TEXAS A&M AGRILIFE RESEARCH & EXTENSION

COTTON ENTOMOLOGY
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TEXAS A&M AGRILIFE RESEARCH, CLIFF LAMB, DIRECTOR
THE TEXAS A&M UNIVERSITY SYSTEM, COLLEGE STATION, TEXAS

COTTON ENTOMOLOGY PROGRAM

RESEARCH ACTIVITY ANNUAL REPORT

2021

SUBMITTED TO:

PLAINS COTTON IMPROVEMENT COMMITTEE
PLAINS COTTON GROWERS, INC.

JAROY MOORE, CENTER DIRECTOR
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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. The team effort of PCG, Texas A&M AgriLife Research and AgriLife Extension Service over several decades resulted in a comprehensive understanding of boll weevil ecology and behavior. 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 two decades.

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 20 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 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 in Lubbock and statewide field crop entomologists, 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. Together, we have maintained the Texas High Plains area as a characteristically low cotton insect-pest prevalence region in the U.S. cotton belt.

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 cotton 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 AND TOBACCO BUDWORM MOTHS IN THE TEXAS HIGH PLAINS

A long-term study is investigating the seasonal moth flight activity patterns of bollworm and tobacco budworm in the Texas High Plains. The regional adoption of cotton and corn cultivars incorporating *Bt* technology has contributed to reduced level of these lepidopteran pests in recent years; however, constant threat of insect resistance to transgenic technology and diminishing underground water availability for irrigation is necessitating lower crop inputs, such as transgenic seed costs, for increasing dryland crop acreage, increasing the importance of these pests.

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

COTTON FLEAHOPPER SUSCEPTIBILITY OF PRE-FLOWER COTTON UNDER LIMITED IRRIGATION PRODUCTION

The objective of this project is to investigate the growth and fruiting response of cotton after cotton fleahopper infestation at three discrete cotton fleahoper susceptible stages (prior to visible squares, 1-2 square, and 3-4 square stages) of cotton under three irrigation water levels. We also quantify cotton compensatory potential following cotton fleahopper induced square loss under phenological stage x irrigation treatments.

Cotton fleahopper augmentation at three crop phenological stages and inspection to determine insect colonization and crop injury

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 two water-deficit (dryland and full-irrigation) conditions (10 pest management scenarios). This study will enable development of research-based action thresholds considering variable yield potential under different water deficit scenarios. These 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

STATEWIDE RESEARCH-EXTENSION PROJECT TO ADDRESS CURRENT COTTON INSECT MANAGEMENT ISSUES

Multi-year statewide studies are being conducted at several Texas locations to represent cotton fields surrounded by variable vegetation/crop complexes and regional insect population pressure in cotton. Study objectives are to evaluate spray tip selection and its impact on insecticide efficacy, cultivar sensitivity to cotton fleahopper herbivory, fleahopper threshold, and cotton bollworm pyrethroid resistance. Research and Extension entomologists from south, central, and north Texas, including IPM agents from throughout Texas cotton production regions collaboratively conduct research to address these project objectives. Lubbock Cotton Entomology Project focuses on cotton fleahopper cultivar susceptibility and threshold.

Field evaluation of spray tip on thrips management

Cotton fleahopper susceptibility and compensatory potential of three distinct phenological stages of pre-flower cotton in water-deficit production scenario

Cotton Incorporated – Core Program
Project Number: 20-246

Megha N. Parajulee
Texas A&M AgriLife Research and Extension Center, Lubbock, Texas

Project Summary

The recent increase in limited-irrigation cotton production in the Texas High Plains has demanded development of pest management strategies at low-input production system. Our current understanding is that cotton fleahoppers can be injurious to cotton during 3-weeks of squaring until about the appearance of first flower. That may warrant possible management of cotton fleahoppers up to three discrete stages of cotton prior to flowering as stated earlier. Impact of cotton fleahoppers on pre-squaring stage, especially when fleahoppers migrate to cotton prior to the occurrence of visible squares, and late squaring/first-flower stage is not quantified. Our earlier work on cotton fleahopper compensation studies suggest that cotton plants can tolerate up to 20% fruit loss. This project aims to investigate the growth and fruiting response of cotton after cotton fleahopper induced square loss at three discrete cotton fleahoper susceptible stages of cotton under deficit-irrigation scenario. The specific objectives of the study were to 1) quantify the damage potential of cotton fleahopper (feeding injury and/or square abortion) at square initiation (prior to visible squares), 1-2-square, and 4-5-square stages of cotton under dryland, deficit irrigation versus full irrigation, 2) determine cotton growth parameters and fruiting profiles as influenced by cotton fleahopper injury at three discrete cotton fleahoper susceptible stages of cotton under deficit-irrigation scenario, and 3) quantify cotton compensatory potential following cotton fleahopper induced square loss under phenological stage x irrigation treatments.

This study is expected to generate a significant amount of data to elucidate the damage potential of cotton fleahoppers at three discrete cotton fleahopper susceptible stages under two drought-stress conditions, including low/supplemental irrigation (drought stress) and full irrigation (no drought stress), and cotton's response to cotton fleahopper injury under each production scenario. The data regarding how the cotton fleahopper injury x drought-stress conditions impact cotton performance at three discrete phenological stages will be useful in making management decisions based on economic models.

Cotton fleahopper infestation at pre-squaring stage reduced cotton lint yield across all three irrigation treatments, although significant only under dryland condition. It is plausible that fleahoppers fed on growing terminals and likely damaged the invisible squares which ultimately reduced the lint yield. Cotton fleahoper infestation also impacted fiber quality, with improved micronaire values under full irrigation. The two-year study clearly suggests that there is an apparent interaction between fleahopper-induced injury to cotton and irrigation water availability for plants to overcome the injury effect, thereby influencing the lint yield and fiber quality. Additional 2-3 years of studies will provide more insight into these results.

Introduction

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

Predominantly, cotton fleahoppers feed upon pinhead-sized or smaller squares, which results in abortion of these young fruits, thereby impacting yields. While cotton fleahopper feeding preferences serve as a baseline for their management in cotton fields, a detailed understanding of cotton plant responses to fleahopper damage remains unachieved. Because cotton vulnerability to cotton fleahoppers spans over a period of 3-4 weeks, information on acute infestation of cotton fleahopper at phenologically-specific crop stages may help cotton producers make appropriate management decisions in low-input, water-deficit production systems. Cotton plant growth is sensitive to numerous environmental and management input factors, particularly irrigation and cultivar traits. Cotton growth responses to various input factors are well-documented and growth models have been developed. However, the specific cotton plant responses to cotton fleahopper injury at phenologically discrete cotton fleahopper susceptible stages remain uninvestigated. This research project proposes to evaluate the cotton crop growth parameters and lint yield following cotton fleahopper acute infestations at three distinct cotton fleahopper susceptible cotton stages (pre-squaring, 1-2-square stage, 4-5-square stage) under deficit-water versus full-irrigation production regimes.

Methodology

The study was conducted at the Texas A&M AgriLife Research farm in Lubbock. A 5-acre subsurface drip irrigation system has been in place for this study. Main-plot treatments included full irrigation, supplemental irrigation, and dryland. The full irrigation water level was created via 90% replenishment of evapotranspiration (ET) requirement for THP, whereas the supplemental irrigation treatment received 30% ET replenishment. Cotton cultivar DP 1820B3XF was planted on 18 May 2020. In 2021, cotton cultivar DP1845B3XF was planted on 18 May, but the crop was destroyed by repeated rain and hailstorm events and the test was replanted on 9 June. Sub-plot treatments included three discrete phenological stages of cotton that is considered susceptible to cotton fleahopper damage: 1) prior to the occurrence of visible squares on seedling cotton or "pre-square" cotton, 2) cotton at 1-2 visible squares stage or early squaring stage, and 3) cotton with 4-5 squares and close to the occurrence of first flower or late squaring).

Two 3-ft sections of uniform cotton were flagged in the middle two rows of each treatment plot (3 irrigation treatments x 3 phenological stages x 2 insect augmentation treatments x 4 replications = 48 experimental units) for insect treatment deployment. At each phenological stages, 5 cotton fleahopper nymphs per plant versus no fleahopper augmentation as control were deployed in these designated row sections to simulate an acute infestation of cotton fleahoppers.

Woolly croton, a cotton fleahopper weed host, was harvested from locations in and near College Station, Texas, in early February and stored in cold storage until fleahoppers were needed for the study. Conditions conducive to cotton fleahopper emergence were simulated in a laboratory environment to induce hatching of overwintered eggs embedded in the croton stems, and emerged cotton fleahoppers were subsequently reared using fresh green beans as a feeding substrate.

Considerable effort was expended to ensure synchronization of rearing efforts with cotton crop development for optimal release timing for each of the three cotton phenological stages. A single release nymphal cotton fleahopper was timed to simulate the acute heavy infestation of cotton fleahoppers (3-4 days of feeding) at each stage. This arrangement ensured significant damage on treatment plots to quantify the variation in damage potential as influenced by cotton phenological stage. The actual release dates in 2020 were 20 June (pre-square), 1 July (early square), and 21 July (late square). Cotton fleahopper rearing cages were installed about a month prior to the first release (e.g., 20 May 2020 for 20 June 2020 release) and staggered the cage installation for the next 4-5 weeks to ensure a continuous supply of cotton fleahopper nymphs for the study. In 2021, actual release dates for pre-square, early square and late-square cotton stages were 2 July, 16 July and 26 July, respectively.

The release was accomplished by manually placing second- to third-instar cotton fleahopper nymphs from the laboratory colony onto the terminals of plants in each treatment plot at the rate of 5 nymphs per plant; the control plots received no fleahoppers and were kept fleahopper-free during the entire study period. Because natural infestation of cotton fleahopper was absent at the experimental farm, the control plots received no insecticidal intervention. An insecticide (acephate 97% 6 oz/acre) was used to kill all remaining cotton fleahoppers after the one-week feeding period in all experimental units to ensure complete removal of released cotton fleahoppers. The entire test was kept insect-free for the remainder of the study to isolate the effect of cotton fleahopper injury only.

Data collection included monitoring of flowering patterns, fruit abscission, and plant height. In 2020, flower monitoring was initiated on 20 July and conducted every 2-3-day intervals with total of 14 sample dates, and in 2021, flower monitoring was started on 7 August and ended on 10 September with a total of 18 sample dates. Harvest aids Boll'd® 6SL (Ethephon [(2-chloroethyl) phosphonic acid] @ 1 qt//acre (boll opener) and Folex® 6 EC (S, S, S-tributyl phosphorotrithioate) 1 pint/a (defoliant) were applied on 12 October in 2020, and in 2021, Boll'd® 6SL (Ethephon [(2-chloroethyl) phosphonic acid] @ 1 qt//acre (boll opener) and Gramoxone® SL 2.0 (Paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) were applied on 25 October and 5 November, respectively, to accelerate opening of matured unopened bolls and begin the defoliation process. Test plots were hand-harvested on 11 and 12 November. Hand-harvested yield samples were ginned, and the samples were analyzed for fiber quality parameters (HVI) at Cotton Incorporated.

Results and Discussion

2020 Study

Cotton fleahopper induced square injuries exerted very low level of square abscission (10-15%). Irrigation water level significantly influenced the cotton lint yield, as expected, with significantly higher yield with increased level of irrigation. Averaged across cotton fleahopper augmentation treatments, dryland produced the lowest lint yield (1102 lb/acre), followed by low water (1420 lb/acre), and the highest lint yield was observed under full irrigation (1691 lb/acre) (Fig. 1). Despite low insect injury, cotton fleahopper infestation at pre-squaring stage (before the onset of visible squares) reduced cotton lint yield across all three irrigation treatments, although the value was statistically significant only under dryland condition (Fig. 2). Even though not significant due to high data variation, lint yields were conspicuously reduced in both supplemental and full irrigation treatments when cotton fleahoppers were augmented at pre-square stage (Fig. 2). It is plausible that fleahoppers fed on growing terminals and likely damaged the invisible squares which ultimately reduced the lint yield. Also, cotton fleahopper infestations at early as well as late squaring (pre-flower) cotton did not reduce lint yield at any of the three irrigation regimes. Figure 2 suggests that cotton compensated or overcompensated (numerically) any fruit loss due to fleahopper-induced injury, ultimately showing no significant effect on lint yield. Early square stage of cotton appeared to be more susceptible to cotton fleahoppers than late squaring cotton under dryland condition; however, irrigated cotton did not show such differential responses. Manual removal of squares (100% squares removed at the time of first flower coinciding with the fleahopper infestation at late squaring stage) significantly reduced the lint yield under dryland condition, but plants compensated the manually removed fruit abscission under both irrigated conditions.

Cotton fleahopper infestation also impacted fiber quality while the plant response to cotton fleahopper injury was influenced by irrigation water level. High water treatment resulted in micronaire values in the premium range for all fleahopper augmentation sub-plot treatments (Fig. 3). Interestingly, lint fiber from the uninfested control plots had micronaire in the premium range, but the micronaire values increased and moved away from premium range to base range for all FH-augmented plots (Fig. 3). All sub-plot treatments resulted in micronaire values at base range under supplemental irrigation. Manual removal of squares resulted in premium micronaire value under dryland and base value under both irrigation regimes. Other fiber quality parameters varied marginally with insect augmentation X irrigation interactions (Table 1). These data clearly suggested an apparent interaction between fleahopper-induced injury to cotton and irrigation water availability for plants to overcome the injury effect, thereby influencing the lint yield and fiber quality.

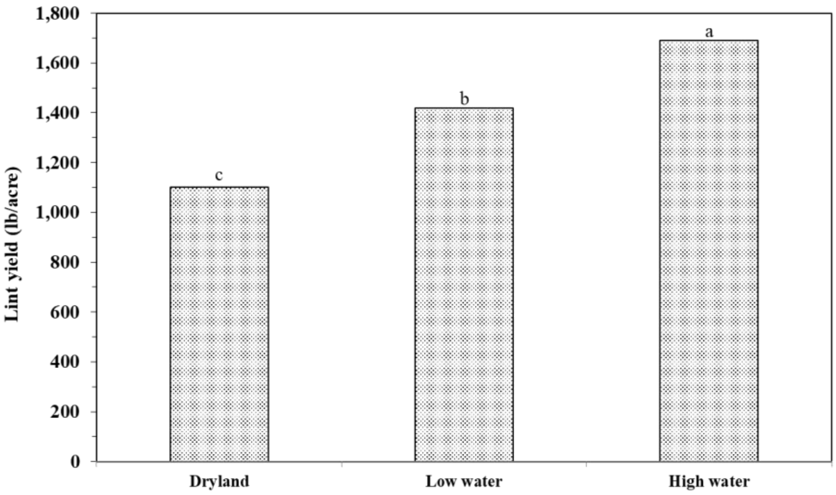

Fig. 1. Average cotton lint yield across cotton fleahopper augmentation treatments under three irrigation water regimes, Lubbock, Texas, 2020. Different lowercase letters indicate treatment means were significantly different from each other.

