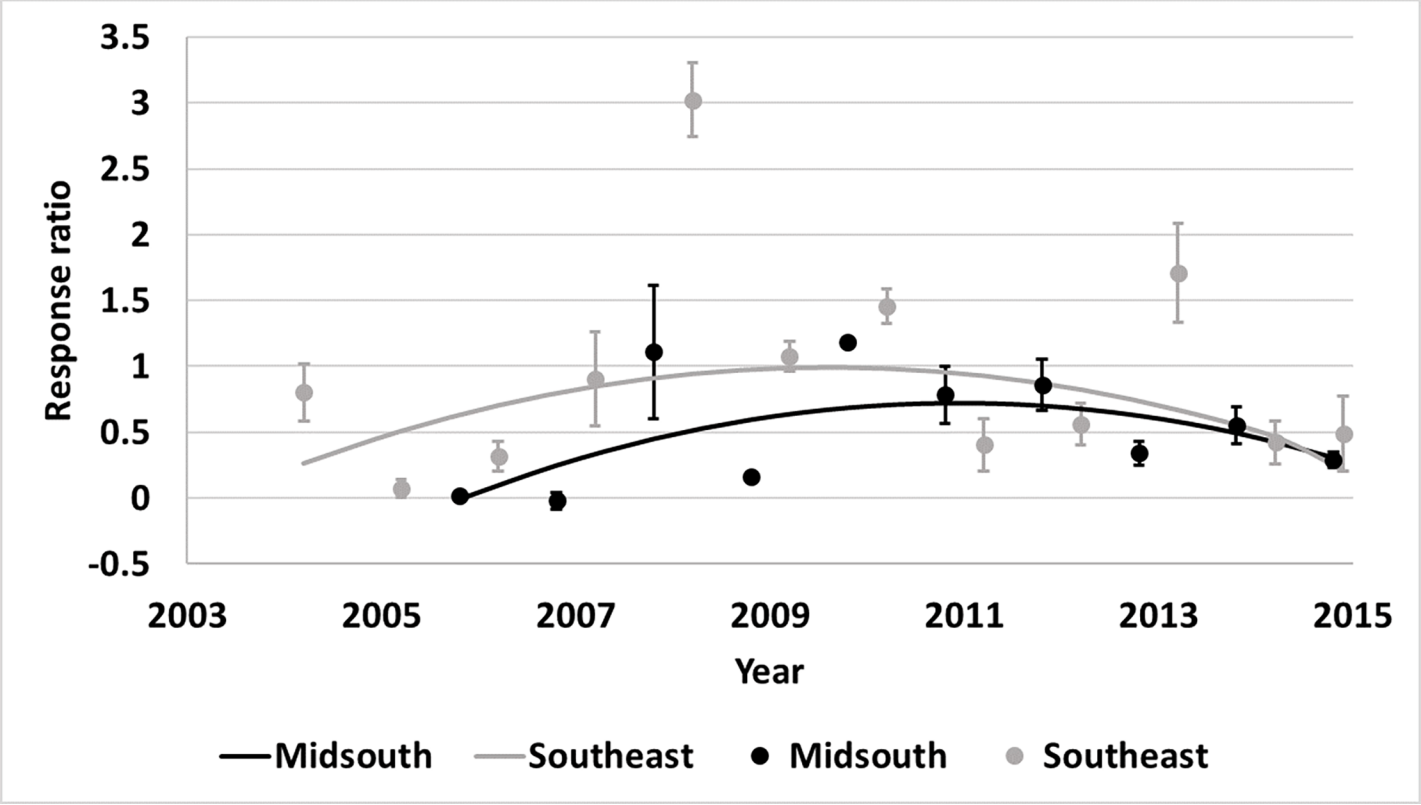

Fig 11. Change over time of yield in Bollgard II cotton by region in trials from the eastern and central Cotton Belt of the United States. Midsouth equation: 0.8941x - 0.02767x2–6.4911; Southeast equation: 0.7171x - 0.02489x2–4.1691. Response ratio = ln ([Bollgard II mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]).

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had differences in relative damage between plant parts, with both providing more protection of bolls and squares than flowers, which is consistent with previous research [51, 52].

Efficacy comparisons between *Bt* technologies

Bollgard II, WideStrike 3, and TwinLink all provided better control of heliothines than WideStrike regarding damage, and WideStrike provided better control than the single-gene product, Bollgard. Among the multi-gene technologies, the lower efficacy of WideStrike was likely due to its reliance on Cry1Ac, which was the first *Bt* gene inserted into commercial cotton varieties and has had resistance documented in *H. zea* [21, 22, 24], and the lack of efficacy of Cry1F against *H. zea* [25]. There was not a difference between WideStrike 3 and either Bollgard II or TwinLink, which was unexpected due to the addition of the Vip3A gene [53]. Only one year of data was available for WideStrike 3 comparisons and more research is needed before drawing conclusions on the impact of this new toxin. Unlike *Bt* to non-*Bt* comparisons where the non-*Bt* variety was normally a close genetic relative of the *Bt* variety, genetic similarity is not expected between *Bt* technologies developed by different companies. Therefore, some of the differences in yield between *Bt* technologies may have been due to differences in yield potential of the germplasm rather than the impact of the *Bt* toxins. Differences between damage on plant parts were observed only between Bollgard and Bollgard II and were consistent with comparisons of these technologies to non-*Bt* varieties. Regional differences between technologies were numerous and followed the same trend as comparisons between *Bt* and non-*Bt* where differences in technologies were greater in the Southeast than in the Midsouth. Taken

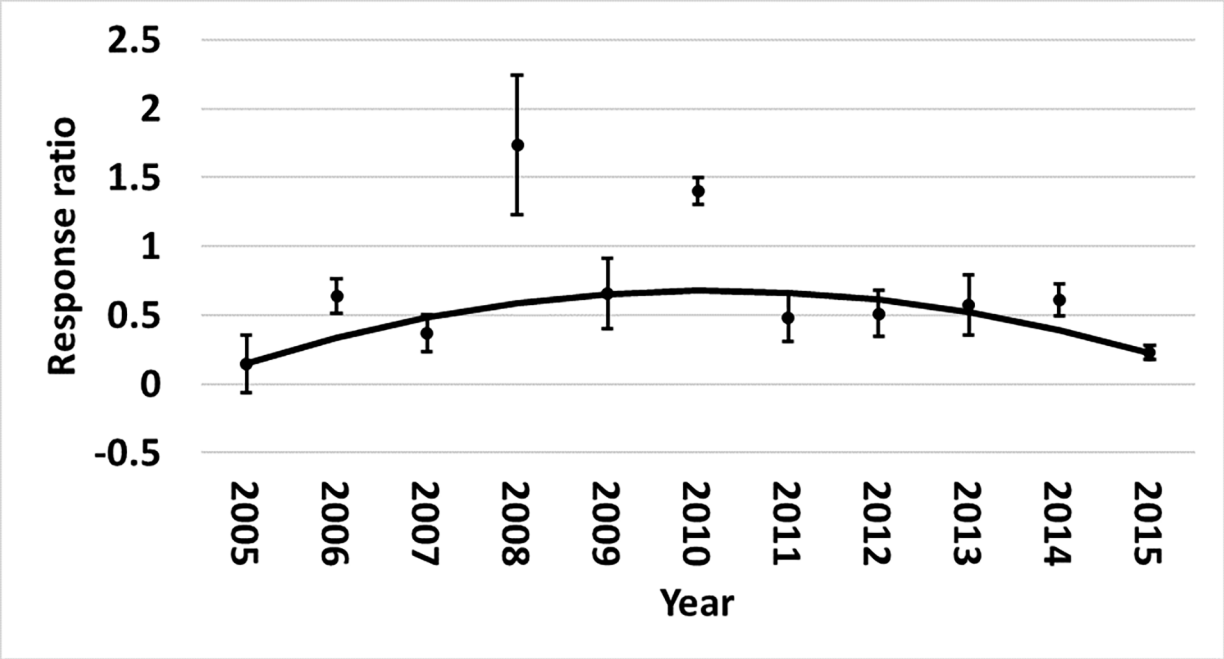

Fig 12. Change over time of yield in WideStrike cotton in trials from the eastern and central Cotton Belt of the United States. Equation: 0.593x - 0.01954x2–3.8209. Response ratio = ln ([WideStrike mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]).

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together, these data reveal that multi-gene technology was superior to single-gene technology, thus demonstrating the need for additional pyramiding of novel *Bt* genes. Also, while performance varied depending on location and the aspect of efficacy being measured, relative performance of the technologies to each other and to non-*Bt* varieties was reasonably consistent.

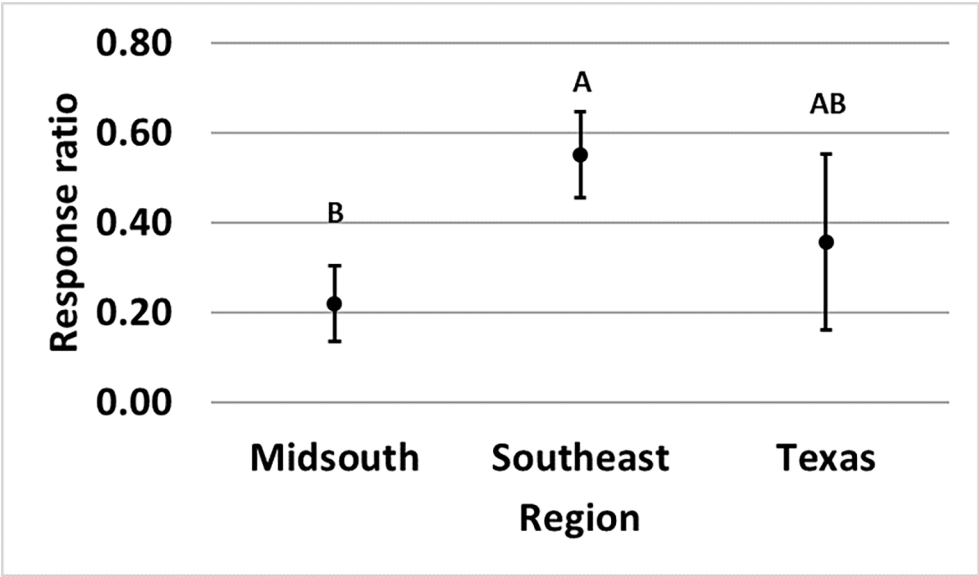

Fig 13. Least square mean ± SE of the response ratio of region of Bollgard cotton yield in trials from the eastern and central Cotton Belt of the United States. Regions not sharing the same uppercase letter are different (Least square means α = 0.05). Response ratio = ln ([Bollgard mean$_x$ + 1] / [non-*Bt* mean$_x$ + 1]).

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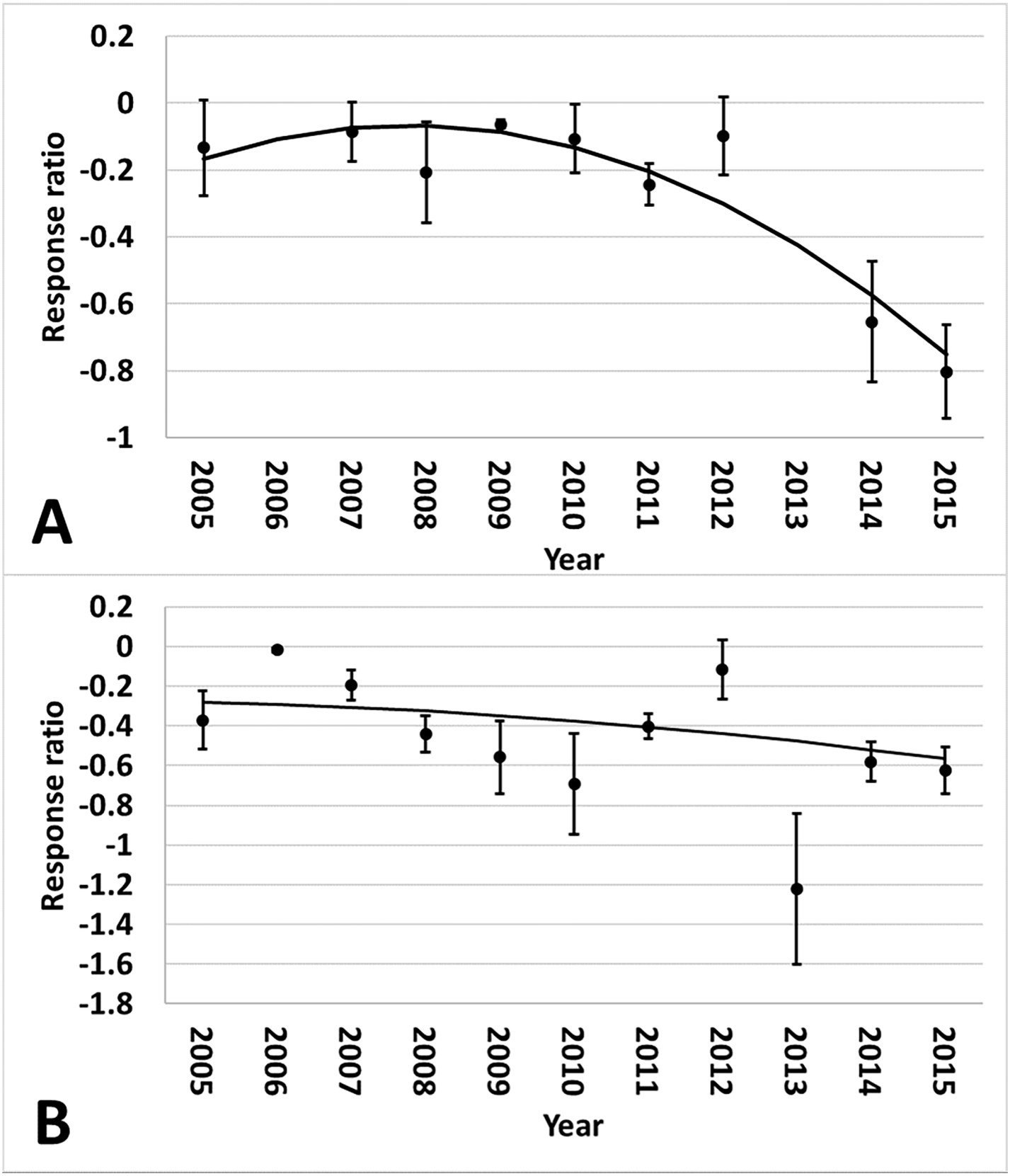
0.2
0
-0.2
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Response ratio
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A
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Response ratio
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Fig 14. Change over time of heliothine counts (A) and damage (B) of the comparison of Bollgard 2: WideStrike cotton in trials from the eastern and central Cotton Belt of the United States. Heliothine counts equation: 0.3345x - 0.01305x2–2.2011, Damage equation: -0.0303x - 0.0581. Response ratio (A and B) = ln ([Bollgard II mean$_x$ + 1] / [WideStrike mean$_x$ + 1]).

https://doi.org/10.1371/journal.pone.0200131.g014

Changes in efficacy and yield over time

Evaluations of heliothine counts and damage over time revealed no changes in Bollgard efficacy from 1996 to 2008; however, a loss of efficacy occurred for both Bollgard II and WideStrike from introduction until 2015 in the Midsouth region (Figs 11 and 12). These technologies rely on three of the oldest commercialized *Bt* toxins (Cry1Ac, Cry2Ab and Cry1F) and resistance to Cry1Ac and Cry2Ab toxins has been documented [20, 22, 24, 54, 55]. Another contributing factor to the apparent loss of efficacy could be a shift to a higher proportion of *H. zea* in the heliothine complex. *Heliothis virescens* is more susceptible to Cry1Ac than *H. zea* [24], and therefore has a lower survival rate in *Bt* cotton. With the widespread adoption to *Bt* crops, population suppression of *H. virescens* may have occurred, resulting in *H. zea* comprising a higher proportion of the heliothine complex in non-Bt cotton [56, 57], resulting in the apparent loss of efficacy in *Bt* cotton even without a change in susceptibility toward either pest. The decline in efficacy reported here supports anecdotal observations of many entomologists in the Midsouth, and highlights the need for additional technologies for *H. zea* control, and the need for continued development of new insecticides and management tactics for lepidopteran pest management in cotton. The reason efficacy in the Southeast had not deteriorated is unknown, but could be related to different landscape diversity reducing selection pressure, a different source population that has experienced less selection, or *H. virescens* comprising a larger proportion of the heliothine complex in the Southeast. Given the mobility of *H. zea* [58–60], resistance developed in one part of the USA can spread rapidly throughout the country, so regional differences are unlikely to persist with this insect.

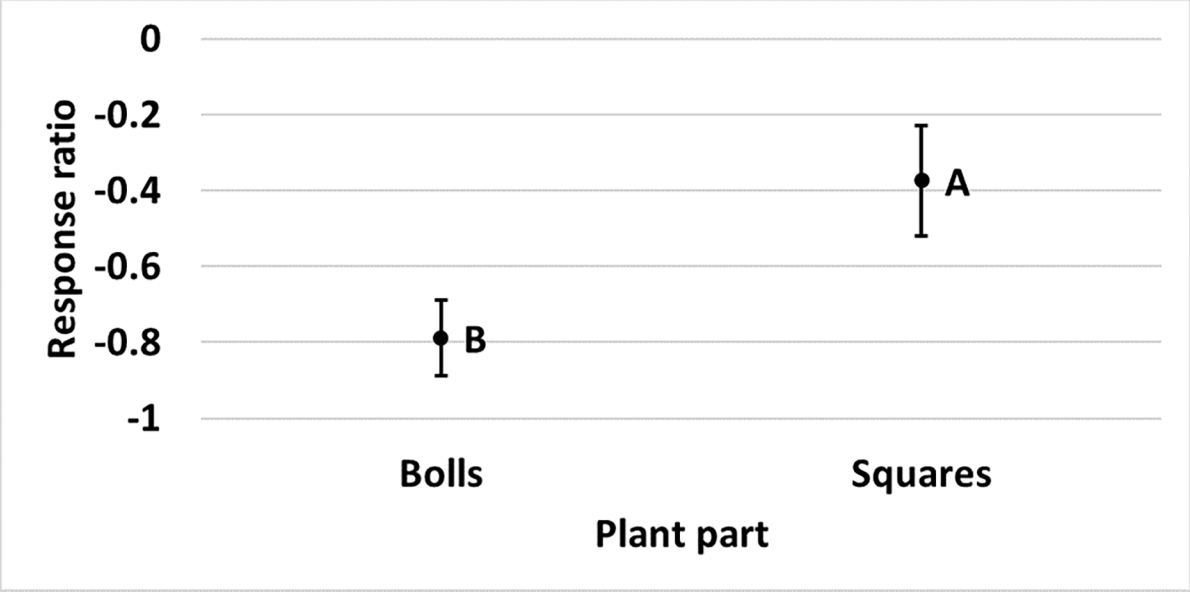

Fig 15. Least square mean ± SE of the response ratio of plant part of the comparison of Bollgard II: Bollgard damage in trials from the eastern and central Cotton Belt of the United States. Plant parts not sharing the same uppercase letter are different (Least square means α = 0.05). Response ratio = ln ([Bollgard II mean$_x$ + 1] / [Bollgard mean$_x$ + 1]).

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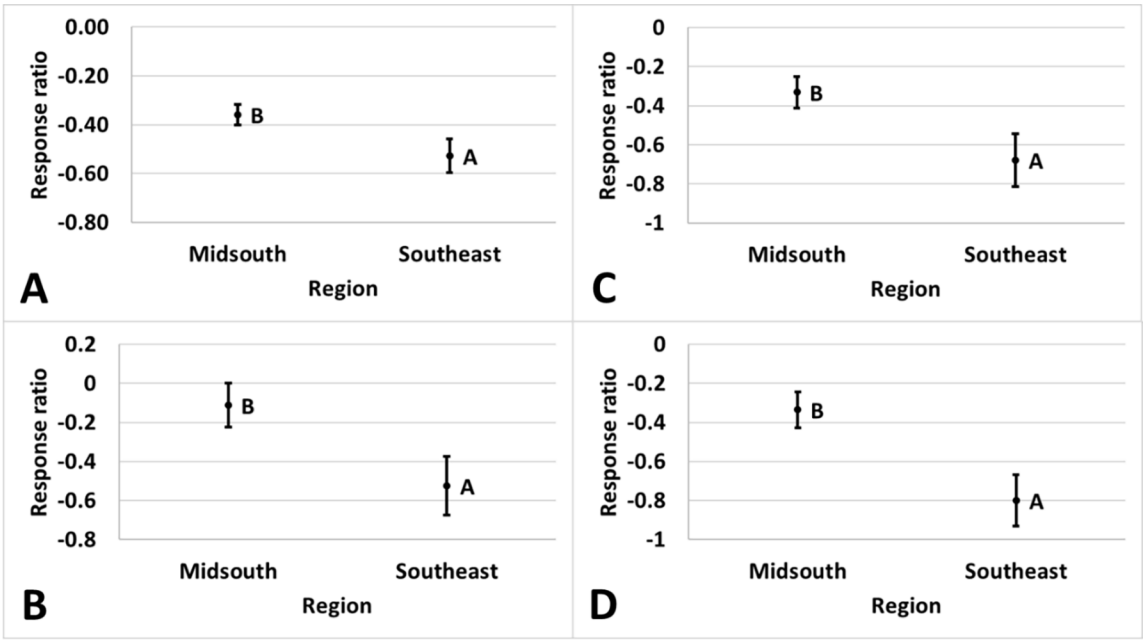

Fig 16. Least square mean ± SE of the response ratio of damage by region of (A) Bollgard II: WideStrike, (B) Bollgard II: WideStrike 3, (C) TwinLink: WideStrike, and (D) WideStrike 3: WideStrike comparisons in trials from the eastern and central Cotton Belt of the United States. Regions not sharing the same uppercase letter are different (Least square means $\alpha = 0.05$). Response ratio (A) = ln ([Bollgard II mean$_x$ + 1] / [WideStrike mean$_x$ + 1]); Response ratio (B) = ln ([Bollgard II mean$_x$ + 1] / [WideStrike 3 mean$_x$ + 1]); Response ratio (C) = ln ([TwinLink mean$_x$ + 1] / [WideStrike mean$_x$ + 1]); Response ratio (D) = ln ([WideStrike 3 mean$_x$ + 1] / [WideStrike mean$_x$ + 1]).

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Evaluations of yield revealed complex changes over time. In both the Midsouth and Southeast regions, yield differences between *Bt* technologies (Bollgard II and WideStrike) and non-

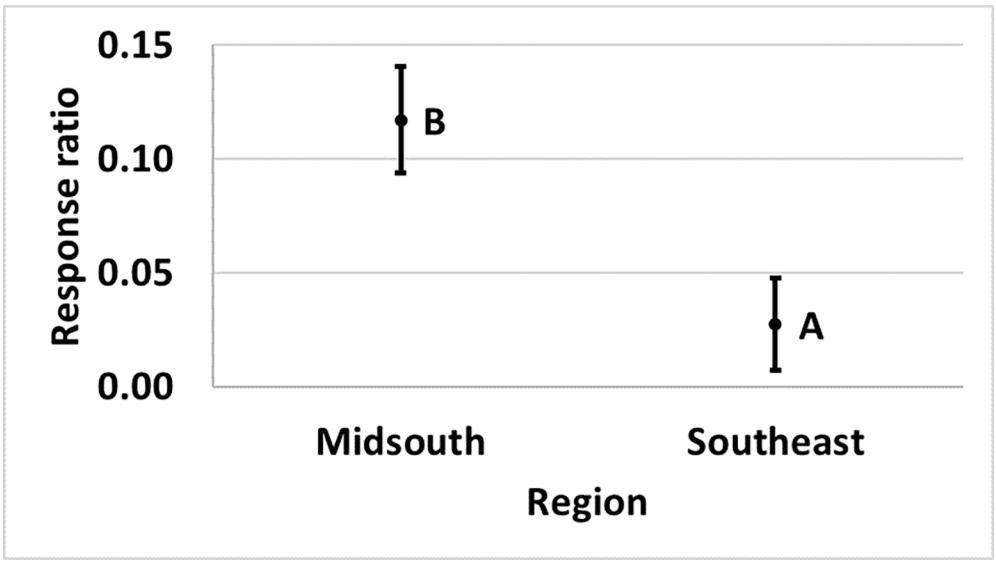

Fig 17. Least square mean ± SE of the response ratio of damage by region of the Bollgard II: WideStrike comparison in trials from the eastern and central Cotton Belt of the United States. Regions not sharing the same uppercase letter are different (Least square means $\alpha = 0.05$). Response ratio = ln ([Bollgard II mean$_x$ + 1] / [WideStrike mean$_x$ + 1]).

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Bt varieties initially increased after commercialization, suggesting improved genetics of the varieties containing *Bt* technologies. After about 2010, these yield differences started to decrease, which is consistent with the increasing damage trends for these technologies in the Midsouth. While damage prevention and yield benefits appear to have decreased, *Bt* technologies still provided some protection from lepidopteran pests through 2015 which resulted in some yield benefits.

Summary

Reductions in insecticide usage occurred with *Bt* cotton, but foliar insecticides were still needed to manage heliothine pests in many cases. *Bt* cotton reduced losses to heliothines and improved yields relative to non-*Bt* varieties, but economic benefits of these changes were not evaluated. Declining yield benefits of *Bt* technologies from around 2010 to 2015 were observed in the Midsouth and Southeast for Bollgard II and WideStrike technologies. Possible reasons for this are a decline in efficacy or a decline in insect pressure. Pheromone trap catches of heliothines would suggest that there is high annual variability in population size, but there was not a consistent trend from 2010–2015 (unpublished data, FRM). A decline in efficacy of *Bt* cotton was observed in the Midsouth, but not in the Southeast. This decline in efficacy could be due to insects becoming resistant to one or more *Bt* toxins or other changes being made in cotton genetics that alter susceptibility to heliothines. Since non-transgenic heliothine resistance is not a known goal for cotton breeders, it is most likely that changes in efficacy were due to insects developing resistance to the commercialized *Bt* toxins. This study was not able to distinguish counts and damage between *H. virescens* and *H. zea*. Since the authors are not aware of any *H. virescens* survival on any *Bt* cotton, it is assumed that changes in efficacy are due to changes in *H. zea* susceptibility. Given the mobile nature of *H. zea*, the resistance that was most pronounced in the Midsouth by 2015 may spread throughout the range of *H. zea*. As resistance becomes more common, the need to introduce new *Bt* technologies and improve other means of managing heliothine pests in cotton will increase. Furthermore, since *Bt* corn and *Bt* cotton use many of the same *Bt* toxins and *H. zea* develops on both crops, resistance management strategies should take both crops into consideration.

Supporting information

S1 Table. List of data sources in this paper used to conduct a meta-analysis of *Bt* cotton technologies.
(PDF)

S2 Table. Summary of the number of *Bt*: Non-*Bt* comparisons by publication type, insecticide use criteria, region, and technology from articles and unpublished data of trials in the eastern and central Cotton Belt of the United States. [1]Arthropod Management Tests; [2]Proceedings of the Beltwide Cotton Conferences; [3]Extension publication; [4]Thesis or dissertation; [5]No data reported from T/D; [6] No data reported for other technologies.
(PDF)

S3 Table. Summary of the number of *Bt*: *Bt* comparisons by publication type, insecticide use criteria, region, and technology from articles and unpublished data of trials in the eastern and central Cotton Belt of the United States. [1]Arthropod Management Tests; [2]Proceedings of the Beltwide Cotton Conference; [3]Extension publication; [4]Thesis or dissertation; [5]No data reported from T/D; [6] No data reported for other comparisons.
(PDF)

S4 Table. Summary of the number of *Bt*: Non-*Bt* comparisons of heliothine counts and damage and cotton yield by region and technology in trials from the eastern and central Cotton Belt of the United States. [1]Data reported as a combination of bolls, flowers, and/or squares; [2]Data reported as a combination of reproductive structures and terminals; [3]No data reported for other technologies.
(PDF)

S5 Table. Summary of the number of *Bt*: *Bt* comparisons of heliothine counts and damage and cotton yield by region and technology in trials from the eastern and central Cotton Belt of the United States. [1]Data reported as a combination of bolls, flowers, and/or squares; [2]Data reported as a combination of reproductive structures and terminals; [3]No data reported for other comparisons.
(PDF)

S6 Table. Statistical results of comparisons of *Bt* cotton technologies for heliothine counts, damage, and cotton yield overall, by plant part, region, year, and their interactions. n/e = not estimated.
(PDF)

S1 Fig. Prisma checklist.
(PDF)

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Host-selection behavior and physiological mechanisms of the cotton aphid, *Aphis gossypii*, in response to rising atmospheric carbon dioxide levels

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ABSTRACT

Rising atmospheric carbon dioxide (CO_2) levels can markedly affect the growth, development, reproduction and behavior of herbivorous insects, mainly by changing the primary and secondary metabolites of their host plants. However, little is known about the host-selection behavior and the respective intrinsic mechanism of sap-sucking insects in response to elevated CO_2. In this experiment, the host-selection behavior, as well as the physiological mechanism based on the analysis of growth, development and energy substances, and the expression of the olfactory-related genes of the cotton aphid, *Aphis gossypii*, were studied under ambient (407.0 ± 4.3 μl/L) and elevated (810.5 ± 7.2 μl/L) CO_2. The results indicated that the aphids reared under ambient and elevated CO_2 did not differ in their level of preference for cotton seedlings, whatever the CO_2 conditions in which the plants developed. However, aphids reared under elevated CO_2 showed a greater ability to respond to the plant volatiles compared to aphids that developed under ambient CO_2 (+23.3%). This suggests that rising atmospheric CO_2 enhances the activity of host selection in this aphid. Compared with ambient CO_2, elevated CO_2 significantly increased aphid body weight (+36.7%) and the contents of glycogen (+18.9%), body fat (+14.6%), and amino acids (+16.8%) and increased the expression of odor-binding protein genes, *OBP2* (+299.6%) and *OBP7* (+47.4%), and chemosensory protein genes, *CSP4* (+265.3%) and *CSP6* (+50.9%), potentially enhancing the overall life activities and upregulating the olfactory ability of *A. gossypii*. We speculated that the rising atmospheric CO_2 level would likely aggravate the damage caused by *A. gossypii* due to the higher potential host selection and increased general activity under future climate change.