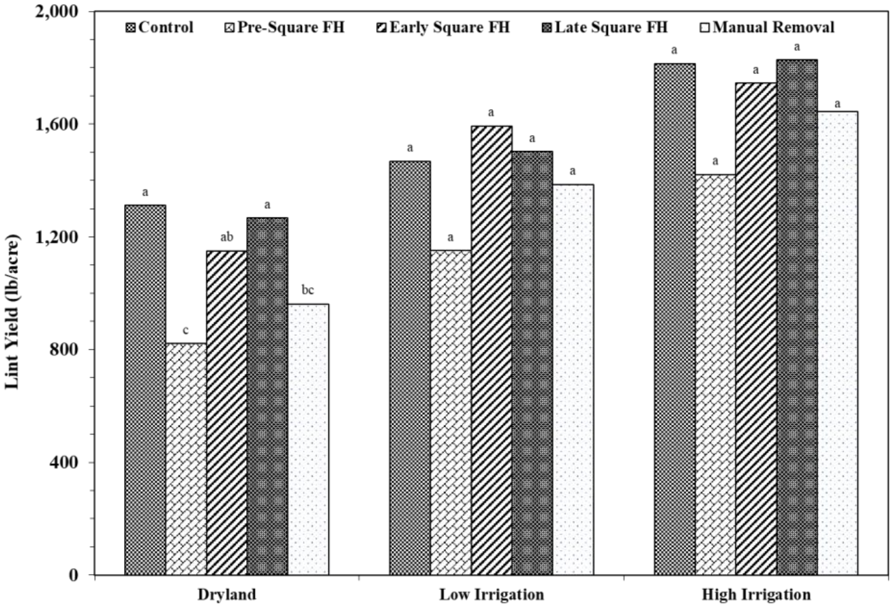

Fig. 2. Cotton lint yield following cotton fleahopper infestations at three cotton phenological stages and manual square removal at first flower under three irrigation water treatments, Lubbock, Texas, 2020. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other. Pre-square FH = fleahoppers augmented prior to the occurrence of visible squares in plants; Early square FH = fleahoppers released at 1-2 visible squares; Late square FH = fleahoppers released when cotton was about to begin flowering; Manual Removal = all visible squares removed from plants at first flower.

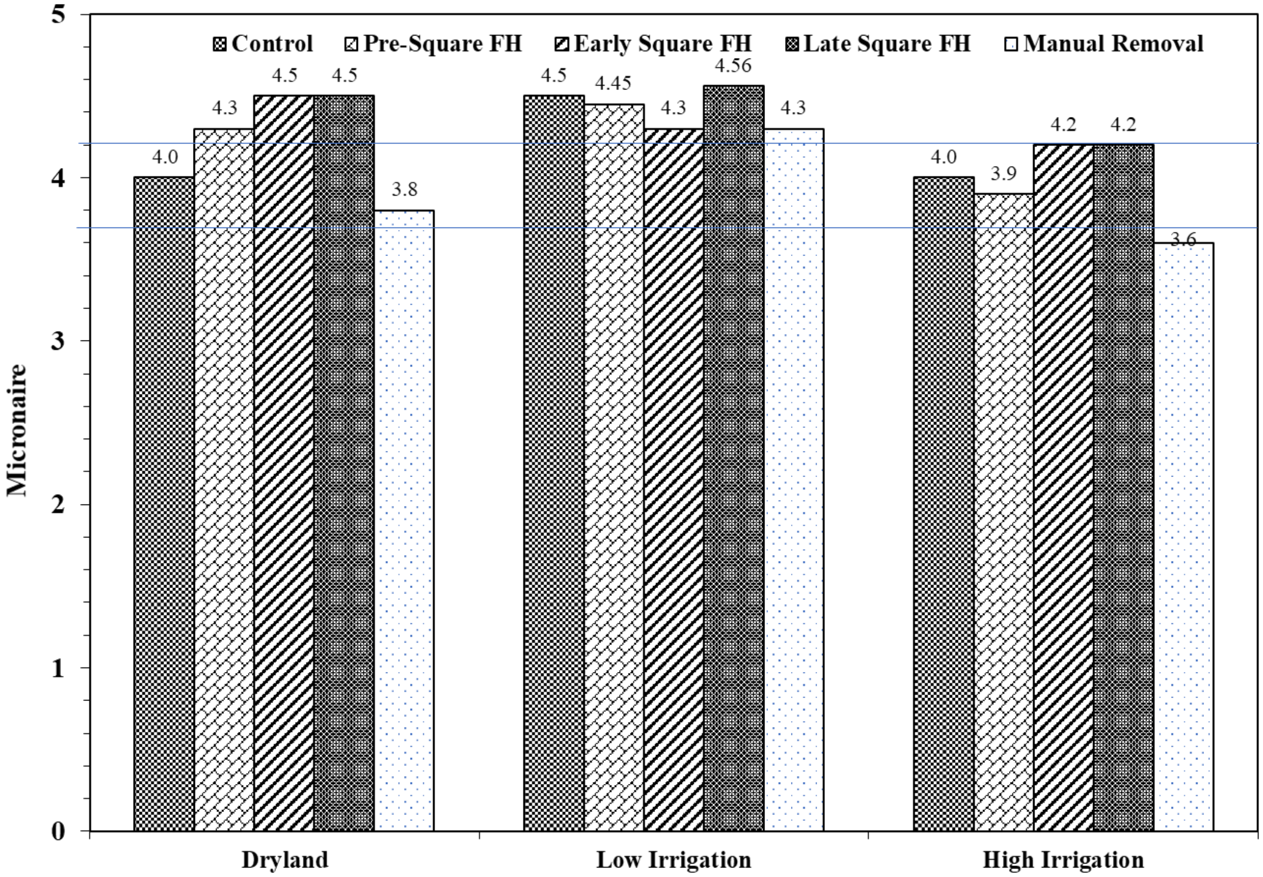

Fig. 3. Cotton fiber micronaire values (units) following cotton fleahopper infestations at three cotton phenological stages and manual square removal at first flower under three irrigation water treatments, Lubbock, Texas, 2020. Two blue lines indicate the region of micronaire values for the premium lint value. Pre-square FH = fleahoppers augmented prior to the occurrence of visible squares in plants; Early square FH = fleahoppers released at 1-2 visible squares; Late square FH = fleahoppers released when cotton was about to begin flowering; Manual Removal = all visible squares removed from plants at first flower.

Table 2. HVI fiber quality parameters influenced by cotton fleahopper augmentation treatments under three irrigation water treatments, Lubbock, Texas, 2020

Fiber Parameters	Irrigation Treatment	Fleahopper Simulation	Uninfested Control	Pre-Square Fleahopper	Early square Fleahopper	Late-square Fleahopper
Micronaire	Dryland	3.08	3.40	4.36	4.51	4.54
Fiber length	Dryland	1.10	1.13	1.14	1.16	1.14
Uniformity	Dryland	80.18	80.43	81.33	81.60	81.50
Strength	Dryland	30.95	31.80	32.13	32.35	32.30
Elongation	Dryland	7.73	7.68	7.65	7.83	7.73
Micronaire	Low	3.43	3.83	4.45	4.30	4.56
Fiber length	Low	1.15	1.16	1.14	1.16	1.16
Uniformity	Low	81.44	81.66	81.55	81.63	82.00
Strength	Low	31.91	31.60	31.88	32.00	31.93
Elongation	Low	7.84	7.99	7.73	7.93	7.85
Micronaire	High	3.00	3.39	3.93	4.24	4.22
Fiber length	High	1.17	1.17	1.20	1.21	1.20
Uniformity	High	80.73	80.94	82.08	82.23	82.60
Strength	High	31.61	31.71	32.15	31.78	31.00
Elongation	High	8.04	8.11	8.28	8.30	8.30

2021 Study

The effect of pre-square cotton fleahopper release was assessed when plants already had significant number of squares on the plant (10 days post-release) which showed 10% square loss, whereas early-square stage had 32% square loss and 21% square loss was observed at late-square stage. Flower initiation began around 7 August and continued beyond 10 September. Peak flower initiation was recorded on 26 August at all water level treatments; however, the highest number of flowers were recorded in dryland plots (Fig. 4) which was largely attributed to incessant rainfall during the cotton flowering stages that likely equalized all irrigation main treatment plots.

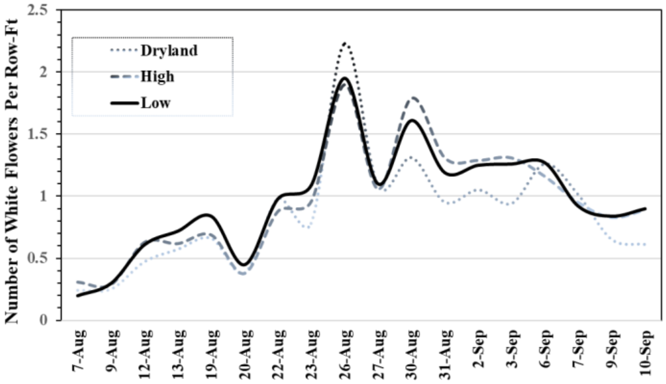

Figure 4. Temporal abundance of white flowers (number of white flowers per row-ft per sample date) recorded from cotton fleahopper infested plots under dryland versus irrigated production conditions, Lubbock, Texas, 2021.

Irrigation level did not significantly influence the lint yield. Replanting of the test delayed the crop maturity and reduced the overall yield. As stated previously, frequent rain events equalized the lint across three irrigation treatments (Fig. 5). Averaged across cotton fleahopper augmentation treatments, dryland produced 570 lb/acre, followed by 763 in low water and 697 in full irrigation treatments (Fig. 1). Insect release treatments significantly affected lint yield in dryland plots, with 627, 453, 793, and 407 lb/acre lint yield in uninfested control, thrips only, cotton fleahoppers only, and thrips+cotton fleahoppers plots, respectively. Even though thrips-induced damage was not apparent during the seedling stage, lint yield was dampened in thrips-release plots in dryland, albeit not statistically significant, and thrips+cotton fleahopper plots had significantly the lowest lint yield (Fig. 6) Lint yield did not vary amongst insect management treatments in low or high irrigation water treatments.

Cotton fleahopper infestation impacted fiber quality while the plant response to cotton fleahopper injury was influenced by irrigation water level (Fig. 7, Table 3). Micronaire values ranged from poor quality (<3.4) to premium (3.7-4.2) fiber across all three water treatments. Two insect-infested treatments in high water treatment had micronaire values in the premium range, but none on low water or dryland plots had micronaire in the premium range. There was no clear explanation for the observed variation in micronaire across treatments. Other fiber quality parameters varied marginally with insect augmentation X irrigation interactions (Table 3). These data suggested an apparent interaction between fleahopper-induced injury to cotton and irrigation water availability for plants to overcome the injury effect, thereby influencing the lint yield and fiber quality.

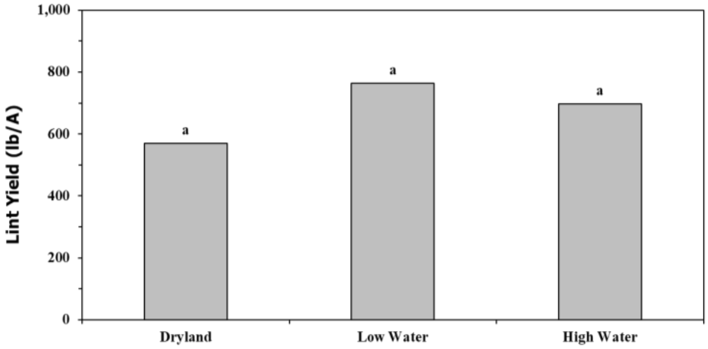

Figure 5. Average cotton lint yield across cotton fleahopper augmentation treatments under three irrigation water regimes, Lubbock, Texas, 2021. Same lowercase letter for each value indicates treatment means were not significantly different from each other.

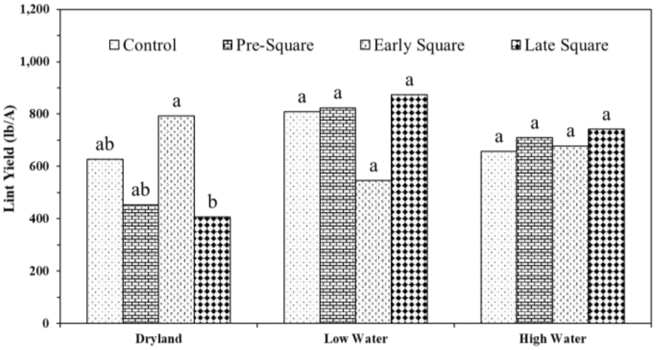

Figure 6. Cotton lint yield following cotton fleahopper infestations at three cotton phenological stages under three irrigation water treatments, Lubbock, Texas, 2021. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

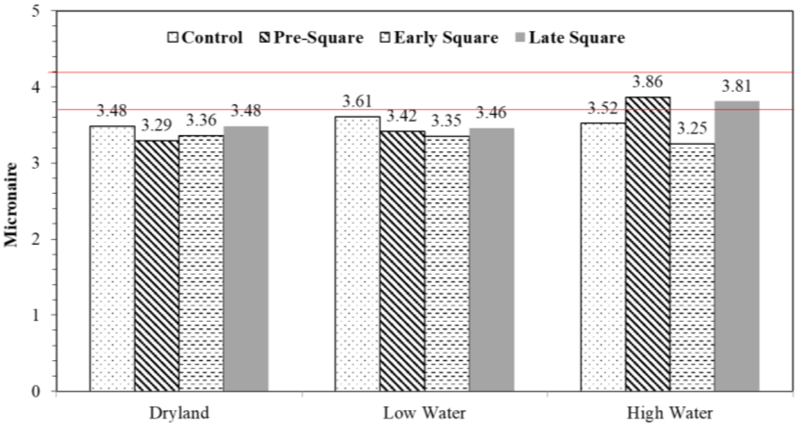

Figure 7. Cotton fiber micronaire (units) values influenced by cotton fleahopper infestation timing under three irrigation treatments, Lubbock, Texas, 2021.

Table 3. HVI fiber quality parameters influenced by cotton fleahopper augmentation treatments under three irrigation water treatments, Lubbock, Texas, 2021.

Parameters	Irrigation	Control	Pre-Square	Early Square	Late Square
Micronaire	Dryland	3.48	3.29	3.36	3.48
Fiber length	Dryland	1.15	1.17	1.18	1.18
Uniformity	Dryland	80.20	79.62	80.77	80.37
Strength	Dryland	31.67	32.07	32.77	31.65
Elongation	Dryland	7.20	7.27	7.37	7.42
Micronaire	Low	3.61	3.42	3.35	3.46
Fiber length	Low	1.16	1.17	1.18	1.16
Uniformity	Low	80.47	81.00	80.10	80.75
Strength	Low	31.42	32.30	32.82	32.52
Elongation	Low	7.75	7.80	7.60	7.47
Micronaire	High	3.52	3.86	3.25	3.81
Fiber length	High	1.19	1.18	1.16	1.18
Uniformity	High	81.20	80.70	80.47	81.95
Strength	High	32.82	30.45	31.77	32.57
Elongation	High	7.80	7.77	7.70	7.67

Acknowledgments

Research funding which facilitated this study came from Cotton Incorporated Core Program and Plains Cotton Improvement Program. Dol Dhakal provided the technical help.

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, causing disproportionate depletion of 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 three 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 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.

Thrips and fleahoppers impacting cotton production risks were evaluated during 2018-2021 with five combinations of single versus sequential infestations under three water-deficit (near-zero deficit or full irrigation, supplemental, and high deficit or dryland) regimes. 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 pests increased the plant apical growth in irrigated plots (2018). Lint yield was similar across all five treatment combinations under dryland condition (2018 and 2019) while sequential infestations of two pests significantly reduced lint yield under dryland in 2020; the sequential infestation of two pests (2018) and cotton fleahopper augmentation (2019) significantly reduced the lint yield under irrigated condition. In 2021, thrips and thrips+fleahopper significantly reduced lint yield compared to fleahopper treatment in dryland, however, yield was similar across insect treatments in low and high-water treatments, indicating the impact of drought conditions on modulating the effect of insect pests as well as the plant's compensatory ability.

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, resulting in disproportionate depletion of underground water and significant shift in the cotton production outlook in THP to even more low input with dryland acreage reaching to about 65%. The shift in cotton production system due to recurring droughts in already a 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 enterprises enterprise (Dhakal et al. 2019, Lascano et al. 2020). While the drought and heat conditions are unpredictable, the anticipated changes in global climate patterns may exacerbate the water-deficit conditions further in the Texas High Plains. 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 and optimal use of inputs. 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 were to: 1) Quantify the impact of four combinations of single versus sequential infestations of two major insects (thrips and cotton fleahoppers) on cotton lint yield and fiber quality under three irrigation water regimes (water-deficit treatments – dryland, low irrigation, and 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 was to integrate production practices and pest management options under numerous cotton management scenarios and the management options are being developed based on breakeven value and net return of each management 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 Texas A&M AgriLife Research farm (Lubbock County, TX).

Irrigation water level treatments. Three irrigation water levels (dryland, supplemental irrigation, and full irrigation) simulated three water-deficit production conditions, including high water-deficit (dryland condition), limited water condition, and no water-deficit. A high-water treatment maintained >90% evapotranspiration replenishment through subsurface drip irrigation throughout the crop growing season, supplemental irrigation maintained about 40% ET replenishment, and the dryland treatment received pre-planting irrigation to facilitate proper seed germination and no additional irrigation for the remainder of the growing season. In 2018, only dryland and full irrigation main plot treatments were available; 2019-2021 had all three water levels.

Planting and field management. The 2018 study followed the conventional tillage system of cotton cultivation and regionally adopted production practices were followed, including pre-planting application of 80 lb N/acre. Cotton cultivar DP 1646 B2XF (seed with no insecticide or fungicide seed treatment) was planted on 31 May 2018. In 2019, wheat was planted on 14 February 2019 as a cover crop to minimize pre-planting soil erosion and prevent cotton seedlings from sandblasting during May/June. Cotton cultivar DP 1646 B2XF was planted on 14 May 2019 and the wheat was terminated on 20 May 2019 with Roundup WEATHERMAX® (48.8% glyphosate) @ 32 oz./acre to facilitate thrips movement to emerging cotton seedlings. Other field management activities included the tank-mixed application of herbicide XTENDIMAX® (48.8% dicamba) @ 22 oz./Acre and Roundup WEATHERMAX® (48.8% glyphosate) @ 32 oz./Acre on 17 June 2019 for weed management, field cultivation on 24 June 2019 for soil aeration and weed management, and fertilizer application (100 lb. N/acre) via side-dressing on 23 July 2019. In 2020, cotton cultivar DP1820B3XF was planted on 18 May 2020 following pre-plant fertilizer application @ 80 lb N/acre. Weed management was achieved via Roundup WEATHERMAX® (48.8% glyphosate) @ 32 oz/acre and XTENDIMAX® (48.8% dicamba) @ 22 oz/acre tank-mix applications on 18 May 2020 and 3 June 2020 and field cultivation on 21 July 2020 for soil aeration and weed management. In 2021, Treflan @ 1qt/acre. was incorporated with field preparation. Wheat was planted on 7 April as cover crop to minimize pre-planting soil erosion and prevent cotton seedlings from sandblasting during May/June. Cotton cultivar DP 1845 B3XF was planted on 12 May 2021 following pre-plant fertilizer application of @ 60 lb N/acre on 22 April 2021. Due to heavy rain events and hailstorm, the first planting crop was damaged, and the same cultivar was replanted on 9 June 2021. Weed management in 2021 was achieved via Roundup WEATHERMAX® (48.8% glyphosate) @ 32 oz/acre and XTENDIMAX® (48.8% dicamba) @ 16 oz/acre tank-mix applications on 14 July and three field cultivation trips during the growing season for weed management.

Insect infestation treatments. Two key insect-pest species (thrips and cotton fleahoppers) impacting cotton production risks were evaluated with five combinations of single versus sequential infestations under three water-deficit (zero, medium, and high) regimes, replicated four times (total 60 plots); only zero and high water-deficit regimes were evaluated in all (2018-2021) studies. Five possible insect infestation scenarios were evaluated where the infestations were simulated during the most vulnerable stage of cotton for each target insect (Table 1). Targeted insect management options were achieved via natural colonization and/or artificial augmentation of insect pests. Because THP 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 fleahoppers at early squaring stage), this study was designed to infest the treatments at the most vulnerable stage of crop for the species infested.

Table 1. Five insect management scenarios evaluated under three irrigation water treatments, Lubbock, Texas, 2018-2021.

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)

2018 study

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 2018 by transferring second-instar fleahoppers 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 2018 and kept insect-free for the remainder of the study to isolate the effect of various treatments.

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 2018 from flagged area and cotton was ginned on 17 December 2018. Lint samples were analyzed at Cotton Incorporated for fiber parameters.

2019 study

Thrips. Wheat cover was terminated on 20 May 2019 with glyphosate to facilitate thrips movement to emerging cotton seedlings to achieve natural infestation of thrips on experimental plots. Uncharacteristic heavy rain events during 23-26 May (4.51” rainfall) with associated small hail event compromised the study field for desired plant stand. Thrips were all dislodged from the wheat cover as well as those already transferred to cotton seedlings. Therefore, thrips were manually augmented on two 5-ft sections per treatment plots on 4 June 2019 via collecting immature thrips from nearby alfalfa terminals and releasing them onto the cotton seedlings, by placing thrips-infested alfalfa terminals at the base of each seedling @ approximately 5 thrips per cotton seedling. This rate of infestation is expected to result in about 1 thrips per seedling after 80% mortality of released thrips. Unexpected storms occurred on 5 and 6 May with additional 1” of rain dislodging all released thrips. We re-released thrips on 7 June 2019, but the ensuing hot and windy days following the second release did not allow thrips to colonize in the experimental plots. Consequently, we assumed no thrips effect on our experimental plots. Nevertheless, we conducted the visual ranking of the experimental plots on 11, 17, and 22 June 2019 to discern if any thrips-induced injury was inflicted on the seedlings. We found no thrips-inflicted injury nor observed any thrips colonization.

Cotton fleahoppers. Woolly croton, with embedded overwintering fleahopper eggs, was harvested from rangeland sites near College Station, Texas, 18 February 2019 and then placed into cold storage. Eighty 1-gallon sheet metal cans, each containing 4 ounces of dry croton twigs per can, were initiated on 10 May 2019 to generate the required number of cotton fleahopper nymphs for the study. 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. Cotton fleahopper emergence began on 19 June 2019. 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 4 July 2019 by transferring second instar fleahopper nymphs from the laboratory colony onto the terminals of plants in each treatment plot at the rate of 5 nymphs per plant. Control plots had no insect activity to warrant any insecticide intervention. Unfortunately, a heavy rainfall occurred on 6 July 2019 (2.75”) and dislodged the released cotton fleahoppers and the treatment deployment was totally ineffective. The field was too wet to re-augment the cotton fleahopper within the next 2-3 days, but another storm passed through west Texas on 11 July 2019 that brought a damaging hail onto our field, causing significant damage to the test plots. Consequently, the crop stand was very poor with significant hail damage to the growing terminals for the crop to perform normally. Nevertheless, we introduced a manual square-removal treatment to selected control plots to evaluate the simulated fleahopper-induced square removal and resulting crop growth profile across three irrigation treatments. However, the unusual rainfall patterns might have already compromised our irrigation treatments. Treatments #1 and #3 were sprayed with BRACKET® 97 (acephate 97%) @ 3 oz./acre on 7 and 17 June 2019 to ensure insect-free plots to isolate the effect of insect-release plots. Square removal treatment was deployed on 26 July 2019 by removing 100% squares from all plants in two 5-row ft sections per plot. Plant mapping was conducted 10 days after cotton fleahopper release to assess the fruit set on all experimental plots.

We also monitored flowering profile by counting number of white flowers in two 5-row ft sections per experimental plots twice a week (23, 26, and 30 July, 2, 5, 9, 12, 16, 19, 23, 26, and 30 August, and 3 and 11 September) during the cotton flowering period (total 14 sample dates). Pre-harvest plant mapping was done on 30 October 2019 and hand harvesting was done on 1 November 2019 from flagged area. Cotton was ginned on 14 November 2019 and the lint samples were sent to Cotton Incorporated for fiber analysis.

2020 study

Thrips. Thrips sampling was performed via whole-plant removal of 10 seedlings per plant in a mason jar for later processing of the samples in the laboratory to extract thrips from plant washing technique. Thrips samplings were done on 29 May, 1 June, 4 June, and 11 June 2020. Treatments #1 and #3 were sprayed with BRACKET® 97 (acephate 97%) @ 3 oz./acre on 29 May and 8 June to ensure insect-free plots to isolate the effect of thrips. Because natural thrips colonization was insignificant, thrips were manually augmented on two 6-ft sections per treatment plots on 20 June 2020 via collecting immature thrips from nearby alfalfa terminals and releasing them onto the cotton seedlings, by placing thrips-infested alfalfa terminals at the base of each seedling @ approximately 10 thrips per cotton seedling. This rate of infestation was expected to result in about 2 thrips per seedling after 80% mortality of released thrips. Thrips-released plots were visually inspected three times to assess for thrips colonization. We found no apparent thrips-inflicted injury on these test plots.

Cotton fleahoppers. Woolly croton, with embedded overwintering fleahopper eggs, was harvested from rangeland sites near College Station, Texas, 2 February 2020 and then placed into cold storage. Forty 1-gallon sheet metal cans, each containing 4 ounces of dry croton twigs per can, were initiated on 15 June 2020 to generate the required number of cotton fleahopper nymphs for the study. 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. Cotton fleahopper emergence began on 24 June 2020. 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 2 July by transferring second-instar fleahoppers from the laboratory colony onto the terminals of plants in each treatment plot at the rate of 5 nymphs per plant. Control plots had no insect activity to warrant any insecticide intervention. Unfortunately, a heavy windstorm occurred in the evening of 2 July and likely compromised the fleahopper colonization in the plant. In addition, we introduced a manual square-removal treatment to selected plots to evaluate the crop growth profile across three irrigation treatments. Plant mapping was performed on July 28 to assess the cotton fleahopper-induced injury.

Temporal flower pattern was monitored for 14 sampling dates, starting on 20 July and conducted every 2-3-day intervals. Harvest aids Boll'd® 6SL (Ethephon [(2-chloroethyl) phosphonic acid] @ 1 qt//A (boll opener) and Folex® 6 EC (S, S, S-Tributyl phosphorotrithioate) 1 pint/A (defoliant) were applied on 12 October to accelerate opening of matured unopened bolls and begin the defoliation process. Test plots were hand-harvested on 23 October. Hand-harvested yield samples were ginned, and fiber analysis was performed at Cotton Incorporated for HVI parameters.

2021 study

Thrips. Visual observation of test plots indicated that we had no thrips colonization in our study site due to late planting (replanted crop) and frequent inclement weather events. Because natural thrips colonization was non-existent, thrips were manually augmented on two 6-ft sections per treatment plots on 18 June 2021 via collecting immature thrips from nearby alfalfa terminals and releasing them onto the cotton seedlings, by placing thrips-infested alfalfa terminals at the base of each seedling @ approximately 10 thrips per cotton seedling. This rate of infestation was expected to result in about 2 thrips per seedling after 80% mortality of released thrips. We again released thrips on all thrips-release plots (T2, T4 plots) as previously released thrips failed to cause noticeable injury to the test plot seedlings. We still found no apparent thrips-inflicted injury on these test plots 7 days after the second release.

Cotton fleahoppers. Woolly croton, with embedded overwintering fleahopper eggs, was harvested from rangeland sites near College Station, Texas, 8 February 2021 and then placed into cold storage. sixty 1-gallon sheet metal cans, each containing 4 ounces of dry croton twigs per can, were initiated on 20 June 2021 to generate the required number of cotton fleahopper nymphs for the study. 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. Cotton fleahopper emergence began on 27 June 2021. 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 16 and 19 July by transferring second-instar fleahopper nymphs from the laboratory colony onto the terminals of plants in each treatment plot at the rate of 5 nymphs per plant. Control plots had no insect activity to warrant any insecticide intervention.

Temporal flower pattern was monitored for 18 sampling dates, from August 7 to September 10. Harvest aids Boll'd® 6SL (Ethephon [(2-chloroethyl) phosphonic acid] @ 1 qt//acre (boll opener) and Folex® 6 EC (S, S, S-Tributyl phosphorotrithioate) 1 pint/acre (defoliant) were applied on 21 October to accelerate opening of matured unopened bolls and begin the defoliation process. Test plots were hand-harvested on 11-12 November and ginned on 23 November 2021. Fiber analysis was performed at Cotton Incorporated for HVI parameters.

RESULTS

2018 study

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.

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.

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 insect augmentation treatments. As expected, the yield threshold in dryland cotton was much lower than that for irrigated cotton and thus the lower yield across all treatments can be partially attributed for lack of insect treatment effect on lint yield.

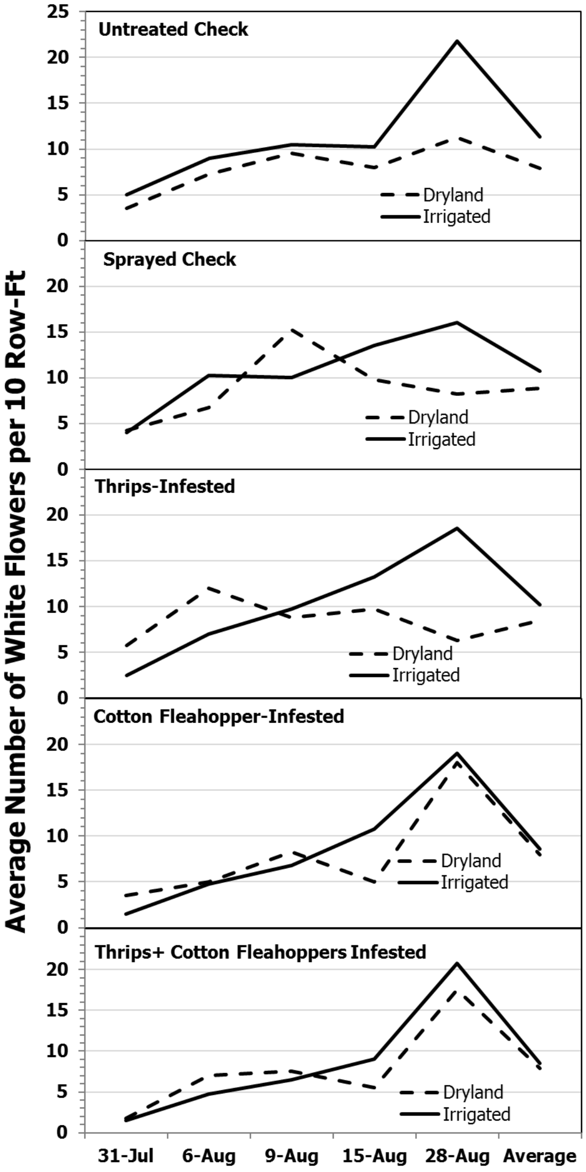

Figure 1. Temporal abundance of white flowers (number of white flowers per 10 row-ft per sample date) recorded from thrips and fleahopper infested plots under dryland versus irrigated production conditions, Lubbock, Texas, 2018.