1. Introduction

Rising atmospheric carbon dioxide (CO_2) levels are one of the most concerning issues about global climate change. CO_2 has continuously risen from 280 ppm before the industrial revolution to 402 ppm at present (Mauna Loa Observatory: NOAA-ESRL), and is expected to reach at least 550 ppm by the middle of the 21st century (Pachauri et al., 2014) and 800 ppm by the turn of this century (Mastrandrea et al., 2011). As an important constituent for plant photosynthesis, especially for C_3 plants, the change in atmospheric CO_2 concentrations directly affects plant photosynthesis and growth (Bazzaz and Catovsky, 2002; Leakey et al., 2009). Elevated CO_2 also indirectly affects the growth and development of herbivorous insects and their physiological metabolism by altering plant biomass and quality, including changes in the composition and content of chemical substances in their host plant tissues (Stacey and Fellowes, 2002; Chen et al., 2004, 2006; Ge and Chen, 2006; Jiang et al., 2016). Because insects account for about half of all living things on the planet and are highly sensitive to global climate change (such as elevated CO_2), the steady increase in environmental CO_2 will likely have a significant negative impact on the maintenance of ecosystem structure and function (Ge, 2011).

Insects and plants have formed complex interrelationships through long evolution, and the breeding of insect populations depends largely on finding suitable host plants and getting appropriate and adequate nutrition (Fan et al., 2014). Sensitive olfactory systems allow insects to accurately perceive chemical signals in the environment (Field et al., 2000). The olfactory receptors of insects are located on the antenna and maxillary palpi; both organs have olfactory receptor neurons (ORNs), which are covered with different types of olfactory receptors (Hallberg, 1982; Sukontason et al., 2007; Liu et al., 2011; Wang et al., 2012). The odor molecules enter the antennal lymph through micropores in the epidermis of the olfactory receptors, and with the help of related

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proteins pass through the hydrophobic lymph to the ORNs where they then produce nerve impulses by the effects of ORN odorant receptors. Different proteins carry different types of odors to complete the insects' initial identification of the host plant (Xia et al., 2008). During this period, the participation of many proteins such as odorant-binding proteins (OBPs), chemosensory proteins (CSPs), odorant degrading enzymes (ODEs), and sensory neuron membrane proteins (SNMPs) are required, and the OBPs and CSPs are the primary peripheral olfactory proteins that play critical roles in odor detection (Zhao et al., 2017). The expression of odorant-binding protein genes (including *OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (including *CSP4* and *CSP6*) are highly expressed in the head of *A. gossypii* (Gu et al., 2013; Xu, 2014; Zhao et al., 2017). Insects need additional basic energy substances during host selection, so they gain energy substances and maintain normal biochemical conditions through breathing. Various energy substances, such as glycogen, fats, and amino acids, produce the necessary energy for insects' life activities according to specific metabolic pathways (Rankin and Burchsted, 1992).

Many experiments have been carried out to study the host-selection behavior of insects. Liu et al. (2002) and Chen et al. (2005) both used leaf selection methods and electropenetrography (EPG) technology to study host selection and specialization in the cotton aphid, *Aphis gossypii*. Wang et al. (2017) used the Petri dish selection method to study the host selection of two kinds of specialized aphids. Geier and Boeckh (1999) used a Y-type tube olfactory to study the host selection of *Aedes aegypti* on a human hand, an extract with human skin residues, L-(C)-lactic acid and CO_2; they found that CO_2 stimulated the flight of *A. aegypti*, and it was more obvious after coordination with lactic acid. Reddy et al. (2004) used a wind tunnel to study the host plant-mediated orientation and oviposition of *Plutella xylostella* on four different brassica host plants. And Rim et al. (2017) used a Y-type tube olfactory to study whether the plant-infestation experience of *Nesidiocoris tenuis* affected its subsequent prey-finding behavior. Moreover, numerous studies confirm that high atmospheric CO_2 concentrations can affect the growth, reproduction, feeding behavior and oviposition of some insects (Guerenstein and Hildebrand, 2008; Sun et al., 2011; Couture et al., 2012). As a phloem-feeding insect, the cotton aphid is one of the most important agricultural pests of cotton production worldwide (Castle et al., 1992; Weathersbee and Hardee, 1994; Birkett and Pickett, 2003). However, the influence of rising atmospheric CO_2 levels on the host-selection behavior of the cotton aphid is not well documented.

In this study, the host-selection behavior, as well as the physiological mechanism based on the analysis of growth, development and energy substances, and the expression of the olfactory-related genes of the cotton aphid were studied under ambient and elevated CO_2. The objectives of this study were to: 1) examine the host-selection behavior of *A. gossypii* as influenced by elevated CO_2 using an olfactometer, 2) quantify the parameters of insect growth and development and accumulated energy substances in insect bodies as a proxy for host-selection behavior influenced by two CO_2 levels, and 3) investigate the expression of odorant-binding protein genes (including *OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (including *CSP4* and *CSP6*) to elucidate the molecular mechanism in the changes in host-selection behavior of *A. gossypii* in response to rising atmospheric CO_2 levels.

2. Materials and methods

2.1. Setup of CO_2 levels

This study was conducted in electronically controlled growth incubators (GDN-400D-4/CO_2; Ningbo Southeast Instrument CO., LTD., Ningbo, China) with a gas-tank system to supply 99% pure CO_2 gas and maintain the desired CO_2 concentrations. In these growth incubators, the environmental conditions were set at 27 °C and 70% RH during the day and 26.5 °C and 70% RH at night. The photoperiod was L14:D10 and the light intensity was 20,000 l× in each growth incubator. Two levels of CO_2 concentration were applied continuously: ambient CO_2 (401.2–411.2 μl/L; mean: 407.0 ± 4.3 μl/L), representing the current level of atmospheric CO_2 concentration, and elevated CO_2 (803.0–818.6 μl/L; mean: 810.5 ± 7.2 μl/L), simulating the predicted level of atmospheric CO_2 concentration at the end of this century (Mastrandrea et al., 2011). Four growth incubators were used as replicates for each CO_2 treatment.

2.2. Host plants and insect colony

The cotton cultivar (cv. C111) was supplied by the Jiangsu Academy of Agricultural Sciences and planted in plastic pots (12 cm in diameter and 15 cm high) filled with nutritional soil (Xingnong Organic Fertilizer CO., LTD., Zhenjiang, China) in the electronically controlled growth incubators. After the emergence of seedlings, the cotton plants were thinned to one plant per pot and exposed to the ambient and elevated CO_2 levels. Potted plants were watered moderately every other day, and no additional chemical fertilizers or insecticides were used throughout the experiment. The experiment consisted of twenty-four plastic pots (i.e., 24 cotton plants) placed in each growth incubator, with a total of 192 cotton plants (24 plants per growth incubator × 4 growth incubators per CO_2 level × 2 CO_2 levels) for the entire experiment. The experimental pots (plants) in each growth incubator were re-randomized once a week to minimize positional effects within the incubator. Thirty days after germination, each experimental cotton seedling (192 total plants) was inoculated with cotton aphids for host-selection behavior studies.

The apterous *A. gossypii* used in this study were provided by the Insect Ecology Group of the Department of Entomology, College of Plant Protection, Nanjing Agricultural University. A single apterous aphid was randomly selected and reared on 30- to 50-day-old cotton (cv. C111) seedlings in electronically controlled growth chambers to establish a standardized colony.

2.3. Growth and accumulation of energy substances by A. gossypii adults

In each growth incubator of ambient and elevated CO_2, 200 newly emerged *A. gossypii* adults (a total of 200 individuals per growth incubator × 4 growth incubators per CO_2 level × 2 CO_2 levels = 1600 newly emerged adults) were randomly selected from the above aphid colony and inoculated singly onto a fully expanded leaf in glass Petri dishes (150 mm diameter; 10 individuals per dish and 20 dishes per growth incubator). Cotton leaves were excised from the 30- to 50-day-old cotton seedlings, and a single aphid was inoculated into each Petri dish and allowed to oviposit for 12 h. Then, all the inoculated aphid adults were removed and all the offspring were reared to adulthood for the following experiments. Forty newborn offspring (10 individuals per growth incubator × 4 growth incubators) in each CO_2 treatment were reared individually until each aphid nymph reached adulthood, and the total nymphal duration for each individual aphid was recorded. Two hundred newly emerged adult aphids (50 individuals per growth incubator × 4 growth incubators) in each CO_2 treatment were randomly selected and divided into 20 replicates (10 adults per replicate) to measure the body weights of cotton aphids using an electronic microbalance with an accuracy of ± 1 μg (Mettler Toledo XP6, Switzerland).

Three types of energy substances, including glycogen, body fat and amino acids, were also measured in the adult aphids. Here, another 60 adult aphids (15 individuals per growth incubator × 4 growth incubators) were randomly selected from each CO_2 treatment and equally divided into three replicates for weighing using the same electronic microbalance; these were ground into a homogenate with 10% trichloroacetic acid, incubated for 3 h at 4–5 °C, and this mixture was then centrifuged at 6000 rpm for 10 min. The supernatant was transferred and 95% ethanol and a drop of saturated Na_2SO_4 were added. The mixture was incubated overnight at 5 °C and centrifuged at 3000 rpm for 20 min. The supernatant was removed and precipitated in the

centrifuge tube for 30 min, and then dissolved in distilled water; this was used to determine the glycogen content in the test aphids using the anthrone method (Yi et al., 2009). In addition, 300 newly emerged adult aphids (75 individuals per growth incubator × 4 growth incubators) were randomly selected from each CO_2 treatment and equally divided into 3 replicates (100 individuals per replicate). These were then weighed using the same electronic microbalance and dried for 48 h using a Christ freeze-dryer (Christ ALPHA 2–4 LD plus; Martin Christ CO. LTD., Osterode, Germany) to determine the constant dry weight (DW). These dried aphids were ground into a homogenate with a mixture of chloroform and methanol (chloroform:methanol = 2:1), the suspension was centrifuged for 10 min at 12000 rpm and the supernatant was removed. This step was repeated once more and the precipitate was then dried to a constant weight (LDW), and the body fat measurement was calculated as DW minus LDW (Colinet et al., 2007). Another 900 newly emerged adults (300 individuals per growth incubator × 4 growth incubators) were randomly selected from each CO_2 treatment and were equally divided into 3 replicates (100 individuals per replicate). These were weighed, dried to a constant weight using the same Christ freeze-dryer, and the hydrochloric hydrolysis method was used for the pretreatment of samples (Sun et al., 2008). The samples were then further processed to quantify the amino acids using the automatic amino acid analyzer (L-8900; Hitachi High-Technologies Corporation, Tokyo, Japan). The glycogen, body fat, and amino acid contents were calculated as μg/mg of fresh weight.

2.4. Measurement of the host-selection behavior of A. gossypii adults

The host-selection behavior of *A. gossypii* adults as influenced by CO_2 level was quantified using a four-chamber olfactometer (PSM4-150; Nanjing Pusen Instrument CO. LTD., Nanjing, China). Each chamber received three 40-day-old cotton seedlings grown under ambient CO_2 (aCotton), elevated CO_2 (eCotton), or ambient air as a control, while the fourth arm was sealed (Fig. 1). In this experiment, an 8 W fluorescent lamp was placed above the four-arm motherboard and the flow meter was adjusted to deliver a consistent airflow of 3 L/min to all three sides. Twenty newly emerged adults from each growth incubator (a total of 80 individuals from 4 growth incubators) were selected from each CO_2 treatment and starved for 2 h, and then released to the center of the four-arm motherboard to observe their host-selection behavior. If the sampled adults reached the nesting area of one arm (Fig. 1) within 6 min, the treatment (*a*Cotton, *e*Cotton, or control) corresponding to that arm was considered as the choice of the released aphids. Test insects that did not reach any nesting area within 6 min of release were considered non-responders (i.e., no choice). To avoid biases in the behavioral observations between tests, the air compressor was turned off for 10 min and wiped with anhydrous alcohol after each test. The intake pipe was also exchanged after each test, and all tests were carried out in a clean, uniform, well-ventilated and relatively closed laboratory.

2.5. Bioassay of expression levels of the odor-binding protein genes and chemosensory protein genes in A. gossypii adults

The expression of the odor-binding protein (*OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*) in adult *A. gossypii* fed on cotton seedlings grown under ambient and elevated CO_2 were assayed through reverse transcription and real-time PCR analyses.

2.5.1. RNA preparation and reverse transcription

A set of 20 newly emerged adult *A. gossypii* from each growth incubator were randomly collected from each CO_2 treatment. Aphids collected from each incubator served as one biological replicate (a total of 4 biological replicates for each CO_2 treatment). The total RNA was extracted from each replicate sample using the TRIzol® reagent (Invitrogen). The concentration and quality of the samples were determined by a NanoDrop™ spectrophotometer (Thermo Scientific) and 1.5% agarose gel electrophoresis. The 1st strand complementary cDNA templates were synthesized with 100 ng of total RNA by using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan). Reverse transcriptase reactions were performed in a final volume of 20 μl.

2.5.2. Real-time PCR analysis

Each cDNA product was diluted twice from 20% to 1.25% solution using RNase-free dH_2O to bring the Ct value within the suitable range of 15–35 based on preliminary experiments. For the fluorescence-based quantitative real-time PCR (qRT-PCR), 2 μl cDNA dilution and 0.2 μM primer were used in 1 × SYBR® *Premix Ex Taq*™ (TaKaRa, Japan) with the 7500 Real-Time PCR Detection System (Applied Biosystems, Foster City, CA) following the supplier's instructions. The reactions were performed in a final volume of 20 μl. This experiment was performed at different concentration of CO_2, thus, it was necessary to use the geNorm algorithm to analyze the stability of potential housekeeping genes (*GAPDH*, *18S*, *RPL7*, *EF1α*, *HSP70* and *β-actin*) of the different samples. The assessment criteria was M < 1.0 (Etschmann et al., 2006). Ultimately, *RPL7* gene was selected as the reference gene in this study (Ma et al., 2016). Then, specific primers were designed using Beacon Designer™ 7.9 software, and the housekeeping gene *RPL7* (Ma et al., 2016) was used as the internal standard to analyze the expression levels of the target genes, including the odor-binding protein genes (*OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*). All primers for qRT-PCR analysis are shown in Table 1. Quantification of the transcript levels of the target genes was conducted following the $2^{-\Delta\Delta Ct}$ normalization method (Livak and Schmittgen, 2001). The expression levels of the internal control gene (i.e., *RPL7*) were examined in every PCR plate to eliminate systematic errors. For each biological replicate

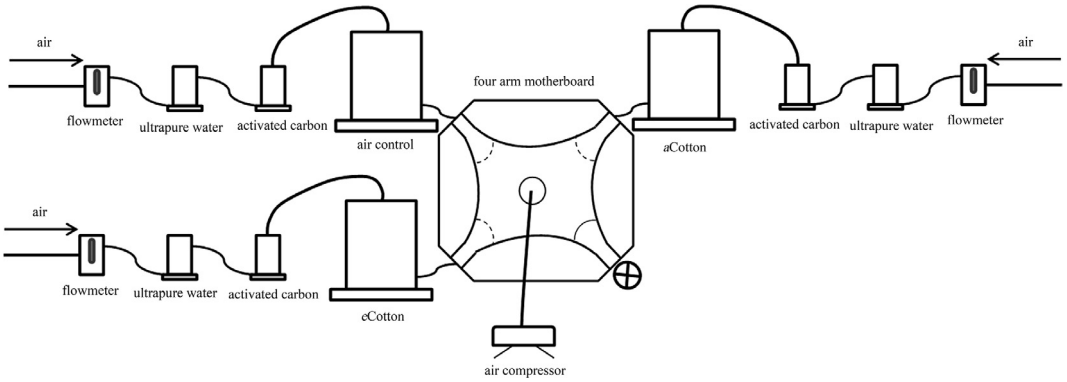

Fig. 1. An olfactometer with a four-arm motherboard (length:width:height = 35 cm:29 cm:4.3 cm) used to measure the host-selection behavior of the cotton aphid, *Aphis gossypii*. Note: The angle between two adjacent arms is 90°; ⊕ indicates that the arm was sealed; *a*Cotton and *e*Cotton are the cotton seedlings grown under ambient and elevated CO_2, respectively).