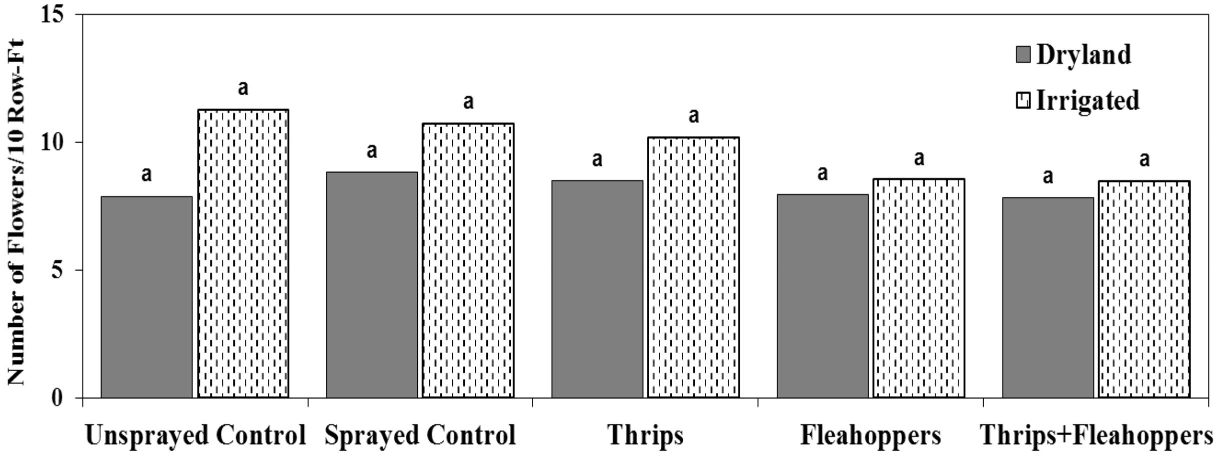

Figure 2. Average abundance of white flowers (number of white flowers per 10 row-ft; n=5 sample dates) recorded from thrips and fleahopper infested plots under dryland versus irrigated production conditions, Lubbock, Texas, 2018. Average values were compared across five treatments within each irrigation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

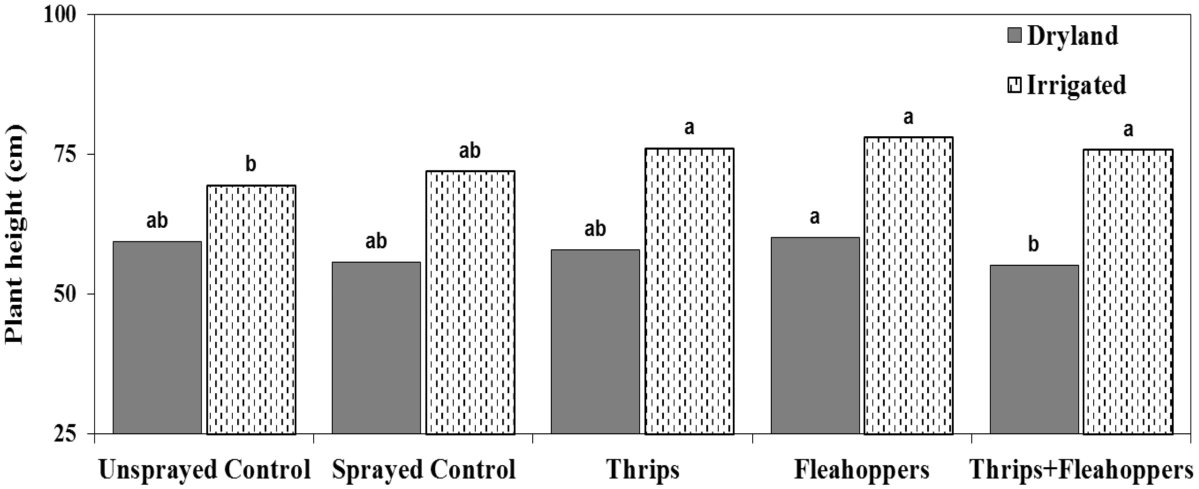

Figure 3. Plant height impacted by thrips and fleahopper infestations under dryland versus irrigated production conditions, Lubbock, Texas, 2018. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly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

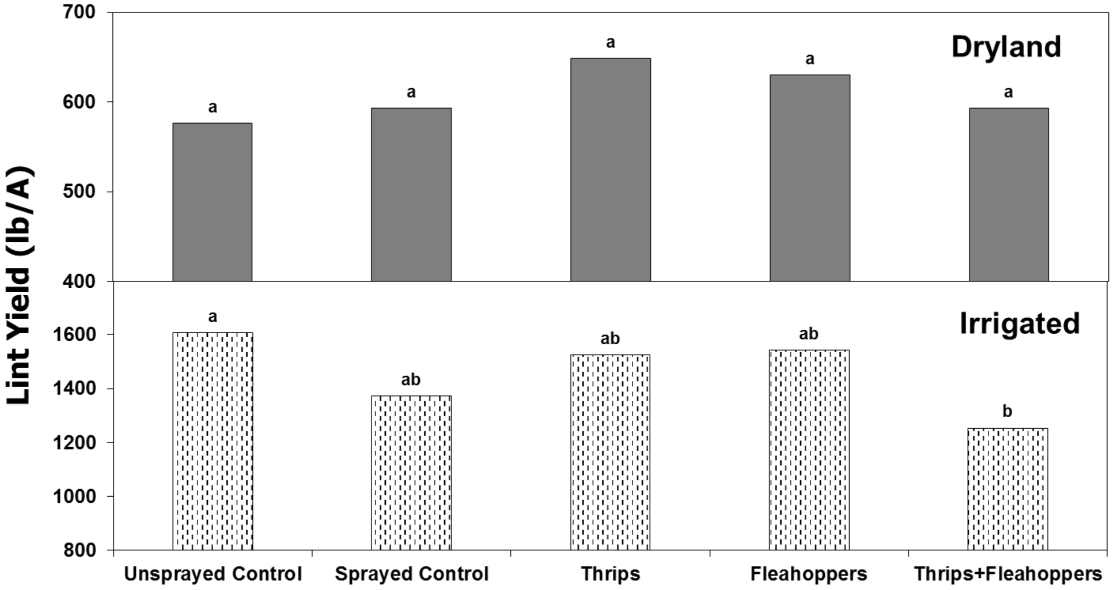

Figure 4. Cotton lint yield losses due to thrips and fleahopper infestation under dryland versus irrigated production conditions, Lubbock, Texas, 2018. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly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

2019 study

Atypical heavy rain events during the pre-squaring stage of cotton with associated small hail event compromised the early season portion of the study. Thrips were all dislodged from the wheat cover as well as those already transferred to cotton seedlings. Manually augmented thrips also suffered from recurring storm events and thrips could not colonize in the study plots. As stated in the Methods section above, we effectively abandoned the possibility of exerting thrips-induced injury effect on seedling cotton. Visual ranking of the experimental plots indicated no evidence of thrips-inflicted injury nor we observed any thrips colonization.

Cotton fleahopper augmentation resulted in 50-55% square abortion compared to 15-20% abortion in control plots; square abortion was similar between dryland and full irrigation plots (Fig. 5). While significant weather events occurred soon after cotton fleahoppers were released, the fleahopper augmentation exerted significant square loss as desired.

Untreated control plots and sprayed control plots showed higher flower densities in both irrigated and dryland cottons compared with that in insect augmented plots; this difference was more pronounced in irrigated plots than in dryland plots (Fig. 6). Full irrigation and supplemental irrigation plots displayed similar flowering patterns throughout the season. The plots with manual square removal to mimic cotton fleahopper-induced square loss displayed synchronized fruiting patterns across irrigation treatments. Overall, average flower abundance was similar amongst unsprayed control, sprayed control, and manual square removal plots, whereas the flower abundance on these three treatments were generally higher than that in all other insect augmented treatments; this trend was similar across all three irrigation water levels (Fig. 6). These patterns clearly indicate that insect infestation, particularly cotton fleahoppers, rendered overall flowering patterns between irrigated and dryland similarly. The average flower abundance was significantly lower in dryland compared to that in irrigated cotton at control plots while other treatments were not consistent across water treatments. These data suggest that the insect infestation during pre-flower stage exerts some significant physiological response to cotton during the flowering stage.

Pre-harvest plant measurement showed that insect augmentation treatments did not result in increased plant heights as observed in 2018. It was expected because the early rain/hailstorm events had severely thinned out the plant stand which allowed plants to grow laterally rather than adding the mainstem nodes following insect infestations. Nevertheless, plots in irrigated cotton had significantly taller plants compared to that in dryland plots as expected.

Lint yield was significantly higher in irrigated cotton (both full and supplemental) compared to that in dryland cotton across all five treatment combinations (Fig. 7). This suggests that the dryland plots were sufficiently water-stressed during the growing season, despite several rainfall events during the early to mid-season; there was a noticeable drought condition during the latter part of the growing season. The highest lint yield under full irrigation treatment was observed in the untreated control treatment (1,268 lb/acre), while the lowest (883 lb/acre) was recorded in the fleahopper infestation treatment (Fig. 7). These were the only treatments that resulted in significant yield difference. Lint yield did not significantly vary across insect augmentation treatments. Under dryland condition, lint yield did not significantly vary across treatments. As expected, the yield threshold in dryland cotton was much lower than that for irrigated cotton and thus the lower yield across all treatments can be partially attributed for lack of insect augmentation treatment effect on lint yield. Also, lint yield was generally similar between supplemental and full irrigation main treatments, owing to frequent rainfall events during early and mid-season that provided sufficient moisture profile in root zones in supplemental irrigation plots to carry the crop's water demand through the season. Thrips only treatment resulted in significantly lower yield under supplemental irrigation compared to that in other treatments (Fig. 8). However, we are unable to speculate the reason for this yield reduction since there were no visible thrips injury during the early growth period of the crop.

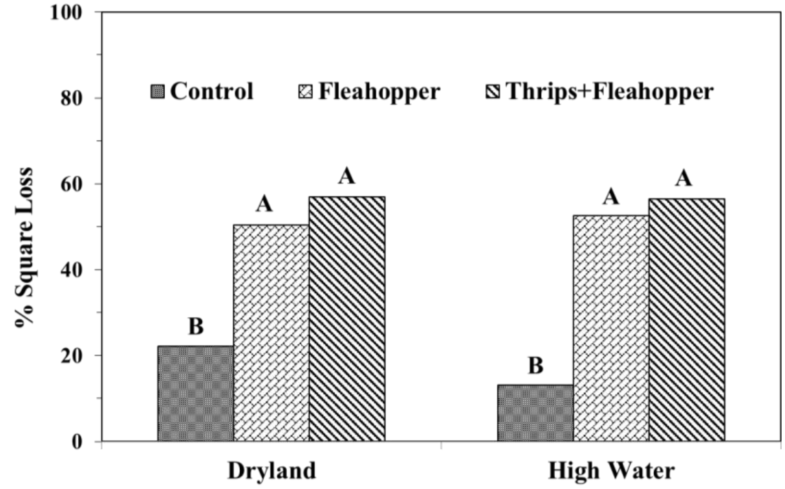

Figure 5. Percentage square loss (number of missing squares with respect to total squares set per plant) recorded following cotton fleahopper infestations in dryland versus full irrigation production conditions, Lubbock, Texas, 2019.

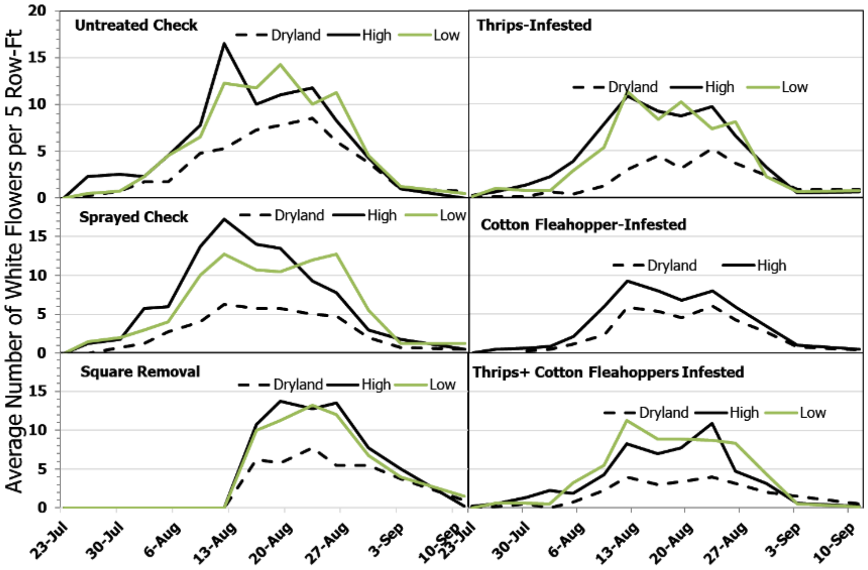

Figure 6. Temporal abundance of white flowers (number of white flowers per 5 row-ft per sample date) recorded from insect-release treatment plots under dryland, supplemental (low), and full (high) irrigation production conditions, Lubbock, Texas, 2019.

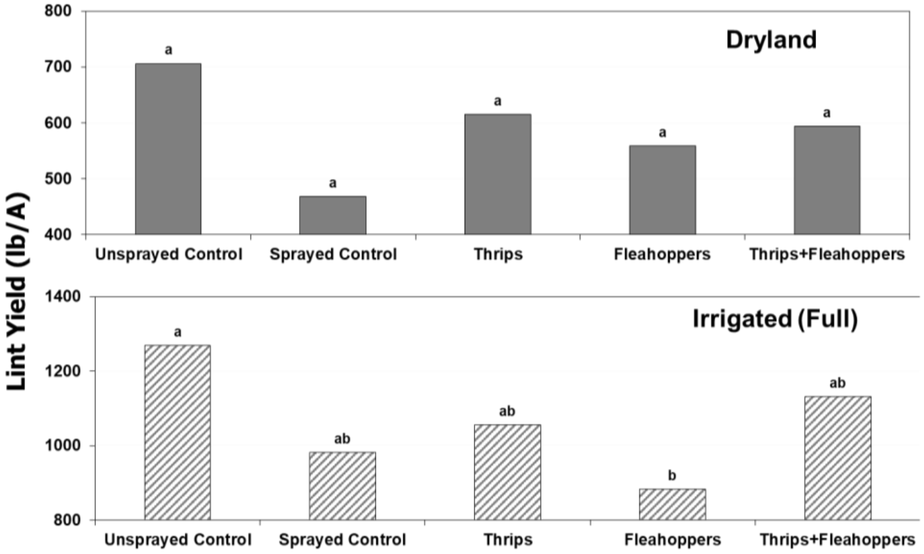

Figure 7. Cotton lint yield losses due to thrips and fleahopper infestations under dryland versus full irrigation production conditions, Lubbock, Texas, 2019. Average values were compared across five treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

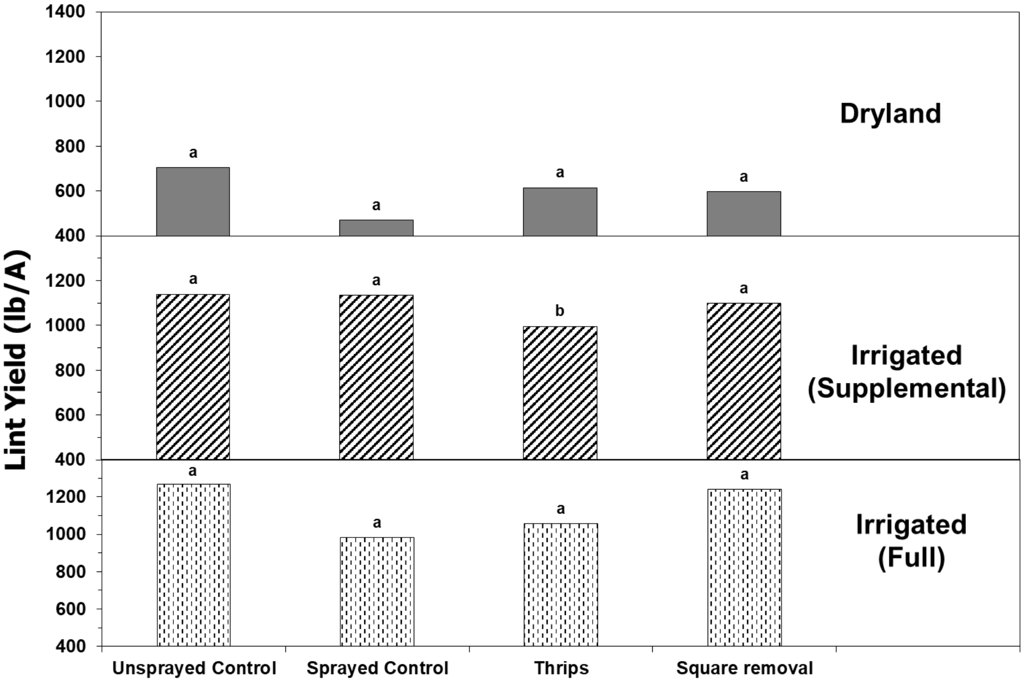

Figure 8. Cotton lint yield losses due to thrips and manual square removal (100% squares pruned at first flower stage to mimic severe cotton fleahopper damage) under three irrigation water regimes, Lubbock, Texas, 2019. Average values were compared across four treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

2020 study

The natural thrips colonization was also insignificant in 2020 as in previous two years. Because natural colonization was inconsequential, thrips were manually augmented per treatment plots. Nevertheless, environmental conditions (e.g., incessant dry wind) did not allow thrips to colonize and exert significant injury to the plants in test plots. Therefore, the manual augmentation did very little to exert injury pressure on cotton plants. Similarly, a heavy windstorm occurred in the evening of 2 July and likely compromised the fleahopper colonization in the plant. As a result, cotton fleahoppers exerted mild injury pressure on plants, which caused about 10-14% square abscission and only increased plant height and more nodes on mainstem compared to that in control plots. The plant height effect, too, was only evident under dryland conditions as the irrigated plots all compensated this low level of early fruit abscission.