Table 1
Primers designed and used in measuring the transcript expression levels of the odor-binding protein genes (*OBP2*, *OBP6* and *OBP7*), chemosensory protein genes (*CSP4* and *CSP6*), and the housekeeping gene (i.e., *RPL7*) of *A. gossypii* in qRT-PCR experiments.

Primers	Sequences (5′ to 3′)	GenBank Accession	Description
OBP2	Forward: CACGGAGCGAACAACTG Reverse: CCATCGTCCACACTGAAC	KC161555.1	Odorant-binding protein gene
OBP6	Forward: TGCGATCATCTGCCAAACA Reverse: AGAGAGCTCGGCATTCATTATC	KC161559.1	
OBP7	Forward: CCGAGAACAACAACAACATA Reverse: GCCAACATCGTCATCTTG	KC161560.1	
CSP4	Forward: CCAGAATTGCAGTAGTCTGTGT Reverse: TGTGGTCGTATTTGGTAGTGTAAG	KC161566.1	Chemosensory protein gene
CSP6	Forward: CGTCTCTATAACTATGACTGTG Reverse: TCTTCGCCTTCTGGTGTA	KC161568.1	
RPL7	Forward: TGCCGGAGTCTGTACTCAA Reverse: TCACACCACGAATACGCA	KP676382	Housekeeping gene

(four replicates per CO_2 treatment), three technical repeats were performed in qRT-PCR analysis.

2.6. Data analysis

The measured indexes, including nymphal duration, adult body weight, and the content of energy substances (glycogen, body fat, and amino acids) and the relative transcript levels of the target genes [odorant-binding protein (*OBP2*, *OBP6*, and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*)] were analyzed by one-way analysis of variance (ANOVA) with two CO_2 levels (ambient CO_2 vs. elevated CO_2) as the source of variability (SPSS v.20.0; IBM Corporation, Armonk, NY, USA). One-way ANOVA was also used to analyze the olfactory response to the three odor sources of *A. gossypii* reared under two CO_2 conditions. Significant differences in the measured indexes between the treatments were analyzed by the LSD test at $P < 0.05$.

3. Results

3.1. Effects of CO_2-modulated cotton seedlings on the host-selection behavior of A. gossypii

A. gossypii adults significantly preferred to select cotton seedlings regardless of the CO_2-rearing levels (ambient CO_2-reared plants, *a*Aphid: +109.09%; *e*Aphid: +170.00% and elevated CO_2-reared plants, *a*Aphid: +136.36%; *e*Aphid: +270.00%) in contrast to the air control treatment ($P < 0.05$; Fig. 2). The aphids reared under ambient and elevated CO_2 did not differ in their level of preference for cotton seedlings, whatever the CO_2 conditions in which the plants developed. However, the number of adult aphids preferred cotton seedlings grown under elevated CO_2 (*e*Cotton) was higher than that of adult aphids preferred cotton seedlings grown under ambient CO_2 (*a*Cotton) for both the ambient CO_2 reared aphids (+13.04%) and elevated CO_2 reared aphids (+37.04%) ($P > 0.05$; Fig. 2).

At the same time, elevated CO_2-reared aphids were significantly more responsive to host odor compared to ambient CO_2-reared aphids and this phenomenon was consistent across all three host odor sources (*a*Cotton, *e*Cotton, and air control) ($P = 0.004 < 0.01$; Fig. 3). Averaged across three odor sources, the number of responding *e*Aphids was significantly higher (+23.3%) than that of responding *a*Aphids ($P < 0.05$; Fig. 3).

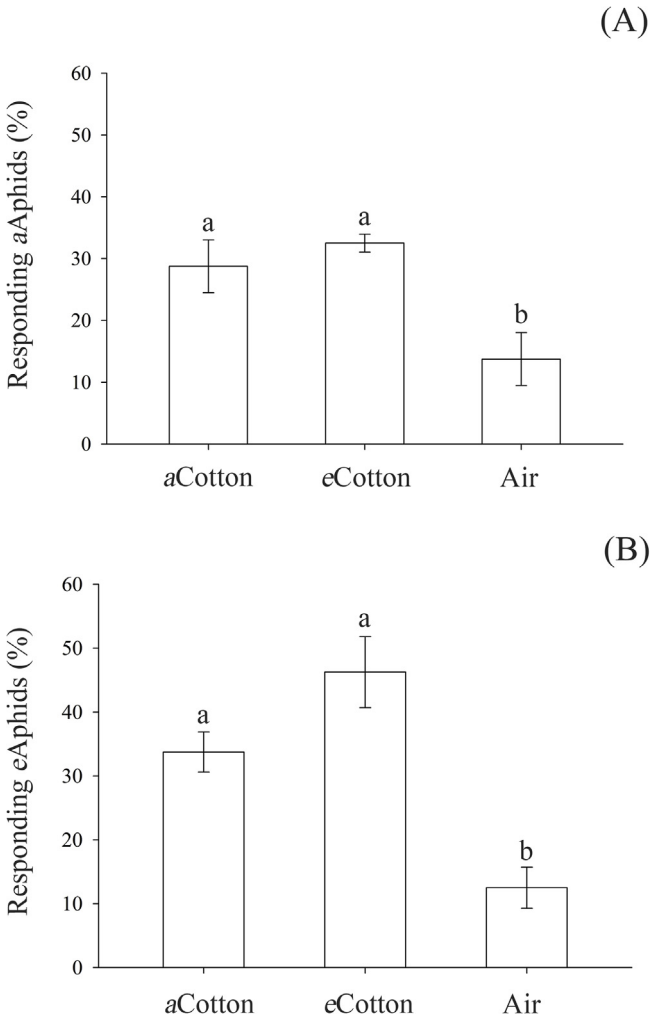

Fig. 2. The percentage of adult *A. gossypii* reared under ambient CO_2 (i.e., *a*Aphids; A) and elevated CO_2 (i.e., *e*Aphid; B) responding to the odor source emanating from cotton seedlings grown under two CO_2 conditions versus an air control deployed in a four-arm olfactometer (Note: each value represents the average (± SE). Aphids – *a*Aphid and *e*Aphid correspond to sampled adult aphids fed on the excised fully expanded leaves from the cotton seedlings grown under ambient and elevated CO_2, respectively; Hosts – *a*Cotton and *e*Cotton correspond to sampled cotton seedlings grown under ambient and elevated CO_2, respectively; Air – the air control treatment; Different lowercase letters indicate significant differences among the host treatments (*a*Cotton, *e*Cotton, and Air) within the same CO_2-reared aphids by the LSD test at $P < 0.05$).

3.2. Effects of CO_2 levels on the growth and energy substances of A. gossypii adults

CO_2 levels significantly affected the adult body weight ($F = 39.49$, $P < 0.001$), and the contents of glycogen ($F = 14.17$, $P = 0.019 < 0.05$), body fat ($F = 10.24$, $P = 0.033 < 0.05$), and amino acids ($F = 129.64$, $P < 0.001$) of the sampled adult aphids fed on cotton grown under ambient and elevated CO_2 (Fig. 4). Compared with ambient CO_2, elevated CO_2 significantly ($P < 0.05$) increased adult body weight (+36.66%; Fig. 4B) and the contents of glycogen (+18.90%; Fig. 4C), body fat (+14.56%; Fig. 4D) and amino acids (+16.78%; Fig. 4E) in *A. gossypii* (see Table 2).

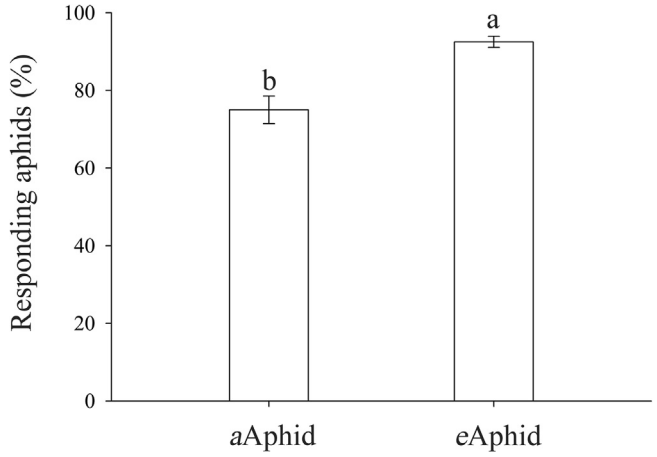

Fig. 3. The percentage of respondent *a*Aphid versus *e*Aphid that were exposed to the three odor sources in an olfactory choice study (Note: each value represents the average (± SE). *a*Aphid and *e*Aphid – the sampled adult aphids fed on the excised fully expanded leaves from the cotton seedlings grown under ambient and elevated CO_2, respectively; Different lowercase letters indicate significant differences between the sampled adult aphids by the LSD test at $P < 0.05$).

3.3. Effects of CO_2 level on the expression levels of odorant-binding protein genes and chemosensory protein genes in A. gossypii adults fed on cotton seedlings grown under ambient and elevated CO_2.

CO_2 levels significantly affected the expression levels of odorant-binding protein genes (*OBP2*: $F = 93.69$, $P < 0.001$; *OBP7*: $F = 11.36$, $P = 0.020 < 0.05$; Fig. 5A) and chemosensory protein genes (*CSP4*: $F = 448.48$, $P < 0.001$; *CSP6*: $F = 7.82$, $P = 0.038 < 0.05$; Fig. 5B) in *A. gossypii* adults fed on cotton seedlings grown under ambient and elevated CO_2. Compared with ambient CO_2, elevated CO_2 significantly ($P < 0.05$) increased the expression levels of *OBP2* (+299.58%; Fig. 3a), *OBP7* (+47.41%; Fig. 3a), *CSP4* (+265.34%; Fig. 3b), and *CSP6* (+50.94%; Fig. 3b).

4. Discussion

Herbivorous insects can identify volatile substances released by host plants during the host selection process through the olfactory receptor (Bartlet et al., 1993; Verkerk and Wright, 1994; Schoonhoven et al., 1998). Possell et al. (2005) detected a significant enhancement of isoprene emissions per unit leaf area in *Mucuna pruriens* under sub-ambient CO_2 concentrations (i.e., 180 µl/L) relative to ambient controls (i.e., 366 µl/L), but not for *Arundo donax*. Loreto et al. (2001) found that the overall emission of monoterpenes at elevated CO_2 will be inhibited because of a concurrent and strong down-regulation of monoterpene synthase activities. Vuorinen et al. (2004) found that volatile organic compound emissions that are induced by the leaf-chewing herbivores will not be influenced by elevated CO_2. This indicates that elevated CO_2 has specific effects on plant volatiles. However, the effects of atmospheric CO_2 concentrations on the volatiles of cotton plants have not been reported. In this study, the results indicated that the aphids reared under ambient and elevated CO_2 did not differ in their level of preference for cotton seedlings, whatever the CO_2 conditions in which the plants developed. However, there is a trend that a greater number of adult aphids preferred *e*Cotton than *a*Cotton for the responding *a*Aphids and *e*Aphid. Whether the future climate change with rising atmospheric CO_2 levels will affect the volatiles of cotton plants and lead to some changes in the host-selection behavior of *A. gossypii* needs further substantiation.

Elevated CO_2 inevitably alters plant metabolites, and this, in turn, would affect the performance of sap-sucking insects by the bottom-up effects of host plants in terms of nutritional status (Awmack and Leather, 2002). For example, elevated CO_2 significantly enhanced the foliar soluble matter of cotton plants, including soluble sugars, free amino acids and fatty acids, which has further positive effects on the population growth of *A. gossypii* in response to elevated CO_2 (Jiang et al., 2016). And the rising atmospheric CO_2 increases the population growth of *Acyrthosiphon pisum* by enhancing food ingestion and improving food quality plasticity, i.e., increasing the contents of amino acids and other nutrient components in host leaves and phloem saps (Guo et al., 2013). As a typical phloem-feeding insect, *A. gossypii* shows much more positive population growth under elevated CO_2 conditions than chewing insects and leaf-mining insects (Bezemer and Jones, 1998; Ge and Chen, 2006; Ge et al., 2010; Sun et al., 2015). In addition, Amsalem and Grozinger (2017) detected that elevated CO_2-treated queen of bumble bees were more active (particularly in terms of flight). In this study elevated CO_2 significantly increased adult body weight, which may provide more energy and favor the life activity of *A. gossypii*. Through oxidative metabolism energy materials such as glycogen, body fat, amino acids, and other constituents serve as primary energy substances in insects, which can use these energy substances singly or in combination as flight fuels (Rankin and Burchsted, 1992). Some short-distance flying insects, such as cockroaches and bees, can use sugars as energy materials (Elliott et al., 1984; Suarez et al., 2005). Body fat also plays an important role in the lifetime of insects. It is a body tissue that contains a variety of metabolic functions. One function is to store and release energy substances to respond to insect energy needs (Arrese and Soulages, 2010), and amino acids are generally used as a supplement for energy. Sun et al. (2008) found a significant increase in the amino acid content in *A. gossypii* under elevated CO_2. The results of this study showed that elevated CO_2 significantly increased adult body weight and the glycogen, body fat and amino acid contents in adult *A. gossypii* compared with ambient CO_2. Therefore, elevated CO_2 can improve the nutritional status of *A. gossypii*, which is more beneficial for the accumulation of energy substances in adult aphids and results in enhanced activity of cotton aphids toward the host crop under rising atmospheric CO_2 conditions.