Because fleahopper-induced square loss was not significant, flowering profile was generally similar across all treatments. Nevertheless, considerable variations existed amongst treatments on temporal flowering patterns. Uninfested and sprayed control plots showed greater flower densities earlier than cotton fleahopper and thrips+cotton fleahopper infested plots (Fig. 9). Clearly, insect infested plots delayed peak flowering and even had slightly fewer total flowers than the uninfested plots. Limited irrigation plots showed greater flower densities in most treatments, but insect-infested treatments had conspicuously lower flower densities for limited irrigation plots during the early reproductive phase of the crop compared to that for uninfested plots (Fig. 9, left versus right panel). High irrigation plots had the lowest flower densities compared to low irrigation or dryland plots under thrips+fleahopper infested treatment. The plots with manual square removal to mimic cotton fleahopper-induced square loss displayed similar fruiting patterns across irrigation treatments. Even at low rate of insect-induced square removal during pre-flower stage, significant physiological responses can be exerted to cotton during the flowering stage.

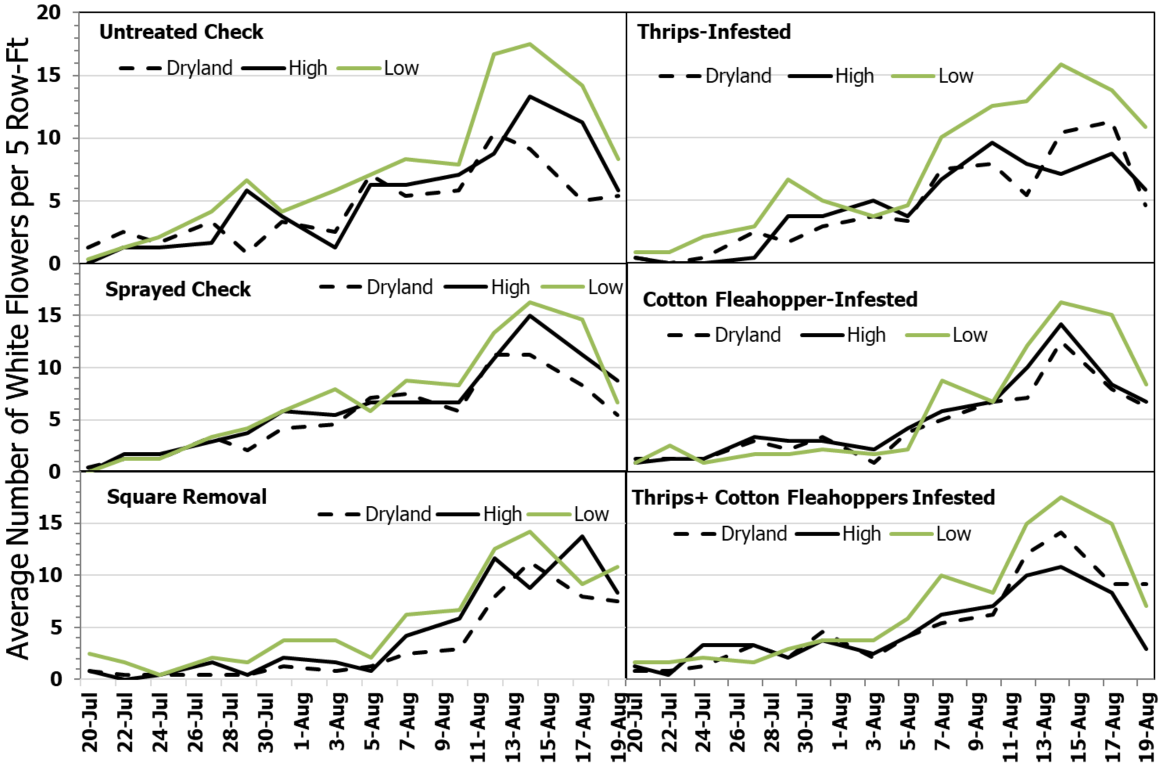

Figure 9. Temporal abundance of white flowers (number of white flowers per 5 row-ft per sample date) recorded from insect-release treatment plots under dryland, supplemental (low), and full (high) irrigation production conditions, Lubbock, Texas, 2020.

As expected, lint yield varied with irrigation treatments. Lint yield was significantly higher in irrigated cotton (High irrigation: 1623 lb/acre; Low irrigation: 1350 lb/acre) compared to that in dryland (1046 lb/acre) cotton across all five treatment combinations (Fig. 10). This suggests that the dryland plots were sufficiently water-stressed during the growing season. The highest lint yield under full irrigation treatment was observed in the uninfested control treatment (1877 lb/acre), while the lowest (890 lb/acre) were recorded in the thrips and thrips+fleahopper infestation treatments (Fig. 10). Overall, thrips+fleahopper treatment resulted in the lowest yield across all three irrigation treatments, although statistically significant only under dryland condition. Another conspicuous trend was that fleahopper alone treatment that exerted only 10-14% square loss did not significantly rendered the yield loss. It is known from the past studies that a low level of fleahopper injury compensates or even overcompensates the insect-induced fruit loss. However, when fleahopper caused even a low-level injury sequentially with a low-level thrips injury, yields were reduced considerably across all irrigation treatments. The lack of statistical significance across sub-treatments under irrigated treatments can be attributed to a large variation in data. Although thrips infestation and thrips-induced injuries were insignificant, lint yields were numerically (irrigated plots) or significantly (dryland) lower across all irrigation treatments.

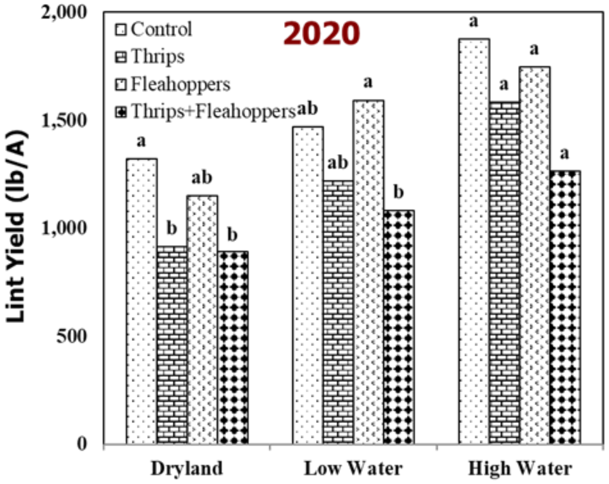

Figure 10. Cotton lint yield losses due to thrips and cotton fleahopper infestations under three irrigation water treatments, Lubbock, Texas, 2020. Average values were compared across four treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

Overall, irrigation treatments did not significantly alter the HVI parameters. However, there was a considerable irrigation x insect infestation interaction in influencing the fiber parameters (Fig. 11). In general, low level of thrips and fleahopper injuries appeared to increase micronaire values, except for low irrigation. In fact, uninfested control plots had the micronaire in the discount range under both dryland and high irrigation treatments, whereas all insect-infested plots had micronaire in premium range (high irrigation) or premium/base range (dryland). It was interesting to note that the micronaire values were at base range for low irrigation treatment for all insect-augmentation treatments. Other fiber parameters, including fiber length, uniformity, strength, and elongation were generally similar across all insect-infestation treatments within each irrigation level (Table 2). Irrigation water treatment had only marginal effect on other HVI parameters.

Table 2. HVI fiber quality parameters influenced by thrips and cotton fleahopper infestation singly as well as sequential infestation of both insects under three irrigation water treatments, Lubbock, Texas, 2020.

Fiber Parameters	**Irrigation Treatment**	**Uninfested Control**	**Thrips**	**Fleahopper**	**Thrips+ Fleahopper**
Micronaire	Dryland	3.40	4.39	4.51	4.24
Fiber length	Dryland	1.13	1.14	1.16	1.14
Uniformity	Dryland	80.43	80.88	81.60	80.90
Strength	Dryland	31.80	31.35	32.35	31.13
Elongation	Dryland	7.68	7.68	7.83	7.70
Micronaire	Low	3.83	4.42	4.30	4.30
Fiber length	Low	1.16	1.15	1.16	1.15
Uniformity	Low	81.66	82.05	81.63	81.90
Strength	Low	31.60	31.63	32.00	31.75
Elongation	Low	7.99	7.90	7.93	7.93
Micronaire	High	3.39	3.96	4.24	4.16
Fiber length	High	1.17	1.20	1.21	1.19
Uniformity	High	80.94	81.35	82.23	82.28
Strength	High	31.71	31.55	31.78	32.03
Elongation	High	8.11	8.15	8.30	8.15

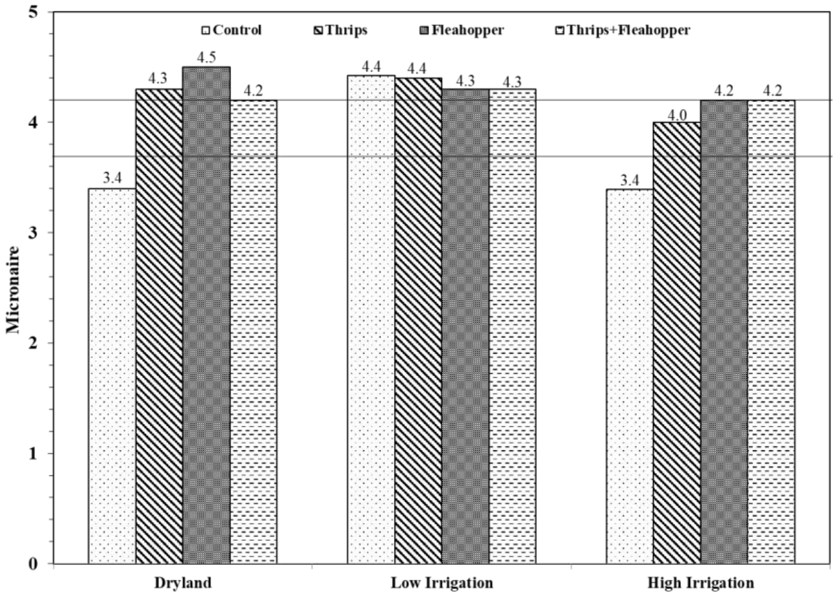

Figure 11. Cotton fiber micronaire (units) values influenced by thrips and cotton fleahopper infestations under three irrigation water treatments, Lubbock, Texas, 2020. Average values between 3.7-4.2 indicate premium cotton fiber.

2021 study

The natural thrips colonization was insignificant in 2021 as in previous two years. Visual observation of test plots indicated that we had no thrips colonization in our study site due to late planting (replanted crop) and frequent inclement weather events. Manually augmented thrips also failed to colonize and exert significant injury to the plants in test plots. Cotton fleahoppers exerted significant injury pressure on plants, which caused about 32% square abscission and increased plant height and more nodes on mainstem compared to that in control plots.

Because cotton fleahopper-induced square loss was significant, variations existed amongst treatments on temporal flowering patterns. Uninfested control plots showed greater flower densities earlier than cotton fleahopper and thrips+cotton fleahopper infested plots (Fig. 12). Clearly, cotton fleahopper infested plots delayed peak flowering than the uninfested plots. Flowering dynamics was influenced by irrigation water and insect infestation treatment interactions.

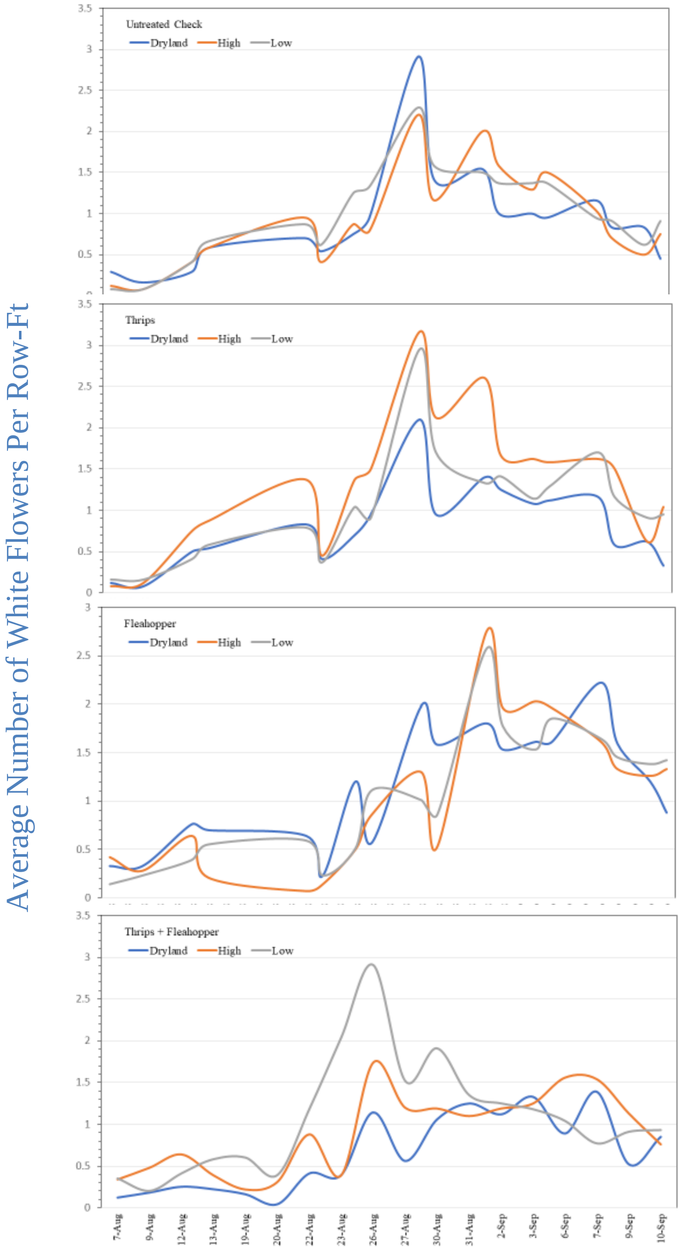

Figure 12. Temporal abundance of white flowers (number of white flowers per row-ft per sample date) recorded from insect-release treatment plots under dryland, supplemental (low), and full (high) irrigation production conditions, Lubbock, Texas, 2021.