Both the odorant-binding protein (OBP) and chemosensory protein (CSP) genes are believed to carry some functional proteins, which are involved in the initial identification of odor sensing, by capturing and transporting hydrophobic odor molecules through the hydrophobic lymph to the olfactory receptor neurons (Honson et al., 2005; Pelosi et al., 2005; Pelosi et al., 2006; Zhou, 2010; Sachse and Krieger, 2011). According to the qRT-PCR analysis, the expression levels of odorant-binding protein genes (*OBP2* and *OBP7*) in *A. gossypii* adults were significantly enhanced under elevated CO_2 compared with ambient CO_2. The combination of OBPs and odor molecules is the first biochemical reaction of herbivorous insects' specificity to identify the external odor substances and is the key component of the first function (You et al., 2012; Hu et al., 2013). Similarly, the chemosensory protein genes (*CSP4* and *CSP6*) in *A. gossypii* adults were also significantly improved under elevated CO_2 compared with ambient CO_2. The CSP genes bear more important functions than the OBP genes; one is to dissolve and transport different chemoreceptor fat-affinity ligands, and certain chemosensory proteins are involved in the functional part of olfaction (Nagnan-Le Meillour et al., 2000; Monteforti et al., 2002). Moreover, the CSP genes play an important role in sensory chemical stimulation (Steinbrecht et al., 1995; Ban et al., 2002; Pelosi et al., 2006). It can be presumed that elevated CO_2 can affect the expression of the partial OBP and CSP genes in adult aphids and then change their host-selection behavior at the molecular level. The peak expression of odorant-binding protein genes in different development duration of insects can be considered as a critical period for their physiological function in regulating insects' host-selection behavior (He et al., 2011; Li et al., 2013). In this study, the *OBP6* expression of *A. gossypii* adults did not differ between the *a*Aphids and *e*Aphids, indicating that the *OBP6* does not contribute to the regulation of the host-selection behavior during the adult stage

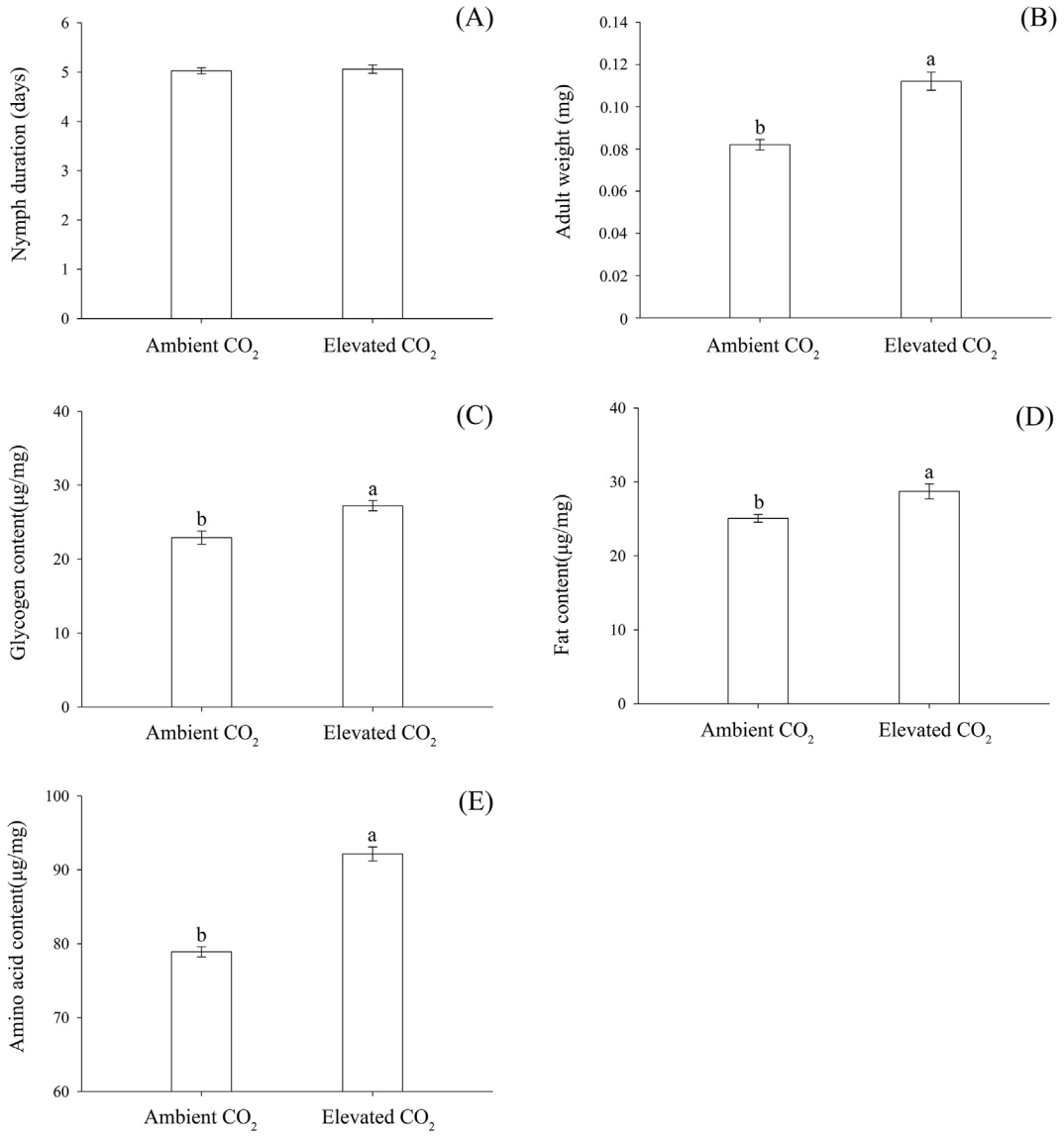

Fig. 4. Nymphal duration (A), adult body weight (B), and the contents of glycogen (C), body fat (D) and amino acids (E) of *A. gossypii* adults fed on cotton seedlings grown under ambient and elevated CO_2. (Note: each value represents the average (± SE). Different lowercase letters indicate significant differences between ambient and elevated CO_2 treatments by the LSD test at $P < 0.05$).

Table 2
One-way ANOVA for the effects of CO_2 levels (ambient vs. elevated) on the growth and development, energy substances, responding aphids, and the expression of odorant-binding protein (*OBP2*, *OBP6* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*) of *A. gossypii* fed on cotton seedlings grown under ambient and elevated CO_2 (*F/P* values).

Measured indexes		*F* value	*P* value
Nymphal duration (days)		0.10	0.75
Adult body weight (mg)		39.49	< 0.001***
Adult body (μg/mg)	Glycogen	14.71	0.019*
	Fat	10.24	0.033*
	Amino acids	129.64	< 0.001***
Responding aphids (%)		21.00	0.004
Odorant-binding genes	*OBP2*	93.69	< 0.001***
	OBP6	0.19	0.68
	OBP7	11.36	0.020*
Chemosensory genes	*CSP4*	448.48	< 0.001***
	CSP6	7.82	0.038*

Note: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

regardless of CO_2 levels.

This study has attempted to elucidate the influence of climate change on the host-selection behavior of *A. gossypii*, that is, elevated CO_2 significantly increased responding aphids in comparison with ambient CO_2, indicating that the host selection activity of *A gossypii* adults can be enhanced under rising atmospheric CO_2 conditions. Two plausible reasons may be proposed for the enhanced host selection under elevated CO_2. First, the increased body weight and enhanced contents of energy substances can improve the general activity of *A. gossypii*. Second, elevated CO_2 increased the expression of odorant-binding protein (*OBP2* and *OBP7*) and chemosensory protein genes (*CSP4* and *CSP6*), which may further enhance the olfactory ability of *A. gossypii*. It is speculated that the rising atmospheric CO_2 level would likely aggravate the damage resulted by *A. gossypii* due to the higher potential host selection and increased general activity under future climate change.

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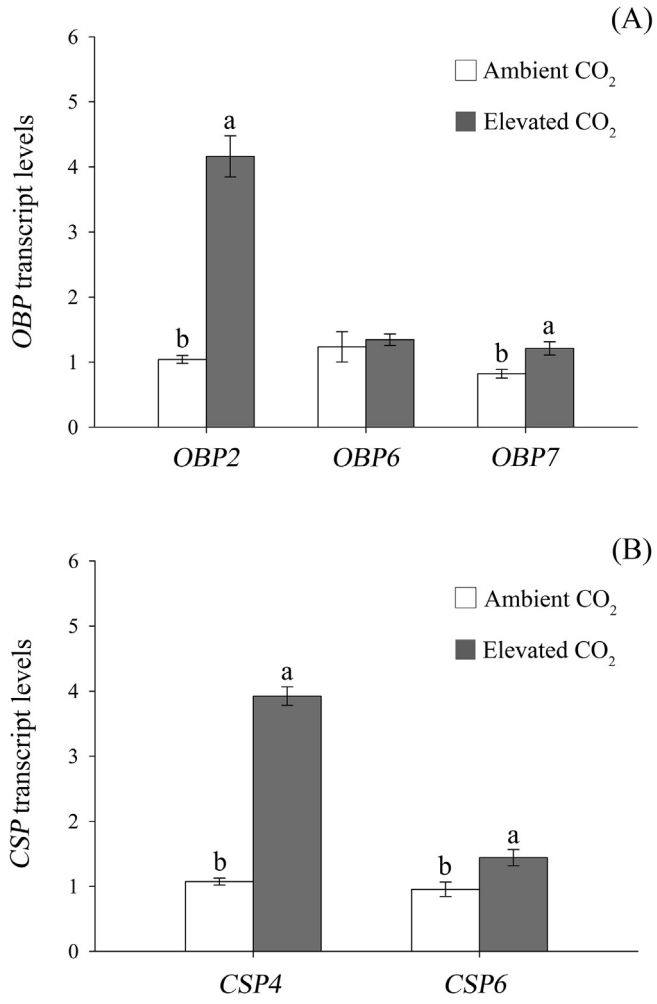

Fig. 5. Transcript expression of odorant-binding protein (A) and chemosensory protein genes (B) of *A. gossypii* adults fed on cotton seedlings grown under ambient and elevated CO_2 (Note: each value represents the average (± SE). Different lowercase letters indicate significant differences between ambient and elevated CO_2 by the LSD test at $P < 0.05$).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jinsphys.2018.05.011.

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Molecular Evidence for the Fitness of Cotton Aphid, *Aphis gossypii* in Response to Elevated CO_2 From the Perspective of Feeding Behavior Analysis

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Rising atmospheric carbon dioxide (CO_2) concentration is likely to influence insect–plant interactions. Aphid, as a typical phloem-feeding herbivorous insect, has shown consistently more positive responses in fitness to elevated CO_2 concentrations than those seen in leaf-chewing insects. But, little is known about the mechanism of this performance. In this study, the foliar soluble constituents of cotton and the life history of the cotton aphid *Aphis gossypii* and its mean relative growth rate (MRGR) and feeding behavior were measured, as well as the relative transcript levels of target genes related appetite, salivary proteins, molting hormone (MH), and juvenile hormone, to investigate the fitness of *A. gossypii* in response to elevated CO_2 (800 ppm vs. 400 ppm). The results indicated that elevated CO_2 significantly stimulated the increase in concentrations of soluble proteins in the leaf and sucrose in seedlings. Significant increases in adult longevity, lifespan, fecundity, and MRGR of *A. gossypii* were found under elevated CO_2 in contrast to ambient CO_2. Furthermore, the feeding behavior of *A. gossypii* was significantly affected by elevated CO_2, including significant shortening of the time of stylet penetration to phloem position and significant decrease in the mean frequency of xylem phase. It is presumed that the fitness of *A. gossypii* can be enhanced, resulting from the increases in nutrient sources and potential increase in the duration of phloem ingestion under elevated CO_2 in contrast to ambient CO_2. In addition, the qPCR results also demonstrated that the genes related to appetite and salivary proteins were significantly upregulated, whereas, the genes related to MH were significantly downregulated under elevated CO_2 in contrast to ambient CO_2, this is in accordance with the performance of *A. gossypii* in response to elevated CO_2. In conclusion, rise in atmospheric CO_2 concentration can enhance the fitness

of *A. gossypii* by increasing their ingestion of higher quantity and higher quality of host plant tissues and by simultaneously upregulating the transcript expression of the genes related to appetite and salivary proteins, and then this may increase the control risk of *A. gossypii* under conditions of climate change in the future.

Keywords: elevated CO_2, *Aphis gossypii*, fitness, feeding behavior, molecular evidence

INTRODUCTION

Global atmospheric carbon dioxide (i.e., CO_2) concentration has continuously risen from about 280 ppm to 408 ppm as on May 2018 (Mauna Loa Observatory: NOAA-ESRL) and future estimations predict an increase up to 550 ppm within a few decades (Pachauri et al., 2014). Rising CO_2 has been an aspect of global climate change, being one great concern for the scientific community, owing not only to its "greenhouse effects" (Tubiello et al., 2000) but also its influences on the physiological and biochemical characters of the plant (Ainsworth and Rogers, 2007). The gaseous form of CO_2 is the direct substrate for photosynthesis in plants (Ziska, 2008), which shows typical increases of photosynthetic rate, biomass, leaf area, and carbon (C): nitrogen (N) ratio, especially in C_3 crops (During photosynthesis, "C" in CO_2 is fixed directly to "C_3" in plants, such as, rice and cotton.) (Ainsworth and Long, 2005; Chen et al., 2005a; Ainsworth et al., 2007). Generally, elevated CO_2 alters plant chemistry by the assimilation and reassignment of C and N resources within plant tissues (Couture et al., 2010). Based on evidence provided by Chen et al. (2004) and Oehme et al. (2013), in spring wheat (*Triticum aestivum*), elevated CO_2 significantly increases the soluble components of plant tissues, such as free amino acids (FAAs), soluble proteins, and glucose. Similar results were also demonstrated by cotton (*Gossypium hirsutum*) plants, that is, elevated CO_2 significantly enhanced foliar soluble matters, including soluble sugars, FAAs, and fatty acids, which had further positive effects on the population growth of the cotton aphid, *Aphis gossypii*, in response to elevated CO_2 (Jiang et al., 2016). As noted in various studies, elevated CO_2 directly affects the primary and secondary metabolites of host plants, which, in turn, indirectly alter the performance of herbivorous insects (Couture et al., 2010; Guo et al., 2014a; Jiang et al., 2016).

Sap-sucking insects have shown consistently more positive responses in fitness to elevated CO_2 concentrations (Sun et al., 2015) than those shown by leaf-chewing insects (Bezemer and Jones, 1998). They feed exclusively on the phloem of their host plants (Douglas, 2003), and the phloem sap mainly contains sucrose (up to 80–85% of the organic components) and soluble proteins (SPs) (Avigad and Dey, 1997). Moreover, sucrose is also recognized as an important transportable sugar in most plant species and as the most effective phagostimulant for herbivorous insects (Hawker, 1985). Moreover, the concentration of SPs in the phloem sap is regarded as a key factor for identifying the nutritional quality of host plants by aphids (Nowak and Komor, 2010). Therefore, since elevated CO_2 inevitably alters plant metabolites, the performance of sap-sucking insects is affected by the bottom-up effects of the host plants in terms of nutritional status (Awmack and Leather, 2002). For example, rising atmospheric CO_2 increases the population growth of *Acyrthosiphon pisum*, owing to enhanced food ingestion and good food-quality plasticity; specifically, it increases amino acids' concentration and other nutrient components in leaves and phloem sap (Guo et al., 2013). Furthermore, the impact of elevated CO_2 on the growth, development, and fecundity of the cotton aphid *A. gossypii* was mainly indirect, which is affected by the nutritional status of the plant (Chen et al., 2005b; Jiang et al., 2016).