The 2021 study suffered with frequent early-season rain events. Replanting of cotton delayed plant growth, fruiting and crop maturity, resulting in overall low lint yield across all main-plot treatments. Lint yield was similar across irrigation treatments as well as insect management treatments, except for the lowest yield in thrips+cotton fleahopper treatment in dryland condition (Fig. 13). Also, thrips and thrips+fleahopper treatments significantly reduced lint yield compared to only fleahopper treatments in dryland, however, lint yield was similar across all insect treatments in low water and high-water treatments, indicating the impact of drought conditions on modulating the effect of insect pests as well as the plant's compensatory ability. Averaged over four years, there was a clear trend that sequential infestations of thrips and fleahoppers reduced the lint yield across all irrigation treatments, but detailed economic analyses are pending.

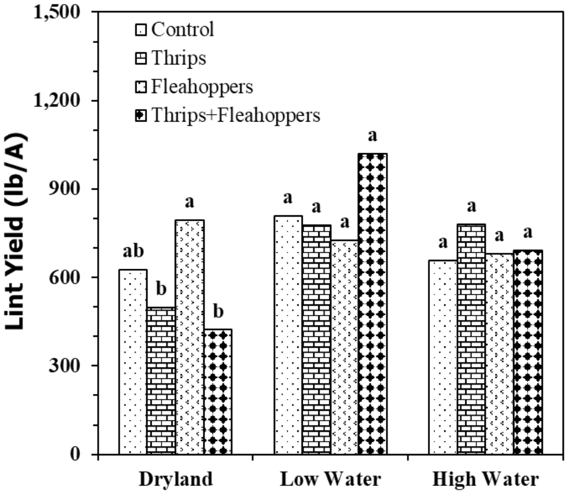

Figure 13. Cotton lint yield losses due to thrips and cotton fleahopper infestations under three irrigation water treatments, Lubbock, Texas, 2021. Average values were compared across four treatments within each irrigation treatment; same lowercase letters indicate treatment means were not significantly different from each other.

Averaged data indicated a decline in yield of 20%, 3%, and 28.7% compared to the uninfested control for dryland under thrips, fleahopper, and thrip and fleahopper pressure, respectively. In the deficit irrigated scenario, yield declined 14%, 1.8%, and 8.3% compared to the control for thrips, fleahopper, and thrip and fleahopper pressure, respectively. In the full irrigated scenario, cotton yield declined by 9%, 11.4%, and 24.6% compared to the control for the thrips, fleahopper, and thrip and fleahopper pressure, respectively. A partial budget analysis will include revenue and cost estimations.

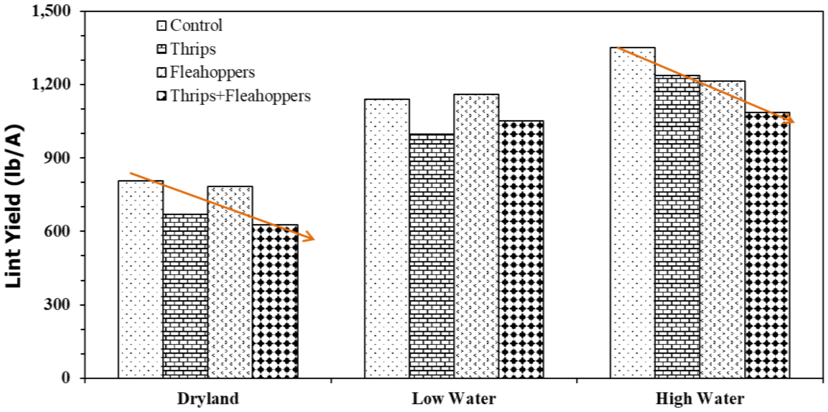

Figure 14. Cotton lint yield, averaged across four years (2018-2021), as influenced by irrigation water x insect infestation treatments.

Maturity delay caused overall decrease in lint quality parameter values across all treatments in 2021. Micronaire values were all in the base range (Table 3, Fig. 15).

Table 3. HVI fiber quality parameters influenced by thrips and cotton fleahopper infestation singly as well as sequential infestation of both insects under three irrigation water treatments, Lubbock, Texas, 2021.

Fiber Parameters	**Irrigation Treatment**	**Uninfested Control**	**Thrips**	**Fleahopper**	**Thrips+ Fleahopper**
Micronaire	Dryland	3.4	3.4	3.3	3.3
Fiber length	Dryland	1.1	1.1	1.1	1.1
Uniformity	Dryland	80.2	80.6	80.7	80.7
Strength	Dryland	31.6	32.9	32.7	33.1
Elongation	Dryland	7.2	7.4	7.3	7.3
Micronaire	Low	3.6	3.3	3.3	3.2
Fiber length	Low	1.1	1.1	1.1	1.1
Uniformity	Low	80.4	80.9	80.1	80.4
Strength	Low	31.4	32.5	32.8	31.9
Elongation	Low	7.5	7.4	7.6	7.4
Micronaire	High	3.5	3.5	3.2	3.3
Fiber length	High	1.1	1.1	1.1	1.2
Uniformity	High	81.2	80.3	80.4	81.1
Strength	High	32.8	32.1	31.7	32.6
Elongation	High	7.8	7.6	7.7	7.7

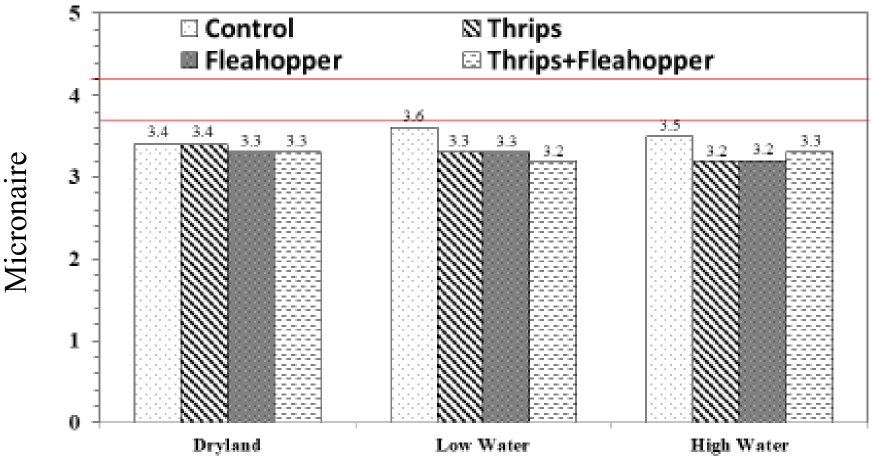

Figure 15. Cotton fiber micronaire (units) values influenced by thrips and fleahopper infestations three irrigation water treatments, Lubbock, Texas, 2021.

We have begun to develop the structure of the profitability model using these four-year data prior. These data will be used to analyze and compare the economics of management of thrips and cotton fleahoppers singly or in sequential combinations under three 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 enterprises). 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 three 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. Although the last three years of data were highly variable and inconsistent between the years, we expect that these data will help us develop the foundation of the model and the final year of data will aid in refining the management model.

Acknowledgments

Research funding which facilitated this study came from Cotton Incorporated Texas State Support Committee. Dol Dhakal provided the technical help.

COTTON YIELD RESPONSE TO SIMULATED COTTON FLEAHOPPER AND WESTERN TARNISHED PLANT BUG INFESTATIONS AS INFLUENCED BY IRRIGATION

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Abstract

Effect of manual removal of early versus late-stage fruits was evaluated on two cotton cultivars as influenced by water level. Experimental design consisted of three fruit abscission treatments (removal of 100% squares prior to the initiation of flowering to mimic cotton fleahopper infestation, 20% bolls removed from the top 1/3rd of the plant canopy at crop cut-out to mimic late season *Lygus* infestation, and control), two water levels (high versus low), and two cultivars (PHY 350 W3FE versus ST 4946 GLB2), replicated three times and deployed in a randomized complete block design (total 36 plots). Significantly higher lint yield was recorded from 'High' water regime (730 lb/acre) compared to that in 'Low' water regime (490 lb/acre). No significant difference in lint yield was recorded between fleahopper simulated treatments and control plots regardless of the water regime. Square removal did not result in significant differences in lint yield between cotton variety PHY 350 W3FE (471 and 683 lb/A) and ST 4946 GLB2 (509 and 779 lb/A) in low and high water, respectively. Lint yield did not significantly vary between 20% late-season fruit loss via manual pruning and control plots, but the yield penalty of 20% late fruit loss was more prominent in low water treatment than in high water regime. Early-season square removal resulted in increased micronaire values at both irrigation regimes, reaching to the discount range under high water regime. The effect of late-season simulated *Lygus*-induced fruit removal did not significantly influence the lint micronaire. The increased irrigation water level (high water regime) increased micronaire values in both cotton cultivars, but PHY 350 W3FE had micronaire in the premium range at both irrigation levels while the micronaire values in ST 4946 GLB2 increased to move away from the premium range to the base range.

Introduction

Cotton fleahopper and Lygus appear to be an emerging concern to the Texas High Plains growers in recent years. Several suitable host plants that support overwintering of these plant bug species exist in the Texas High Plains. The shift in cotton production system from 60:40% irrigated: dryland to 40:60% in the last two decades has altered how we grow cotton. This shift from irrigated to dryland farming warranted to manage cotton pests effectively to increase profitability. Plant bugs have a general inclination to attack the stressed plants and cause significant damage. Cotton plant responses to cotton fleahopper and Lygus injury under a range of irrigation regimes remain uninvestigated. The overall goal of this study was to characterize the effects of simulated cotton fleahopper and western tarnished plant bug on cotton lint yield and fiber quality in relation to available irrigation water levels.

Materials and Methods

Effect of manual removal of early-stage fruits versus control was evaluated on two cotton cultivars, PHY 350 W3FE and ST 4946 GLB2, as influenced by irrigation water level. Experimental design consisted of three fruit abscission treatments (removal of 100% squares prior to the initiation of flowering to mimic cotton fleahopper infestation, 20% bolls removed from the top 1/3rd of the plant canopy at crop cut-out to mimic late season Lygus infestation, and control), two water levels (high versus low), and two cultivars (PHY 350 W3FE versus ST 4946 GLB2), replicated three times and deployed in a randomized complete block design (total 36 plots). The experimental unit of each fruit abscission treatment was a 10-ft section of a uniform cotton row flagged in the middle of a 4-row x 300-ft plot. Square abscission treatments, 1) control (zero square removal) and 2) manual removal of 100% squares, were deployed when cotton was highly vulnerable to fleahopper injury (2-3 weeks into cotton squaring). The test plots were monitored for the occurrence of any other insects, but no such occurrences were observed throughout the growing season. At crop cut-out, 20% bolls from the top of cotton plants were removed from *Lygus* injury simulated plots.

Results and Discussion

Simulation of cotton fleahopper infestations. Combined over two cultivars, significantly higher lint yield was recorded from 'high' water regime (730 lb/acre) compared to that in 'low' water regime (490 lb/acre) (Fig. 1). No significant difference in lint yield was recorded between fleahopper simulated treatments and control plots regardless of the water regime (Fig. 1). Square removal did not result in significant differences in lint yield between cotton variety PHY 350 W3FE (471 and 683 lb/A) and ST 4946 GLB2 (509 and 779 lb/A) in low and high water, respectively.

Simulation of late-season boll abortion. Lint yield did not significantly vary between 20% late-season fruit loss via manual pruning and control plots, but the yield penalty of 20% late fruit loss was more prominent in low water treatment than in high water regime (Fig. 2). Also, PHY 350 WFE was more susceptible to late-season fruit loss than ST 4946 GLB2 (Fig. 2). Both in 'low' and 'high' water regimes, significantly higher micronaire was recorded between fleahopper simulated treatments and control plots; however, no significant differences in micronaire were detected between *Lygus* simulated treatments and control plots both in 'low' and 'high' water regimes.

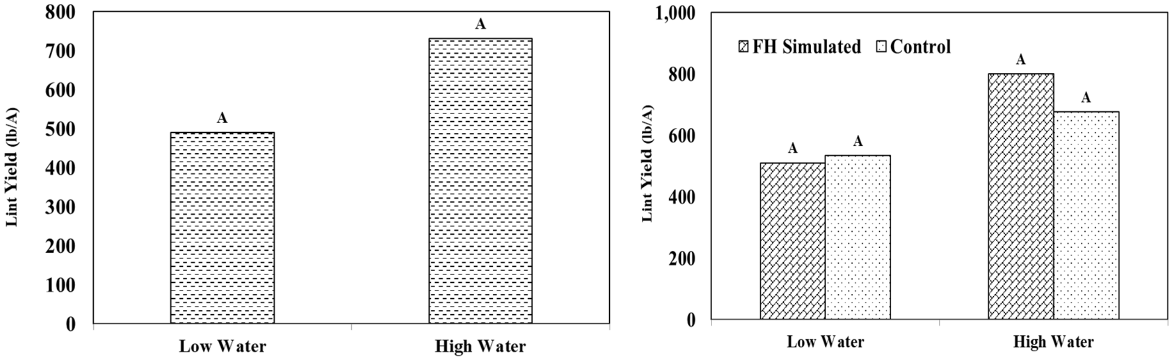

Figure 1. Average lint yield under high and low water regimes (left) and the yield following manual removal of 100% squares prior to first flower versus control plots, Lamesa, Texas, 2019.

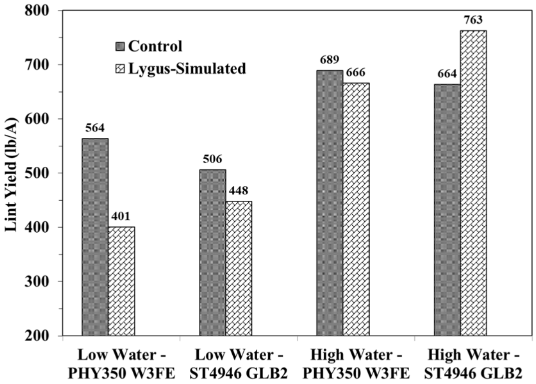

Figure 2. Average lint yield influenced by simulated *Lygus*-induced fruit removal in late season in two cotton varieties under high and low water regimes, Lamesa, Texas, 2019. Average values were not statistically significant due to high variation in data.

Averaged over two cotton cultivars, early-season square removal resulted in increased micronaire values at both irrigation regimes, reaching to the discount range under high water regime. The effect of late-season simulated *Lygus*-induced fruit removal did not significantly influence the lint micronaire. The increased irrigation water level (high water regime) increased micronaire values in both cotton cultivars, but PHY 350 W3FE had micronaire in the premium range at both irrigation levels while the micronaire values in ST 4946 GLB2 increased to move away from the premium range to the base range (Fig. 3).

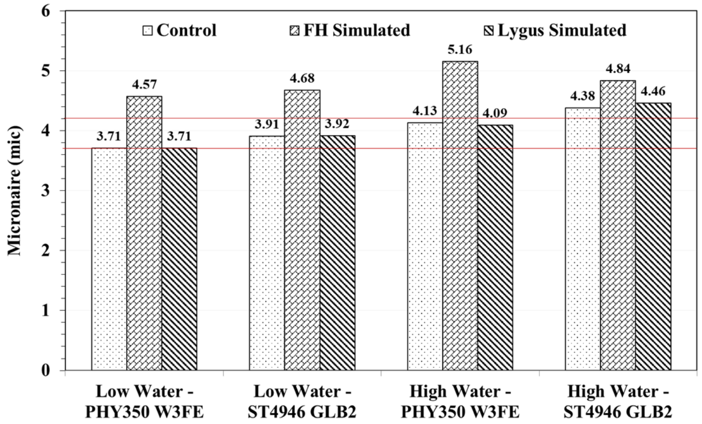

Figure 3. Average micronaire values influenced by simulated early-season cotton fleahopper damage and late-season *Lygus*-induced fruit removal in two cotton cultivars under high and low irrigation regimes, Lamesa, Texas, 2019. The area enclosed by two red lines (3.7-4.2) indicates the micronaire values for premium quality cotton lint.