Electrical penetration graph (EPG) technique, which monitors the stylet penetration behavior via variation in electrical recording signals, is a well established and effective experimental method to quantify the sap-feeding behavior of aphids (McLean and Kinsey, 1964; Tjallingii, 1988, 1990; Jiang et al., 2015). Our previous study indicated that elevated CO_2 promoted the ingestion efficiency of the cotton aphid *A. gossypii* and simultaneously increased the leaf turgor and foliar soluble constituents of cotton plants (Jiang et al., 2015). Although the feeding behavior of aphids in response to elevated CO_2 has been well established, the underlying molecular mechanism of elevated CO_2-induced changes in the ingestion in aphids remains largely unknown. It has been documented that the feeding behavior of insects is regulated by neuropeptide F (i.e., NPF) and angiotensin-converting enzymes (i.e., ACE) related to appetite (Nassel and Wegener, 2011; Wang et al., 2015). Wu et al. (2003) reported that the expression of NPF was high in the larvae of *Drosophila melanogaster* that were attracted to food, whereas its downregulation coincided with food aversion and hyperactivity of older larvae; the the over-expression of NPF in older larvae conversely promoted feeding and suppressed hypermobility and excessive behaviors. Numerous invertebrates, for example, *Litopenaeus vannamei* and *Melicertus marginatus* (Christie et al., 2011), *Caenorhabditis elegans* (de Bono and Bargmann, 1998), *Periplaneta americana* (Mikani et al., 2012), *Latrodectus hesperus* (Christie, 2015), and *Schistocerca gregaria* (Van Wielendaele et al., 2013), exhibit the fact that NPF has a function in the modulation of feeding behavior. Likewise, it was demonstrated that ACE modulates the aphid–plant interactions by affecting feeding behavior and survival of aphids, through evidence obtained from the knockdown of ACE genes (Wang et al., 2015). Previous studies showed that the salivary sheath protein and C002 play a critical role in the process of stylet penetration and food ingestion in aphids (Mutti et al., 2008; Bos et al., 2010; Abdellatef et al., 2015). Here, the question is what are the underlying molecular mechanisms that elicit the positive responses in the fitness of sap-sucking insects to elevated CO_2.

The Cotton aphid, *A. gossypii*, as a typical phloem-feeding insect, is known as one of the most problematic insect pests

of cotton plants worldwide. In this study, an EPG experiment was carried out with cotton (*G. hirsutum*) plants and the cotton aphid *A. gossypii* under ambient and elevated CO_2 in open-top chambers; simultaneously, an assay to identify the foliar soluble constituents of cotton plants and the molecular biology analysis of the genes related to appetite and salivary proteins of cotton aphids were conducted. The purpose was to examine the effects of elevated CO_2 on stylet ingestion and fitness of phloem-feeding insects on host plants as well as elucidate the molecular mechanisms of feeding behavioral response of phloem-feeders when the host plant is exposed to rising atmospheric CO_2 concentrations.

MATERIALS AND METHODS

CO_2 Levels and Condition Setting

This study was conducted in six identical electronically controlled growth incubators (GDN-400D-4/CO_2; Ningbo Southeast Instrument Co., Ltd., Ningbo, China) with a gas-tank system that maintained the desired CO_2 concentration. In these growth incubators, a periodic regime was maintained at 26°C and 70% RH during the day, 25°C and 70% RH at night, and L14: D10 photoperiod with light at 20000 Lux supplied by LED lamps. The CO_2 concentrations in the three growth incubators mentioned above were set at the current atmospheric CO_2 level (i.e., 400 ppm), and the rest of the three growth incubators were set at an elevated CO_2 level (i.e., 800 ppm), which was the predicted CO_2 level at the end of the 21st century (Mastrandrea et al., 2011). During the experiment, the six growth incubators were alternated by switching CO_2 concentration rates as well as swapping the entire content of each growth incubator every 5 days in order to equalize the possible bias on the cotton plants and aphids due to the incubator-specific growth conditions.

Host Plants and Cotton Aphids

Cotton (cv. C111) was planted in white plastic pots (12 cm diameter, 15 cm high) filled with nutritional soil (Xingnong Organic Fertilizer Co., Ltd., Zhenjiang, China). After the seedlings' emergence, cotton plants were thinned to one plant per pot and exposed to the above mentioned (about 400 ppm) and elevated (about 800 ppm) CO_2 conditions. The cotton plants were watered moderately every day; no additional chemical fertilizers or insecticides were used. At least 60 pots were randomly placed in each growth incubator (i.e., a total of 180 pots of cotton plants per CO_2 treatment) and re-randomized once a week to minimize position effects within each growth incubator.

The colony of the apterous cotton aphid *A. gossypii* used in this study was provided by Prof. Xiangdong Liu from the Department of Entomology, Nanjing Agricultural University. To obtain a standardized aphid colony for this experiment, only one clone in this colony was selected to establish an experimental population of *A. gossypii*. The colony was maintained on 35- to 60-day-old cotton seedlings planted in the same white plastic pots filled with the same nutritional soil in the same electronically controlled growth incubators mentioned above for the following experiments.

Foliar Soluble Constituents of Cotton Seedlings

For the quantitative analysis of foliar soluble nutrition of cotton seedlings, 30 fully expanded leaves on the third to fourth main stem nodes were randomly selected and excised from the potted cotton seedlings in the above mentioned growth incubators of ambient and elevated CO_2 treatments, respectively. Cotton leaves were ground into a fine powder with a mortar and pestle in liquid nitrogen. For the determination of foliar FAAs, the leaf powder (accurately weighed 200–300 mg) was transferred to a 50 ml centrifuge tube, and then, it was diluted to a 10 ml solution by 0.02 mol/L HCl solution. The extraction buffer was sonicated for 15 min at 4°C, and then centrifuged for 15 min at 4,000 rpm/min (RCF = 1503 g) at 4°C to obtain the supernatant containing FAAs, 700 ml of the supernatant was transferred to a 1.5 ml microtube for deproteinization by an equal volume of 4% sulfosalicylic acid solution, then centrifuged for 15 min at 4,000 rpm/min (RCF = 1503 g) at 4°C. The supernatants of the all the samples were individually filtered through 0.22 μm hydrophilic membranes, and, finally, the measurement of FAA concentrations was performed using an automatic FAA analyzer (L-8900; Hitachi High-Technologies Corporation, Tokyo, Japan). The values of FAAs were expressed as mg/g fresh weight.

The above obtained leaf powder was collected, approximately 30–40 mg fresh weight was transferred into a 1.5 ml microtube, and 0.9% saline was used as an extraction buffer at a ratio of 1:9 (tissue weight in g and buffer volume in ml) for the measurement of the foliar SP content. The supernatant of extraction buffer was used as a protein solution for the following test. The foliar SP content was determined by following the instructions of the corresponding diagnostic kit A045-2 (Jiancheng Bioengineering Institute, Nanjing, China). For sucrose determination, 30–40 mg of above obtained leaf power was collected in a 5 ml centrifuge tube with distilled water at a ratio of 1:10 (tissue weight in g and buffer volume in ml), and the mixture was boiled for 10 min and centrifuged at 4,000 rpm/min (RCF = 1503 g) for 10 min. The supernatants were used for assaying the foliar sucrose content according to the corresponding diagnostic kit for the determination of plant sucrose content (Jiancheng Bioengineering Institute, Nanjing, China). There were three replicates for assaying the foliar contents of soluble constituents (including FAA, SP, and sucrose) of cotton seedlings.

Aphid Infestation

Life History Parameters of Cotton Aphids

A total of 45 newborn first instar nymphs were selected from the above mentioned aphid colony of *A. gossypii* and individually reared on fully expanded leaves, which were excised from the 35- to 60-day-old cotton seedlings grown under ambient and elevated CO_2, respectively, in glass culture dishes (150 mm in diameter; one nymph per leaf × one leaf per dish × 15 dishes per growth incubator ×3 growth incubator per CO_2 treatment). Aphid nymphs were monitored twice a day to record molting until they developed into adults. The exuvia was removed, and the ecdysis time was recorded to quantify the nymphal duration of *A. gossypii*. Moreover, the number of offsprings laid per adult

was recorded twice a day, and all the nymphs were removed until the adult aphid died to determine fecundity. The life history parameters of the reproductive period and adult longevity were also, finally, calculated and recorded. In this experiment, eight nymphs of the ambient CO_2 treatment and four nymphs of the elevated CO_2 treatment died in the rearing process, and actually, there were 37 and 41 individuals of *A. gossypii* in the treatments of ambient and elevated CO_2, respectively. But, for assessing survival rate, we added data to 45 replicates in two CO_2 treatments.

Mean Relative Growth Rate (MRGR)

A total of 30 newborn first instar aphids were randomly selected from the above mentioned aphid colony and weighed (i.e., W1) using a precision scale with an accuracy of ±1 μg (Mettler Toledo XP6, Switzerland) and then individually reared using the same protocol for the measurement of life history parameters of *A. gossypii* (i.e., one nymph per leaf × one leaf per dish × 10 dishes per growth incubator × 3 growth incubator per CO_2 treatment). These tested aphid nymphs were reweighed (i.e., W2) by using the same precision scale after 5 days of rearing, and the mean relative growth rate (i.e., MRGR) of *A. gossypii* nymphs was calculated based on the method described by Hodge et al. (2005): MRGR = (lnW2 − lnW1)/t, where W1 is the initial weight, W2 is the final weight, and t is the rearing time (here, 5 days) of *A. gossypii* nymphs. In this experiment, two nymphs of the ambient CO_2 treatment and five nymphs of the elevated CO_2 treatment died in the rearing process, and actually, there were 23 and 20 individuals of *A. gossypii* in the treatments of ambient and elevated CO_2, respectively.

Electrical Penetration Graphs (EPG) to Monitor Aphid Feeding

To monitor the feeding behavior of the cotton aphid *A. gossypii*, 300 newborn first instar nymphs were randomly selected from the above mentioned aphid colony and reared on fully expanded leaves, which were excised from cotton seedlings grown under ambient and elevated CO_2 conditions, respectively, in 30 culture dishes (150 mm in diameter; 10 nymphs per leaf × one leaf per dish × 10 dishes per growth incubator × 3 growth incubator per CO_2 treatment). Once the newborn adult aphids emerged, they were randomly selected and used for the following EPG test.

The feeding activities of the cotton aphid *A. gossypii* were studied by using a Giga-8 DC-EPG amplifier system with 1 GΩ input impedance, 50× amplification, and <1 pA input bias current (Wageningen University, Wageningen, Netherlands). The above mentioned newborn adult aphids were individually connected to a gold wire (0.5 mm diameter, 3 cm long) with conductive silver glue on their dorsum. After 1 h of starvation, the wired adult aphids were carefully placed on the abaxial surface of the fully expanded leaf in the same culture dishes mentioned above, and the other side of the gold wire was connected to the amplifier of the Giga-8 DC-EPG amplifier system. The experiment was conducted in a greenhouse at 26.5 ± 1°C, 70 ± 10% RH, and L14:D10 photoperiod. Based on previous studies, probing behavior was continuously recorded for 5 h, and the 4-h effective records (which contained enough effective information for data analysis) from the beginning of the feeding test were analyzed using the EPG Stylet software (EPG Systems, Wageningen, Netherlands). All recorded signals were analyzed, including non-penetration period (i.e., the NP waveform indicating aphid walking and stylet not probing the host substrate), pathway phase (i.e., the C waveform indicating aphid stylet probing the host substrate to locate the feeding site), phloem phase (i.e., the E waveform, including two events: the E1 waveform showing salivation into phloem sieve elements; the E2 waveform showing ingestion of the phloem content), and xylem phase (i.e., the G waveform indicating ingestion of the xylem sap). In this study, there were eight types of EPG recordings, including the waveforms of NP, C, E1, E2, G, first E1, first E2, and E2 > 8 min (seen in **Table 1**). The waveform parameters of the first E1 waveform and the first E2 waveform indicated the duration of the first occurrence of E1 and E2, respectively; the waveform of E2 > 8 min indicated sustained phloem ingestion for more than 8 min (Kimmins and Tjallingii, 1985; Davis and Radcliffe, 2008).

TABLE 1 | The electrical penetration graphs (EPG) of the cotton aphid *Aphis gossypii* and the respective correlated stylet penetration activities.

EPG waveform	Definition
NP	Non-penetration period
C	Stylet pathway activity (salivary sheath deposition)
E1	Saliva secretion to phloem tissues
E2	Ingestion from phloem tissues
G	Xylem ingestion
First E1	The first occurrence of E1
First E2	The first occurrence of E2
E2 ≥ 8 min	Sustained phloem ingestion for more than 8 min

RNA Preparation and Reverse Transcription

The newborn adults of *A. gossypii* sampled for the molecular test were randomly selected from the tested adult aphids used for the above mentioned EPG test. Once the newborn adults emerged, 20 of them were randomly collected from each growth incubator and mixed as one biological replicate, and there were three biological replicates for the treatments of ambient and elevated CO_2, respectively. Total RNA was extracted from sampled newborn adult aphids by using the TRIzol® reagent (Invitrogen). The concentration and quality of samples were determined by using the NanoDrop™ spectrophotometer (Thermo Scientific) and 1.5% agarose gel electrophoresis. The first-strand complementary cDNA templates were synthesized with 100 ng of total RNA by using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan). Reverse transcriptase reactions were performed in a 20 μl final volume reaction.

Real-Time PCR Analysis

Each cDNA product was diluted from 5× to 80× by diluting twice using RNase-free dH_2O, in order to make the Ct value

TABLE 2 | The primers used for the qRT-PCR analysis of the related target genes of neuropeptide F (NPF), angiotensin converting enzyme (ACE), salivary proteins (C002a and C002b), salivary sheath protein (SHP), molting hormone (MH), and juvenile hormone (JHAMT and JHEH) of the cotton aphid *A. gossypii*.

Primer		Sequence	Description
RPL	Forward	TGCCGGAGTCTGTACTCAA	Housekeeping gene
	Reverse	TCACACCACGAATACGCA	
NPF	Forward	CTATCACAACACCGAGATTAC	Neuropeptide F
	Reverse	AACAGCATGTCATACAAGTC	
ACE	Forward	AGTTCAATGCCTCAATCT	Angiotensin converting enzyme
	Reverse	TAATCCTATAATCTTGTCTGTTG	
C002a	Forward	CCAAGATTAGAGCACGACT	Salivary protein
	Reverse	AAATGTCTAAAGAAACGTCCA	
C002b	Forward	CCGATTAGCCAGAGTGTT	Salivary protein
	Reverse	TGGAAGGAGTGTTGGTAAG	
SHP	Forward	CCTTGTGATTCTACCGATT	Salivary sheath protein
	Reverse	AGCGACCGTATATTCTCT	
MH	Forward	GCAGCGTGTTCGTATCTA	Molting hormone
	Reverse	TTATTCCAGCGGCAATGTA	
JHAMT	Forward	CAGTTGGTTGGTGTTGATAA	Juvenile hormone-III synthase
	Reverse	GCATACTACGCAAGGAATC	
JHEH	Forward	TTTCCGAACGAAATACCGAT	Juvenile hormone epoxide hydrolase
	Reverse	ATCTCGTAAACTGTCGACCA	

to fall within the suitable range of 15–35 based on preliminary experiments. For fluorescence-based quantitative real-time PCR (qRT-PCR), 2 μl of cDNA dilution (100 ng/μl) and 0.2 μM of primers were used in 1× SYBR® *Premix Ex Taq* (TaKaRa) with the 7500 Real-Time PCR Detection System (Applied Biosystems), following the supplier's instructions. Reactions were performed in a 20 μl final volume. Specific primers for testing the genes were designed by Beacon Designer™ 7.9 software, and the housekeeping gene *RPL* was used as the internal standard to analyze the expression levels of target genes, including appetite related genes [i.e., NPF and ACE], salivary protein genes [i.e., C002a, C002b and salivary sheath protein (SHP)], molting hormone (MH) gene (i.e., CYP314A1) and juvenile hormone genes (i.e., JHAMT and JHEH) of the cotton aphid *A. gossypii*, All the primers used for the qRT-PCR test are shown in **Table 2**. Quantification of the transcript levels of target genes was conducted by following the $2^{-\Delta\Delta Ct}$ normalization method. The expression levels of the internal control gene were examined in every PCR plate to eliminate systematic errors. Four biological replicates were made for each treatment in the qRT-PCR analysis, and each biological replicate contained three technical repeats.