Acknowledgments

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THRIPS DAMAGE POTENTIAL IN TEXAS HIGH PLAINS COTTON

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Abstract

The western flower thrips, *Frankliniella occidentalis* Pergande were field-collected and released on seedlings of six cotton varieties in the greenhouse at 1-2 true-leaf stage @ 0.5, 1, and 2 thrips per plant. Three weeks after releasing thrips, seedlings were carefully clipped, preserved in ethyl alcohol and washed to recover thrips. Visual ranking of the seedlings was conducted, and chlorophyll readings were recorded. Leaf area was measured using a LI-COR® leaf area meter. Adult thrips numbers retrieved after three weeks of study were the highest in the 0.5 and 1 thrips per plant treatments while the lowest numbers were observed in control. No significant differences in thrips densities were detected between cultivars. Thrips density augmentation significantly influenced the degree of seedling injury as reflected in visual ranking of the cotton seedlings. Thrips density treatments did not affect chlorophyll readings.

Introduction

The western flower thrips (WFT), *Frankliniella occidentalis,* Pergande (Thysanoptera: Thripidae), is an important polyphagous pest of many crops including greenhouse crops throughout the world. WFT is an important pest of seedling cotton throughout the United States cotton belt. In Texas, WFT is considered as the most significant growth stressor for seedling cotton. WFT can cause stunted growth, loss of apical dominance, leaf area destruction, delayed maturity, and reduced lint yields. Excessive feeding of thrips leads to the browning of leaves on the edges, development of a silvery leaf surface color, or curling upward from the edges. WFT can be found in cotton throughout the growing season, but cotton is the most vulnerable to thrips damage for the first three to four weeks following planting and cotyledon emergence or 3-4 true leaf stage. In the U.S., thrips infested 9.4 million acres in 2016 while in Texas, thrips infested 5.4 million acres causing yield loss of approximately 6,720 bales (Williams 2017)**.** Early infestations of thrips can reduce as much as 50% of the leaf area but cotton plants can regain lost leaf area once thrips infestations cease (Lei and Wilson 2004). Gaines (1934) documented that the plants injured by thrips set bolls two weeks later and produced 44 percent fewer bolls per plant than normal plants; however, it is pertinent to observe that relatively small number of studies indicating no yield losses even in presence of heavy thrips presence (Harp and Turner 1976). This may be due to the ability of cotton varieties to compensate for early season damage in favorable environmental conditions (Kerns et al. 2009, Vandiver et al. 2009, Cook et al. 2011). Limited information is available on the impact of different densities of thrips on seedling cotton in the Texas High Plains. The objective of this study was to evaluate the impact of thrips augmentation on seedling cotton health.

Materials and Methods

A 3-year greenhouse study (2013-2015) was conducted at the Texas A&M AgriLife Research and Extension Center, Lubbock. Six cotton cultivars (07-7-1001 CT-1206, 07-7-1407 CT-1205, PHY367 WRF, SSG HQ212 NCT, FM 1740B2RF and ST 5458B2RF) were planted in 16-oz Styrofoam® cups. The study was deployed in a completely randomized block design with six cultivars, four thrips densities, and four replications. Field-collected immature thrips, provisioned for 24 h on green beans in the laboratory, were released using a small camel brush to dislodge thrips from the green beans onto the cotton seedlings. Thrips densities released included: no thrips (*Control*); ½ thrips per plant (e.g., one thrips per two plants) (*Density 0.5*); one thrips per plant (*Density 1*); and two thrips per plant at the 1- to 2-true leaf stage (*Density 2*). Orthene® 97 was sprayed to manage thrips on control treatments.

Visual leaf tissue damage rankings of all plants were recorded prior to plant clipping 21 days after the deployment of thrips augmentation treatments. Thrips damage ranking was based on a scale of 1-10: 1-2) no or insignificant damage (<5% damage), 3-4) slight damage (<25% damage), 5-6) moderate damage (<50% damage), 7-8) severe damage (<75% damage), 9) <90% leaves and terminals damaged, and 10) 100% leaves damaged and plant stunted or dead. Leaf area from each treatment was recorded using a leaf area meter to test whether leaf surface area was reduced by thrips density treatments. Chlorophyll readings were also recorded using a chlorophyll meter to determine if thrips densities and/or tested cotton varieties influenced the chlorophyll level. Three weeks after initial releases, seedlings were clipped near the soil surface and placed into a mason jar containing 75% denatured ethyl alcohol, and the adult and juvenile thrips were quantified (Rummel and Arnold 1989). Adults and juveniles were counted using a microscope at a 10X or higher magnification.

Results

In 2013 greenhouse study, several highly significant factors were observed between thrips densities released and thrips numbers recovered. Thrips density augmentation significantly increased thrips densities in sampled seedlings compared to that in control, indicating that the thrips movement across treatments was minimal (Fig. 1). In 2013 study, total thrips retrieved were the highest at 1 thrips per plant treatment, followed by 2 thrips per plant, and the lowest numbers were in 0.5 thrips per plant, all significantly different from each other. In 2014, highest densities were recovered from Density 2 treatment; Density 0.5 and 1.0 had similar thrips densities. In 2015, thrips colonization was much higher than in 2013 and 2014 (Fig. 1).

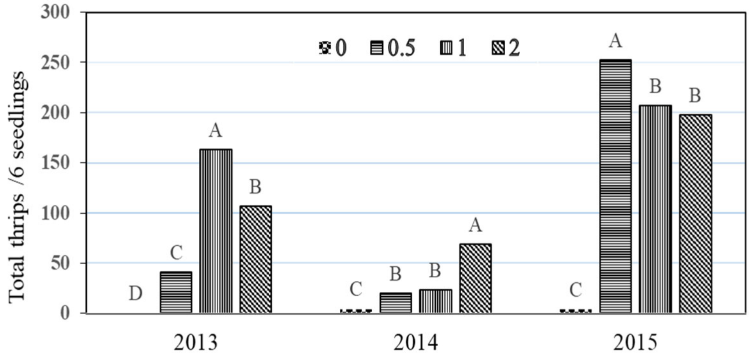

Figure 1. Numbers of total thrips (adults plus larvae) recovered from six-seedling sample unit in the greenhouse using a plant washing technique, 2013-2015.

Thrips augmentation reduced leaf surface area in two of the three years of the study, whereas the effect was not clearly separated in 2015 (Fig. 2). Additionally, leaf area did not vary across the six cultivars evaluated. Overall, visual injury rankings increased with increased thrips densities, clearly suggesting that the density of 0.5 to 1 thrips per seedling can exert significant injury to the young cotton seedlings (Fig. 3).

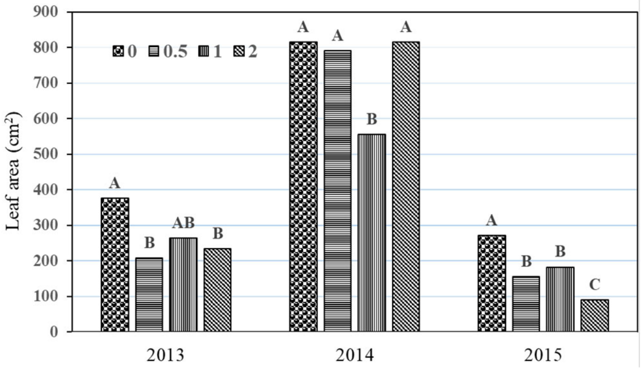

Figure 2. Leaf area per 6-cotton seedlings as affected by varying densities of western flower thrips in the greenhouse.

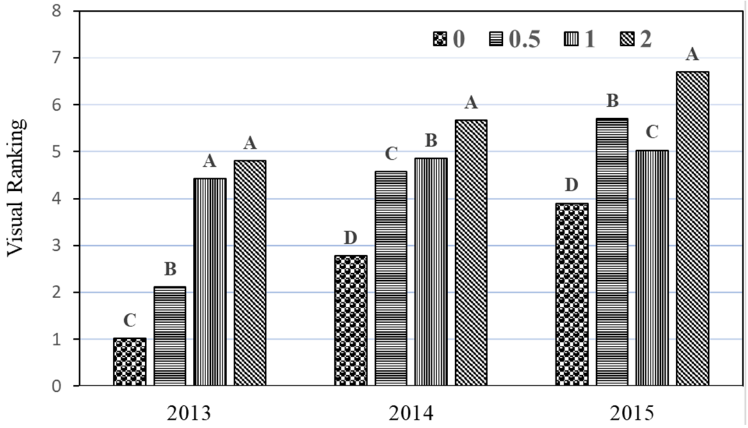

Figure 3. Visual damage ranking (1=no damage to 10= extreme damage and death of the seedling) of the cotton seedlings influenced by varying densities of western flower thrips in the greenhouse.

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RESEARCH LETTER

Soil acidification in a continuous cotton production system

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Abstract

Effects of nitrogen (N) fertilization on soil organic carbon (SOC) and total N are well established, but their effects on soil acidification and emerging soil health indicators such as labile N and carbon (C) pools are not adequately documented. This research evaluated soil N and C pools and soil pH with long-term N management in continuous cotton (*Gossypium hirsutum* L.) production. Residual soil inorganic N, potentially mineralizable N and C, total N, SOC, pH, and electrical conductivity were measured after 17 yr of continuous N application. Comparison of five N rates (0, 56, 112, 168, and 224 kg ha^{-1}) showed an increase in residual inorganic N pools and decrease in pH with an increase in N application rate, while other parameters did not change significantly. Soil acidification was significant with 168 and 224 kg N ha^{-1} rates. Soil pH dropped by 0.039 per kilogram increase in residual inorganic N. Optimizing N rate that minimizes residual inorganic N can reduce soil acidification.

1 | INTRODUCTION

Nitrogen (N) is a primary nutrient essential for all crops. It is added to soil as fertilizer and manure and through atmospheric deposition, biological fixation by legume crops, and mineralization of crop residues and organic amendments. All of the applied N is not used by crops. The inorganic N remaining in the soil after the crop harvest is residual inorganic N. In semiarid environments, large amounts of residual inorganic N are present in soils because moisture limitations often constrain plant assimilation and soil nutrient movement. Residual soil N can be credited to the recommended N rate to a subsequent crop. If the N rate is not adjusted to meet the crop demand, continuous use of chemical N fertilizer at high rates can increase soil acidification, affecting nutrient availability and crop production (Ghimire et al., 2017).

Soil acidification has become one of the significant environmental problems associated with N addition, challenging agricultural sustainability and profitability (Guo et al., 2010; Limousin & Tessier, 2007). Ion-exchange reactions strongly buffer soils, and it typically takes 100 to 1,000 yr to change soil pH under natural conditions (Chadwick & Chorover, 2001; Singh et al., 2003). However, a large amount of soil N not used by plants and microorganisms in arid and semiarid regions remains in the soil profile as a residual N (Ghimire et al., 2017). Therefore, there is a high chance of rapid soil acidification in arid and semiarid environments with high residual inorganic N. Specifically, ammoniacal N remaining in the profile decreases soil pH.

Soil acidification could affect cotton (*Gossypium hirsutum* L.) production in the Texas High Plains, one of the highly productive agricultural ecosystems in the United States, with ~1.7 million ha planted in 2019 (NASS, 2021). High N fertilizer costs already challenge cotton farmers. Nitrogen management alone accounts for 15–20% of cotton production costs

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Abbreviations: EC, electrical conductivity; PMC, potentially mineralizable carbon; PMN, potentially mineralizable nitrogen; SOC, soil organic carbon.

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(Bronson et al., 2004; Dhakal et al., 2019). Improved knowledge of the N fertilization rate and the extent of soil acidification will help sustain cotton farming in the Texas High Plains. The main objective of this study was to evaluate soil N pools, soil pH, and acidification rate with long-term N management in a continuous cotton production system.

Core Ideas

- N rates of 168 and 224 kg ha^{-1} resulted in 73–167% higher residual inorganic N than lower rates.
- Soil pH decreased by 0.039 per kg increase in residual N at 0-to-15-cm depth.
- Optimizing N rate that minimizes residual N while maintaining yields can reduce soil acidification.

2 | MATERIALS AND METHODS

A long-term field study was established in 2002 at the Texas A&M AgriLife Research farm near Plainview, TX (34.15 N, 101.95 W, 1072 m asl). The study site has a Pullman clay loam soil (fine, mixed, superactive, thermic Torrertic Paleustoll) (Soil Survey Staff, 2020) and a hot, dry, semiarid environment, with annual mean maximum and minimum temperatures of 23 and 7.3 ˚C, respectively, and average annual precipitation of 506 mm.

The study consisted of a randomized complete block design with five N rate treatments (0, 56, 112, 168, and 224 kg ha^{-1}) and four replications from 2002 to 2018. The treatments were fixed so that each plot received the same rate of N fertilizer and conventional tillage each year. Individual plots were 12.2 m by 36.6 m. Each plot received pre-bloom side-dressed N from urea ammonium nitrate (32:0:0) using a soil knife injection applicator based on the treatment N rate. Residual inorganic N was monitored in June 2019 at 0-to-15-cm and 15-to-30-cm depths, right before applying N treatments. The experimental detail, 2002–2015 carryover NO_3-N, and cotton lint yield are summarized in Dhakal, Lange, Parajulee, and Segarra (2019), which analyzed soil residual inorganic N (NO_3-N and NH_4-N) along with other soil health indicators to understand the drivers of acidification. Regionally adapted commercial cotton cultivars were used throughout this study. Cotton was planted in the third week of May using a John Deere four-row planter and harvested in November using a John Deere cotton stripper with a field cleaner. The seeding rate was 138 k seeds ha^{-1} in 0.76-m rows. All plots were irrigated with a subsurface drip irrigation system. Standard chemical management protocols were followed to manage weeds, insect pests, and diseases.

Soil samples were collected in June 2019, after 17 yr of experiment establishment, from four random locations within each plot using a Giddings hydraulic probe of 2-cm inner diameter. Soils were collected at 0-to-30-cm depth, and each core was divided into 0-to-15-cm and 15-to-30-cm depth increments. The four cores from each plot were composited by depth, homogenized, bagged, and transported to the laboratory. A separate set of four subsamples was collected from 0-to-15-cm and 15-to-30-cm depths of each treatment using a bulk density probe (i.d. 2.1 cm) and composited to determine soil bulk density.

In the laboratory, gravimetric water content was determined by oven-drying 20-g soil samples from each plot for 24 h at 105 ˚C. Soil residual inorganic N was determined by extracting 5-g subsamples with 25-ml 1 M KCl solution and analyzing for NO_3-N and NH_4-N contents in an automated Timberline Ammonia Analyzer (Timberline Instruments). Potentially mineralizable carbon (PMC) was determined by 4-d aerobic incubation of 20-g soil samples (Zibilske, 1994) and subsequent measurement of the headspace CO_2-C using LI-820 Infrared Gas Analyzer (Li-COR Biosciences). The potentially mineralizable N (PMN) content was determined by extracting 5-g incubated samples on Day 4 and analyzing NO_3-N and NH_4-N as for residual inorganic N. The PMN was also estimated by extracting another set of 5-g soil samples in hot-KCl (Gianello & Bremner, 1986). Soil organic C (SOC) and total N were determined in a dry combustion analyzer (LECO Corporation). Soil bulk density samples were collected using 2-cm-diam. soil cores and oven-drying of soil samples for 24 h at 105 ˚C. Bulk density was calculated by dividing the oven-dried soil by the core volume.

Data were tested for and met the criteria for normality of residuals and homogeneity of variance. Data on all soil properties were analyzed using a MIXED model procedure of a statistical analysis system (SAS v.9.4, SAS Institute). For all analyses, treatments were considered a fixed effect and replications as a random factor. Treatment means were separated at $p < .05$ unless otherwise stated. Simple linear regression analysis was performed to explain the relationship of inorganic N with soil pH. Since the same rate of N was applied for the last 17 yr, the sum of total N applied for the entire study period was used to calculate the acidification rate.