Data Analysis

Statistical analysis of all data was performed by using the SPSS v.20.0 software (IBM Corporation, Armonk, NY, United States). One-way analyses of variance (ANOVAs) were used to analyze the effects of CO_2 levels on the foliar contents, on the insect life history parameters and feeding behavior, and on the relative transcript levels of the target genes. Also, the least significant difference (LSD) test was used to analyze the significant differences between the treatments of ambient and elevated CO_2 at $P < 0.05$. Survival data were calculated using the Kaplan–Meier survival curve and were compared using the log-rank test with a significance threshold of $P < 0.05$. Each experiment was compared with a control group, and all experiments were conducted independently at least three times.

RESULTS

Effects of Elevated CO_2 on the Foliar Contents of Soluble Constituents of the Cotton Seedlings

The levels of CO_2 significantly affected the contents of foliar SPs (ie., SPs; $F = 25.59$, $P \leq 0.001$; **Figure 1A**) and sucrose (ie., sucrose; $F = 7.13$, $P \leq 0.05$; **Figure 1B**), whereas these levels failed to significantly affect the content of total FAAs (ie., FAA; $F = 1.09$, $P \geq 0.05$; **Figure 1C**). The order of increase in SPs and sucrose was over 115% and 56% (**Figure 1**), respectively, in elevated CO_2 treatment when compared with ambient CO_2 treatment ($P < 0.05$; **Figures 1A,B**).

Moreover, CO_2 levels significantly affected the foliar serine (Ser) content ($F = 13.54$, $P < 0.01$), whereas these levels failed to significantly affect the contents of other FAAs ($F \leq 4.90$, $P \geq 0.05$; **Table 3**). Compared with ambient CO_2, elevated CO_2 significantly decreased the foliar Ser content of cotton seedlings (−30.23%; **Figure 2**).

Effects of Elevated CO_2 on the Growth, Development, Fecundity, and Survival Rate of the Cotton Aphid *A. gossypii*

The levels of CO_2 failed to significantly affect the development duration of the first, second, and fourth instar nymphs, total nymphal stages ($F \leq 3.58$, $P \geq 0.062$), reproductive period ($F = 0.07$, $P > 0.05$), whereas these levels significantly affected

TABLE 3 | One-way analyses of variances (ANOVAs) for the effects of CO_2 levels (i.e., ambient vs. elevated) on the foliar contents of soluble constituents of cotton seedlings, and the transcript levels of the target genes related to growth, development, and fecundity of the cotton aphid *A. gossypii* fed on the fully expanded leaves excised from the 35- to 60-day-old cotton seedlings grown under ambient and elevated CO_2 conditions.

Measured indexes		*F*-Values	*P*-Values
Cotton seedlings (mg/g fresh weight)	Foliar soluble proteins	25.59	0.000***
	Foliar sucrose	7.13	0.018*
	Foliar free amino acids (FAA)	1.09	0.328
Foliar FAA (mg/g fresh weight)	Asp	0.02	0.895
	Thr	4.90	0.058
	Ser	13.54	0.006**
	Glu	0.82	0.392
	Gly	3.36	0.104
	Ala	0.10	0.762
	Cys	0.05	0.83
	Val	0.81	0.395
	Phe	0.14	0.722
	Lys	2.13	0.183
Transcript levels of target genes in *A. gossypii*	Neuropeptide F (NPF)	10.65	0.017*
	Angiotensin converting enzyme (ACE)	11.62	0.014*
	Salivary protein (C002a)	2.87	0.141
	Salivary protein (C002b)	7.89	0.031*
	Salivary sheath protein (SHP)	34.57	0.001**
	Molting hormone (MH: CYP314A1)	9.32	0.022*
	Juvenile hormone (JHAMT)	1.31	0.295
	Juvenile hormone (JHEH)	5.30	0.061

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

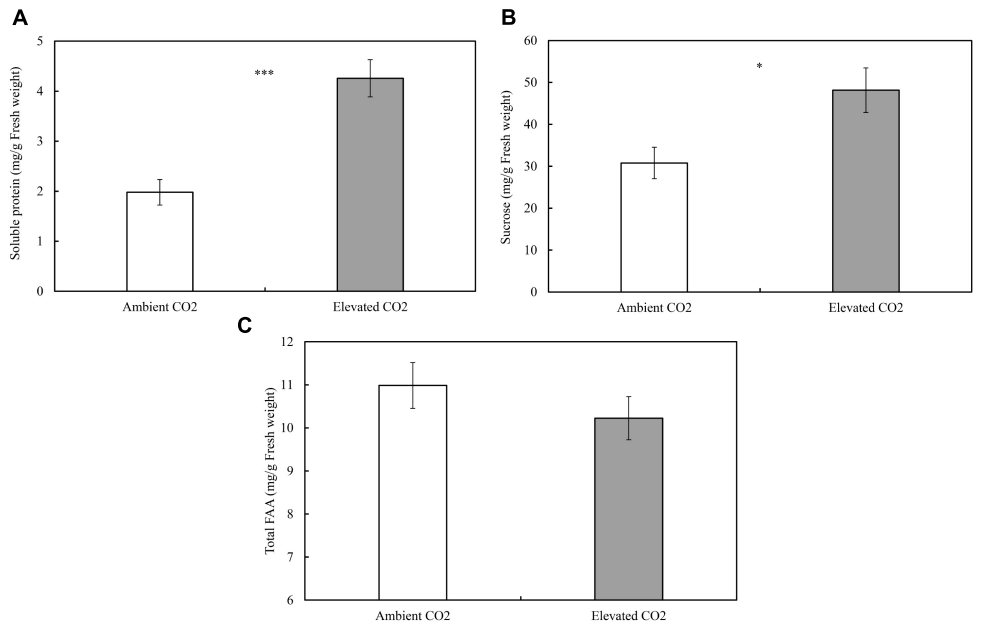

FIGURE 1 | Impacts of elevated CO_2 on the foliar contents of soluble constituents of cotton seedlings (**A**: soluble proteins; **B:** sucrose; **C**: total free amino acids; asterisks indicate a significant difference between the treatments of ambient and elevated CO_2 by the LSD test at $P < 0.05$).

the development duration of the third instar nymph ($F = 4.07$, $P < 0.05$), adult longevity ($F = 4.95$, $P < 0.05$), the whole life span ($F = 5.02$, $P < 0.05$), the fecundity ($F = 4.23$, $P < 0.05$), and the MRGR ($F = 27.69$, $P < 0.001$) of *A. gossypii* (**Table 4**).

Compared with ambient CO_2, elevated CO_2 significantly shortened the development duration of the third instar nymph by 7.56% ($P < 0.05$) and significantly prolonged the adult longevity and whole life span of *A. gossypii* by 14.24% and

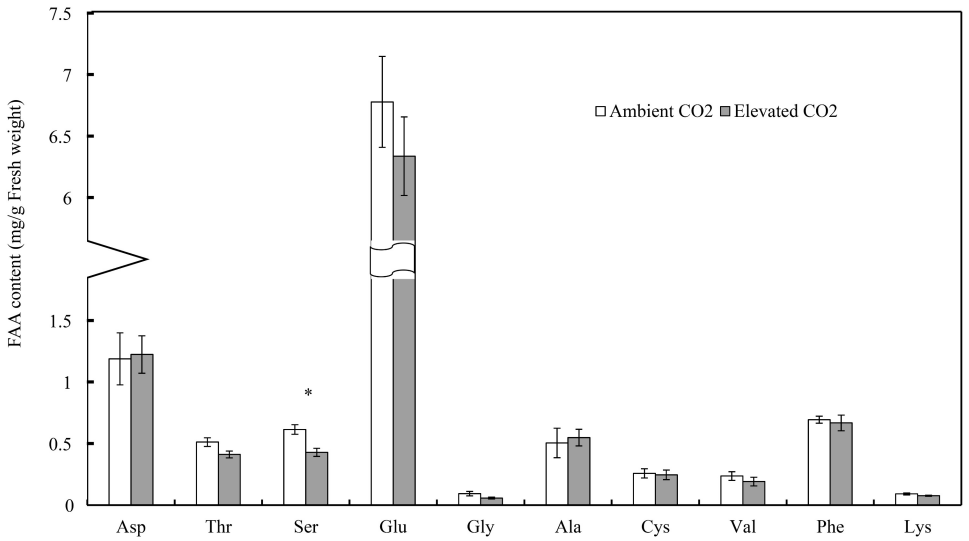

FIGURE 2 | Impacts of elevated CO_2 on the contents of different types of foliar free amino acids of cotton seedlings (Asterisks indicate a significant difference between the treatments of ambient and elevated CO_2 by the LSD test at $P < 0.05$).

TABLE 4 | Mean (±SE) values of the development indexes (including nymph duration, adult longevity, and whole life span), fecundity (including number of offsprings laid per adult and reproductive period), and mean relative growth rate (MRGR) of the cotton aphid *A. gossypii* fed on the fully expanded leaves excised from the 35- to 60-day-old cotton seedlings grown under ambient and elevated CO_2 conditions.

Measured indexes	*df*	CO_2 levels		One-way ANOVAs	
		Ambient CO_2	Elevated CO_2	*F*	*P*
Nymph duration (days)					
The first instar	1, 76	1.59 ± 0.033	1.72 ± 0.055	3.58	0.062
The second instar	1, 76	1.08 ± 0.041	1.12 ± 0.042	0.48	0.491
The third instar	1, 76	1.11 ± 0.034	1.02 ± 0.024	4.07	0.047*
The fourth instar	1, 76	1.24 ± 0.042	1.20 ± 0.039	0.72	0.399
Total nymph stage	1, 76	5.03 ± 0.061	5.06 ± 0.084	0.10	0.749
Adult longevity (days)	1, 76	17.84 ± 0.922	20.38 ± 0.696	4.95	0.029*
Whole life-span (days)	1, 76	22.86 ± 0.905	25.44 ± 0.725	5.02	0.028*
Reproductive period (days)	1, 76	11.91 ± 0.489	12.09 ± 0.480	0.07	0.794
Number. of offspring laid per adult	1, 76	52.14 ± 2.222	57.56 ± 1.506	4.23	0.043*
Mean relative growth rate (MRGR)	1, 41	0.54 ± 0.007	0.60 ± 0.009	27.69	0.000***

Asterisks indicate a significant difference between the treatments of ambient and elevated CO_2 by one-way ANOVAs at $P < 0.05$. Different lowercase letters indicate significant difference between the treatments of ambient and elevated CO_2 by the LSD test at $P < 0.05$.

11.26% ($P < 0.05$), respectively, and simultaneously enhanced the number of offsprings per adult and the MRGR of *A. gossypii* by 10.41% and 10.80%, respectively ($P < 0.05$; **Table 4**). The survival rate of *A. gossypii* from the newborn stage to the death of adult maintained under elevated CO_2 condition was significantly longer ($P = 0.011$), 24.42 ± 0.87 days, than that seen under ambient CO_2 condition 20.82 ± 0.98 days (**Figure 3**).

Impacts of Elevated CO_2 on the Feeding Behavior of the Cotton Aphid *A. gossypii*

The EPG data were used to infer possible changes in feeding behavior of *A. gossypii* under ambient and elevated CO_2 conditions. The data analysis (**Table 5**) indicated that CO_2 levels significantly affected the frequency of G phase ($F = 5.81$, $P < 0.05$) and the mean time from the start of the EPG experiment to the first E1 waveform ($F = 6.77$, $P < 0.05$) and the first E2 waveform ($F = 4.76$, $P < 0.05$), whereas these levels failed to significantly affect the frequency of the other EPG waveforms ($F \leq 0.81$, $P \geq 0.38$) or the total duration of the EPG waveforms ($F \leq 2.89$, $P \geq 0.10$) of the cotton aphid *A. gossypii*.

In contrast to ambient CO_2, elevated CO_2 significantly reduced the frequency of the G waveform by 75.99% ($P < 0.05$) and significantly shortened the time from the start of the EPG experiment to the E1 and E2 waveforms by 48.95% and 40.36%, respectively ($P < 0.05$; **Table 5**). Moreover, an increase in the total duration of the E2 (+33.12%) and E2 ≥ 8min (+29.58%) waveforms was found for the elevated CO_2 treatment in contrast to the ambient CO_2 treatment, respectively ($P > 0.05$; **Table 5**).

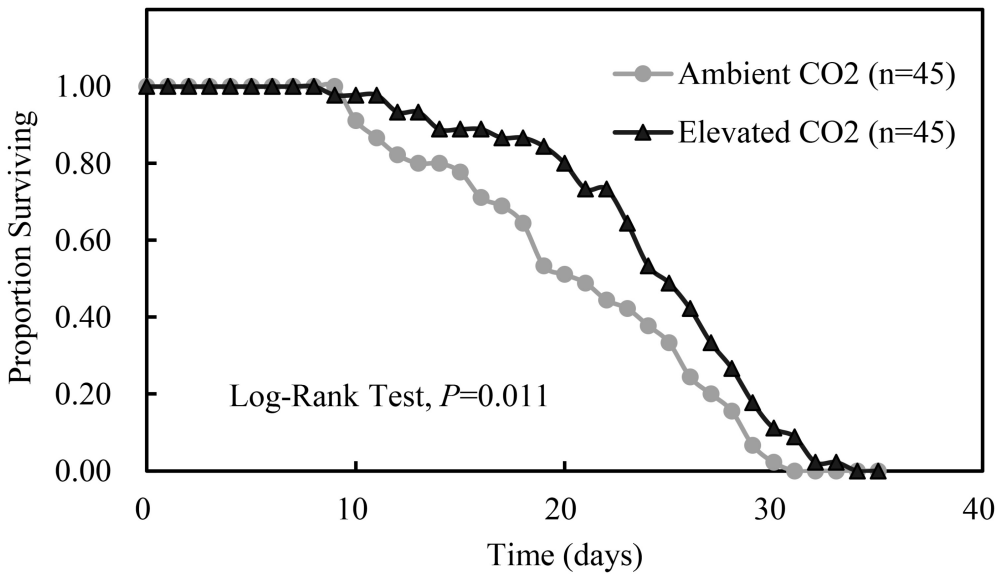

FIGURE 3 | Kaplan–Meier survival curves of *Aphis gossypii* fed on the cotton plant under different CO_2 levels (ambient vs. elevated) (The significant difference between the treatments of ambient and elevated CO_2 were obtained by log-rank test at $P < 0.05$).

TABLE 5 | Mean (±SE) values of the feeding behavior parameters of the cotton aphid *A. gossypii* fed on fully expanded leaves excised from the 35- to 60-day-old cotton seedlings grown under ambient and elevated CO_2 conditions.

Measured indexes of the EPG waveforms	*df*	CO_2 levels		One-way ANOVAs	
		Ambient CO_2	Elevated CO_2	*F*	*P*
Mean frequency of the EPG waveforms					
NP	1, 27	8.8 ± 1.5	8.6 ± 1.4	0.01	0.91
C	1, 27	13.2 ± 2.3	12.8 ± 1.9	0.02	0.89
E1	1, 27	6.3 ± 1.2	7.8 ± 1.0	0.81	0.38
E2	1, 27	4.1 ± 0.9	4.1 ± 0.8	0.004	0.95
G	1, 27	3.9 ± 1.2	0.9 ± 0.3	5.81	0.023*
Mean total duration of the EPG waveforms (min)					
NP	1, 27	7.8 ± 3.2	5.2 ± 1.7	0.47	0.49
C	1, 27	106.6 ± 11.4	114.5 ± 13.9	0.20	0.66
E1	1, 27	25.0 ± 6.6	27.1 ± 6.4	0.06	0.82
E2	1, 27	54.9 ± 15.9	73.0 ± 17.1	0.61	0.44
E2 ≥ 8 min	1, 27	47.8 ± 15.0	61.9 ± 16.8	0.40	0.54
G	1, 27	45.9 ± 12.6	20.1 ± 7.9	2.89	0.10
Mean time from the start of the EPG experiment to the E1 and E2 waveforms (min)					
The first E1	1, 27	72.1 ± 10.6	36.8 ± 8.2	6.77	0.015*
The first E2	1, 27	99.2 ± 15.9	59.2 ± 8.3	4.76	0.038*

NP, non-penetration period; C, stylet pathway activity; E1, saliva secretion to phloem tissues; E2, ingestion from phloem tissues; G, xylem ingestion; The first E, the first occurrence of E1; The first E2, the first occurrence of E2; E2 ≥ 8 min, sustained phloem ingestion for more than 8 min. Asterisks indicate a significant difference between the treatments of ambient and elevated CO_2 by one-way ANOVAs at $P < 0.05$.

Impacts of Elevated CO_2 on the Expression of the Target Genes Related to Growth, Development, Reproduction, and Feeding of the Cotton Aphid *A. gossypii*

The levels of CO_2 significantly affected the expression levels of the appetite related genes of NPF ($F = 10.65$, $P < 0.05$) and ACE ($F = 11.62$, $P < 0.05$), the salivary protein genes of C002b ($F = 7.89$, $P < 0.05$) and SHP ($F = 34.57$, $P < 0.01$), and the MH gene of CYP314A1 ($F = 9.32$, $P < 0.05$), whereas these levels failed to significantly affect the expression levels of the salivary protein gene of C002a ($F = 2.87$, $P > 0.05$), the JH genes of JHAMT ($F = 1.31$, $P > 0.05$) and JHEH ($F = 5.30$, $P > 0.05$) in the cotton aphid *A. gossypii* fed on fully expanded leaves excised from the 35- to 60-day-old cotton

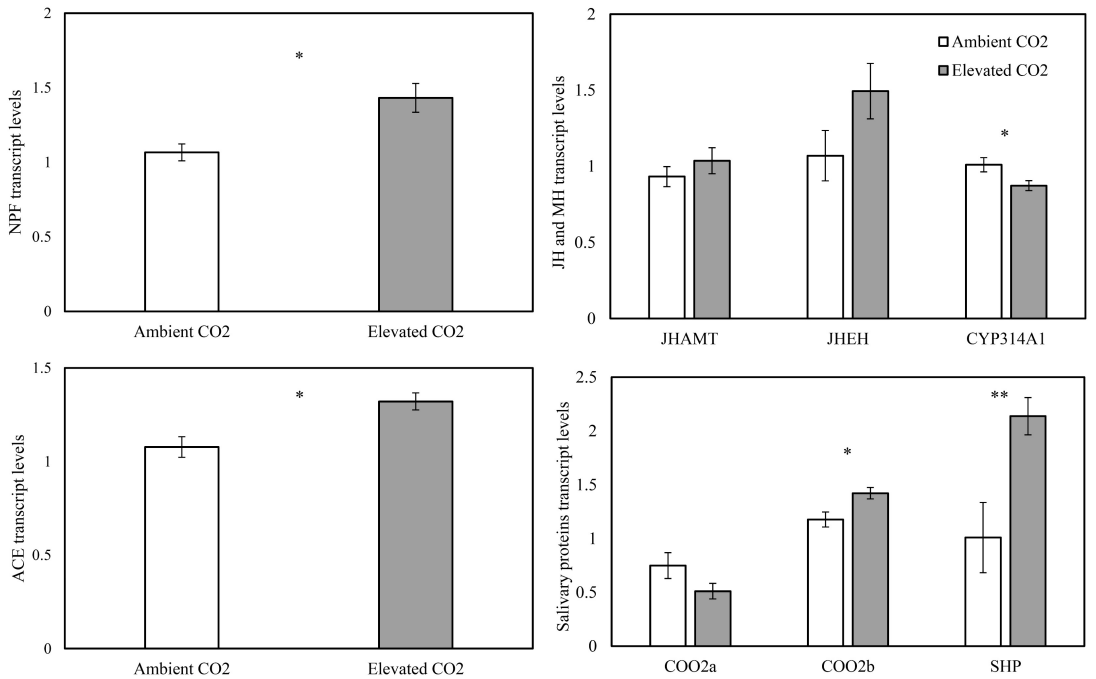

FIGURE 4 | Relative transcript levels (Mean ± SE) of the appetite genes of neuropeptide F (NPF) and angiotensin converting enzyme (ACE), the salivary protein genes of C002a, C002b, and salivary sheath protein (SHP), and the molting hormone (MH) gene of CYP314A1, and the juvenile hormone (JH) (JH) genes of JHAMT and JHEH of the cotton aphid, *Aphis gossypii* fed on the fully expanded leaves excised from the 35- to 60-day-old cotton seedlings grown under ambient and elevated CO_2 (Asterisks indicate a significant difference between the treatments of ambient and elevated CO_2 by the LSD test at $P < 0.05$).

seedlings grown under ambient and elevated CO_2 conditions (**Table 3**).

As compared with ambient CO_2, elevated CO_2 significantly upregulated the relative transcript levels of the salivary protein genes of C002b and SHP by 20.80% and 111.85% and the appetite related genes of NPF and ACE by 34.27% and 22.66%, respectively ($P < 0.05$), simultaneously, downregulating the relative transcript level of the salivary protein gene of C002a by 31.80% ($P > 0.05$; **Figure 4**). Moreover, elevated CO_2 also upregulated the expression levels of the JH genes of JHAMT and JHEH by 11.20% and 39.64%, respectively ($P > 0.05$), simultaneously, significantly downregulating the expression levels of the MH gene of CYP314A1 by 13.60% ($P < 0.05$; **Figure 4**).

DISCUSSION

Currently, the global atmospheric CO_2 concentration continues to rise, standing now at 400 ppm and possibly reaching 800 ppm by the end of this century (Pachauri et al., 2014). As the main factor responsible for global warming, elevated CO_2 directly induces changes in plant growth, development, metabolism, and plant chemistry (Dader et al., 2016; Jiang et al., 2016); meanwhile, insects are sensitive to these environmental variations, which cause changes in their behavior, growth, development, fertility, and the occurrence of populations as a result of metabolic rate fluctuation (Sun et al., 2015, 2017; He et al., 2017). With the elevated CO_2 condition, Oehme et al. (2013), in their study, observed that the concentrations of fructose and glucose in spring wheat showed a significant increase, whereas the total amino acid concentration was not altered. These changes in plant chemistry positively affect the relative growth rate (RGR) of aphids. In this study, we found that elevated CO_2 had significant effects on the soluble nutrients of cotton, which, thereby, were beneficial to the performance of *A. gossypii* because of the bottom-up effects of the plant, which was in accordance with previous studies (Guo et al., 2013, 2014a). Moreover, the qPCR results also indicated that elevated CO_2 induced a certain degree of upregulation in JH transcription and a significant downregulation in MH transcription, whereas the transcription of genes related to appetite (NPF and ACE) and salivary proteins (C002b and SHP) was significantly upregulated under elevated CO_2; all these molecular evidences determined here supported our findings well.

In general, elevated atmospheric CO_2 generally presents positive effects on foliar soluble nutrition of plants, especially in C_3 plants (Chen et al., 2005a; Wu et al., 2007; Guo et al., 2013). These alterations on the quality of plant host tissue can directly affect the performance of herbivorous insects. However, the response to elevated CO_2 varies between insects that have piercing and chewing mouthparts (Coll and Hughes, 2008; Sun et al., 2016). A recent meta-analysis examining the effects of elevated CO_2 on the life history traits of insects found that while the abundance of foliage feeders tends to decrease, phloem feeders on average tend to perform better under elevated CO_2 (Robinson et al., 2012). Generally, elevated CO_2 shows

negative effects on chewing insects with a decline in the foliar nitrogen content of host plants; as a recent study on the cotton bollworm, *Helicoverpa armigera*, showed that larval durations were significantly prolonged by elevated CO_2, additionally, female pupal weight, fecundity, and total population size under elevated CO_2 were lower than ambient CO_2 (Liu et al., 2017). In contrast, aphids, as a kind of phloem feeders, are considered the only feeding guild that positively responds to elevated CO_2. Our previous study has shown that, according to four successive generation data, elevated CO_2 significantly increases fresh body weight, fecundity, and population abundance of *A. gossypii* (Jiang et al., 2016). In regard to *Rhopalosiphum padi* reared on *Hordeum vulgare*, which was maintained under elevated CO_2, there was a significant increase in aphid abundance and intrinsic rate of population increase; however, there were no statistically significant effects on fecundity and development time of the aphid, such beneficial performance of *R. padi* results from plant biochemical response; under elevated CO_2 (Ryan et al., 2015). However, piercing-sucking insect seems to have a species-specific response; in terms of studying the population responses of five aphid species to elevated CO_2, one species showed an increase (*Myzus persicae*), one showed a decrease (*A. pisum*), and the other three remained unaffected (*Aphis nerii*, *A. oenotherae*, *Aulacorthum solani*) (Hughes and Bazzaz, 2001). *Bemisia tabaci* under elevated CO_2 treatment had a neutral response with no alterations in its life span, sex ratio, and fecundity (Sun et al., 2011). So, as CO_2 is the substrate for plant photosynthesis, elevated CO_2 may directly alter physiological and biochemical processes in plants. Furthermore, this indirectly affects insect physiological metabolism by changing plant nutrition and plant defense (Todgham and Stillman, 2013; Guo et al., 2014a,b).

According to our study on the expression of key genes of the JH and MH pathways, it indicated that elevated CO_2 slightly decreased MH transcription and mildly increased JH transcription. The JH and the main ecdysteroid (20E), known as highly versatile hormones, regulate many aspects of insect physiology, such as development, growth, reproduction, and aging (Riddiford, 1994; Flatt et al., 2008). Recent research suggested that JH was also involved in the regulation of final insect size and growth rates (Mirth et al., 2014). Studies on the tobacco hornworm *Manduca sexta* showed that a decline in circulating JH initiates the first step in the hormonal cascade that begins with the attainment of critical weight, and ends, after a terminal growth period (TGP), with the rise in circulating ecdysone that stops body growth (Fain and Riddiford, 1975; Cymborowski et al., 1982). Intriguingly, similar result was observed in *D. melanogaster*; additionally, it was demonstrated that the effect of JH on growth rate and final body size was mediated by ecdysone synthesis via the regulation of the insulin/insulin-like growth factor (IGF) signaling (IIS) pathway by JH, without affecting the developmental timing (Colombani et al., 2005; Mirth et al., 2014). Our research speculated that JH might be cooperating with MH on regulating MRGR in *A. gossypii* through the key effector of the IIS pathway, like the results obtained of Mirth et al. (2014). These data were in line with previous studies that speculated a cross talk between JH and IIS in *A. gossypii*.

In this study, for the purpose of matching the higher growth rate observed under elevated CO_2, *the A. gossypii* aphids needed to increase their food intake to obtain enough nutrition for growth. So, our EPG recordings showed that *the A. gossypii* aphids had higher efficiency of stylet penetration under elevated CO_2 compared with ambient CO_2. In our previous study, one reason for this result might be that the increase in leaf turgor and soluble constituents of the leaf favored ingestion in *A. gossypii* (Jiang et al., 2016). Sun et al. (2015) also provided the evidence for increased ingestion under elevated CO_2; that is, originally, *A. pisum* infestation triggered the abscisic acid (ABA) signaling pathway to decrease the stomatal apertures of *Medicago truncatula*, which consequently decreased leaf transpiration and helped to maintain the leaf water potential. Furthermore, elevated CO_2 upregulates an ABA-independent enzyme, carbonic anhydrase, which led to a further decrease in the stomatal aperture of aphid-infested plants. Thus, the effects of elevated CO_2 accentuated stomatal closure and synergistically increased leaf turgor in plants, resulting in enhanced aphid feeding. The second case might be that elevated CO_2 alters plant resistance. For piercing-sucking insects, the results obtained by Sun et al. (2013) indicated that the JA-regulated defense against M. persicae was more effective than the SA-regulated defense in *Arabidopsis* and that elevated CO_2 tends to enhance the ineffective SA signaling pathway and reduce the effective JA signaling pathway against aphids. Later, similar studies in regard to *A. pisum* reared on *M. truncatula* also demonstrated that elevated CO_2 enhances the SA-dependent defense pathway and suppresses the JA/ethylene-dependent defense pathway (Guo et al., 2014b, 2016). A recent research on elevated plant resistance in response to CO_2 indicated that the heat shock protein 90 plays a critical role in plant resistance against the aphid under elevated CO_2 (Sun et al., 2017). Taken together, elevated CO_2 increased host water potential and decreased plant resistance against piercing-sucking insects, which favored ingestion and growth of *A. gossypii*.

With respect to the appetite of *A. gossypii*, our results indicated that elevated CO_2 significantly increased aphid appetite and further regulated the feeding behavior. As known, SHP and C002 play important roles in the stylet probing phase and phloem feeding phase, respectively, in piercing-sucking insects. Insect stylet movement is accompanied by the secretion of gel saliva, which forms a salivary flange on the epidermis and an enveloping salivary sheath in the apoplast, both of which may provide stability, lubrication, and protection during feeding, while the latter also seals the plasma membrane at stylet penetration sites (Will and van Bel, 2006; Will et al., 2012). Abdellatef et al. (2015) showed that silencing the expression of SHP causes transgenerational feeding suppression in *Sitobion avenae*, additionally, reduced SHP expression correlates with a decline in growth, reproduction, and survival rates. The *A. pisum* aphids inject the protein C002 into the host plant during feeding to increase the acquisition of phloem sap. Knockdown of C002 in this aphid causes a decrease in the time that it spends in contact with the phloem sap (Mutti et al., 2008). In the current experiment, our results have revealed for the first time that the increased expression of the salivary protein genes was induced by the high expression of the insect appetite related genes and that

this then leads to the higher efficiency of ingestion under elevated CO_2 condition. But, in the present study, still only little was known about how elevated CO_2 impacts the appetite of aphids; it needs further studies in the future. Overall, elevated CO_2 could increase plant phloem nutrition, which, in turn, favored the fitness of aphids via enhanced ingestion due to improved appetite. All the supporting evidences might point to the fact that the rising CO_2 concentration increases the risk of pest control under the conditions of climate change in the future.

AUTHOR CONTRIBUTIONS

All the authors listed have made a substantial, direct, and intellectual contribution to the work and have approved its publication. SJ and FC designed the study. SJ, YD, YqL, and YmL performed the experiments. SJ and FC analyzed the data. SJ wrote the manuscript. SJ, FC, MP, SF, and MB reviewed and polished the manuscript.

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