3 | RESULTS

Soil N pools were significantly different among N rates. Specifically, N application rates of 168 and 224 kg ha^{-1} had greater residual inorganic N than the lower N rates at both 0-to-15-cm and 15-to-30-cm soil depths (Table 1). Soil PMN analyzed using an aerobic incubation method and hot-KCl extraction showed the same trend, higher PMN contents with

TABLE 1 Soil properties under different N rate treatments at 0-to-15-cm and 15-to-30-cm soil depths

Depth	N rate	Residual inorganic N	PMN–incubation	PMN–hot-KCl	TN	SOC	C/N ratio	Soil pH	EC	Db
cm		kg ha^{-1}			Mg ha^{-1}				dS m^{-1}	g cm^{-3}
0–15	0	22.2±1.76b	16.9±0.62b	15.9±1.42b	0.63±0.04b	5.32±0.39	8.40±0.18a	7.85±0.17a	0.16±0.03	1.33±0.05
	56	26.5±1.46b	21.6±1.56b	18.9±0.51b	0.67±0.04ab	5.74±0.33	8.58±0.12a	7.85±0.17a	0.14±0.01	1.33±0.04
	112	29.8±1.04b	23.9±1.09b	22.8±0.73b	0.69±0.03ab	5.85±0.31	8.48±0.11a	7.53±0.30a	0.13±0.01	1.33±0.02
	168	59.0±15.0a	39.4±8.93a	39.7±10.9a	0.72±0.05a	5.91±0.43	8.18±0.18b	6.45±0.42b	0.18±0.04	1.38±0.02
	224	59.3±20.0a	41.7±12.1a	39.3±11.5a	0.74±0.06a	5.89±0.49	8.00±0.16b	6.45±0.61b	0.20±0.07	1.32±0.03
15–30	0	17.9±0.28b	17.3±1.64b	15.7±1.74b	0.70±0.05a	4.91±0.36	7.01±0.17	7.93±0.11	0.20±0.02bc	1.50±0.02
	56	20.8±1.62b	16.3±1.43b	16.0±1.15b	0.61±0.02b	4.42±0.32	7.24±0.24	8.10±0.11	0.21±0.02b	1.49±0.03
	112	24.0±1.87b	21.2±2.89b	18.6±2.50b	0.58±0.05b	4.16±0.32	7.18±0.15	8.00±0.04	0.17±0.02c	1.47±0.03
	168	41.5±6.77a	35.3±7.51a	33.0±6.79a	0.63±0.05b	4.61±0.41	7.34±0.10	7.78±0.23	0.27±0.02a	1.46±0.02
	224	46.9±6.14a	40.5±6.02a	34.6±2.92a	0.72±0.08a	5.31±0.85	7.27±0.35	7.75±0.15	0.26±0.01ab	1.48±0.04

Note. Db = bulk density; EC = electrical conductivity; PMN = potentially mineralizable N; SOC = soil organic C; TN = total N. Mean values followed by different lowercase letters in a column indicate a significant difference between N rate treatments within a soil depth ($p \leq .05$).

168 and 224 kg ha^{-1} rates than with the other treatments. Soil total N at 0-to-15-cm depth was significantly greater with the 224 kg N ha^{-1} rate than with 0 kg N ha^{-1}, and other treatments remained intermediate between these rates. At 15-to-30-cm depth, soil N was greater with 0 and 224 kg N ha^{-1} rates than the other N rates. Soil PMN measurement using aerobic incubation and hot-KCl extraction showed 98% similarity in variation in PMN with increasing N rates.

The SOC content did not increase with increasing N rate in either soil depth, while the C/N ratio was lower with the 168 and 224 kg ha^{-1} rates than other rates at 0-to-15-cm depth (Table 1). At 15-to-30-cm depth, the C/N ratio was not significantly different among N rate treatments.

Soil pH at 0-to-15-cm depth varied significantly across N augmentation treatments (Table 1). The long-term application of N at high rates (>112 kg ha^{-1}) reduced soil pH. Although regression analysis between different N pools revealed a strong relationship between residual inorganic N and two methods of PMN measurements (Figure 1a,b,c), residual inorganic N was the best predictor of soil acidification (Figure 1d,e). Soil pH decreased by 0.039 and 0.01 per kilogram increase in residual inorganic N at 0-to-15-cm and 15-to-30-cm depths, respectively. Soils under different N rates received 0 to 3,808 kg N ha^{-1} during 2002–2018. The soil pH decline rate was 0.4 pH units per 1,000 kg N addition at 0-to-15-cm depth and 0.07 units per 1,000 kg N addition at 15-to-30-cm depth.

Soil electrical conductivity (EC) did not differ among N rates at 0-to-15-cm depth nor did soil bulk density at either depth (Table 1). Soil EC at 15-to-30-cm depth was greater with the 168 and 224 kg ha^{-1} rates than with the 0 and 112 kg ha^{-1} N rate treatments. Soil EC with the 56 kg N ha^{-1} treatment was lower than soil EC with the 224 kg ha^{-1} rate but not significantly different from that of 168 kg ha^{-1} rate.

4 | DISCUSSION AND CONCLUSION

Evaluation of different N pools after 17 yr of five rates of N augmentation treatments in continuous cotton showed more residual soil inorganic N and PMN at higher N rates than soils receiving 0 fertilizer N. High residual inorganic N suggests greater N availability at 0-to-30-cm soil depth than N needed for the crop. Consistently high residual inorganic N accumulation in the soil profile of semiarid soils, where N rates are not adjusted based on residual inorganic N and where the N is not lost through drainage and runoff, can cause gradual acidification of soil (Tang et al., 2002 ; Ghimire, Machado, & Bista, 2017). We observed more than 59.0 kg ha^{-1} residual inorganic N and 39.3 to 41.7 kg ha^{-1} PMN in the surface 0-to-15 cm in those soils that received more than 112 kg N ha^{-1}, which were correlated with significant soil acidification. The rate of soil acidification decreased as soil residual

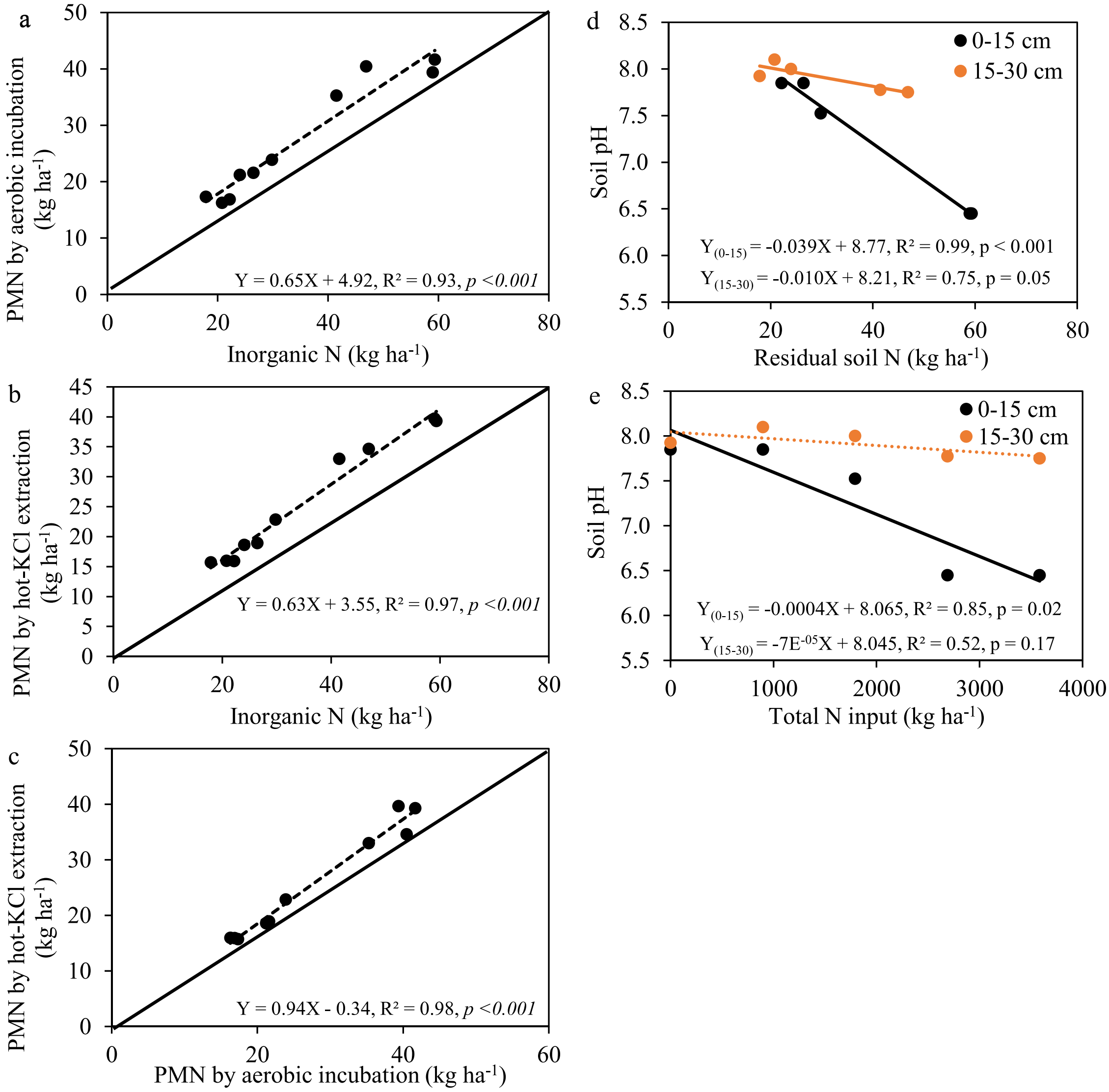

FIGURE 1 Relationship between different N pools and soil pH; (a) inorganic N and PMN by aerobic incubation, (b) inorganic N and PMN by hot-KCl extraction, and (c) between two methods of PMN estimation, (d) residual soil inorganic N and soil pH in 2019, and (e) total N inputs during 2002–2018 and soil pH in 2019. PMN = potentially mineralizable N

inorganic N content decreased. The 15-to-30-cm depth had a lower acidification rate than did surface soil. When acidification rate with reference to cumulative N input was calculated, soil pH decreased by 0.4 and 0.07 per 1,000 kg N addition at 0-to-15-cm and 15-to-30-cm depths, respectively. This acidification rate is greater than the 0.2 to 0.3 pH unit per 1,000 kg N addition seen in winter wheat (*Triticum aestivum* L.)–fallow rotations in the U.S. Pacific Northwest (Ghimire, Machado, & Bista, 2017) and in the southwestern part of Australia (Singh, Odeh, & McBratney, 2003).

The PMN is often considered a good indicator of biologically available N and serves as a better predictor of crop yield than inorganic N alone (Sharifi et al., 2007). The relative response of PMN measured by hot-KCl extraction and aerobic incubation showed a similar decrease in pH with increasing PMN contents. Although both methods were equally effective in estimating soil acidification with an increasing N rate, residual inorganic N was the best predictor of soil acidity in the cotton production system.

In contrast to N pools and soil pH, higher N rate treatments did not affect PMC and SOC. An increase in N rate is expected to increase biomass production and thereby increase labile and total SOC content. Cotton lint yield was higher at high N rate treatments (Dhakal, Lange, Parajulee, & Segarra, 2019), but a corresponding increase in biomass formation on a scale that could be measured as change in SOC was not observed. Greater residual inorganic N under high N rate treatments may have increased the decomposition rate, leading to a higher SOC turnover in treatments receiving a high amount of N. Reduced C/N ratio at higher N rate treatments also suggests

N accumulation with no change in SOC. Slight acidification of alkaline soils increases nutrient availability. However, continuous acidification decreases crop production through several changes in soil reactions. Continuous N fertilizer addition significantly decreased soil pH and affected crop production in drylands of the inland U.S. Pacific Northwest (Mahler, 2002; Ghimire, Machado, & Bista, 2017), the rainfed agroecosystems of South Asia (Ghimire & Bista, 2016), major crop production regions of China (Guo et al., 2010), and the semiarid regions of Australia (Singh, Odeh, & McBratney, 2003). The change in soil pH in this study is small. However, previous studies showed that when soil pH decreased to below 5.5, cotton plants began showing Al and Mn toxicity symptoms and affect lint quality (Singh, Odeh, & McBratney, 2003). Because soil pH is measured on a logarithmic scale, even a small change in pH indicates a large increase in H^+ ions and consequently affects soil health and nutrient availability. Plant and microbial uptake of N are expected to decrease with increasing soil acidity (Motavalli et al., 1995), further increasing residual inorganic N and accelerating the rate of acidification.

Continuous monitoring of N management effects on residual inorganic N and soil acidification will determine the rate of N that maximizes cotton production while reducing negative impacts on soil health and sustainability. Results of this study suggest that adding more than 112 kg N ha^{-1} annually in a continuous cotton production system may reduce soil pH below the critical limit for cotton production, affecting both crop production and the environment of the largest cotton-producing area in the United States.

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AUTHOR CONTRIBUTIONS

Rajan Ghimire: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Software; Writing-original draft. Megha N. Parajulee, Conceptualization; Funding acquisition; Investigation; Project administration; Writing-review & editing. Pramod Acharya: Data curation; Investigation; Methodology; Writing-review & editing. Dol P. Dhakal: Data curation; Investigation; Writing-review & editing. Abdul Hakeem: Data curation; Investigation; Writing-review & editing. Katie L. Lewis: Funding acquisition; writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Novel-iridoviral kinase induces mortality and reduces performance of green peach aphids (*Myzus persicae*) in transgenic *Arabidopsis* plants

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Abstract

Insect pests are a serious threat to agricultural productivity. Genetically-modified crops offer a promising alternative, and *Bacillus thuringiensis* endotoxin genes have played a major role in this respect. However, to overcome insect tolerance issues and to broaden the target range, it is critical to identify alternative insecticidal toxins working through novel mechanisms. In our previous study, a kinase from *Chilo* iridescent virus (CIV) that has insecticidal activity was identified and designated as Iridovirus Serine/Threonine Kinase (ISTK). A 35 kDa truncated form of ISTK, designated iridoptin, was obtained during expression and purification of ISTK in the yeast system. This yeast-expressed CIV toxin induced 50% mortality in cotton aphids and 100% mortality in green peach aphids (GPA). In this study, codon optimized *ISTK* gene and *iridoptin* fragment (designated *oISTK* and *oIRI,* respectively) were designed by altering the codon usage features that are seldom present in plant exons. Codon-optimized gene(s) cloned into plant expression vectors were used to stably transform *Arabidopsis* plants. PCR analysis of genomic DNA of transformed plants confirmed the presence of the DNA insert (*oISTK*/*oIRI*) in selected transgenic lines. Further screening was performed by selecting the PCR positive lines, which showed expression of respective toxins at the polypeptide level, using Western blot analyses. Codon-optimized gene constructs resulted in significant improvement in levels of expression of ISTK and iridoptin polypeptide and confirmed its stability in planta. The stable lines expressing either of the two toxin forms induced moderate to very high mortality in GPAs and significantly affected GPA development and fecundity.

Keywords Transgenic insect-resistant plants · Green peach aphids · *Chilo* iridescent virus · Serine/threonine kinase · Plant-incorporated protectants

Introduction

Insect pests are a key factor in reduction of agricultural productivity. The economic impact of insect pests exceeds $200 billion globally (Pimental 2009) and is $33 billion for the U.S. (USBC 2001). Extensive use of chemical insecticides dominates the current insect control strategies. Genetically modified (GM) crops offer a promising alternative toward reducing the insecticide load in agricultural systems. Insect-resistant transgenic crops commercialized thus far almost always have utilized genes derived from the bacterium *Bacillus thuringiensis (Bt)*.

Aphids (Order: Hemiptera) are sap-sucking insects and a global crop pest. They cause serious economic damage via direct feeding and via transmission of viral diseases, resulting in crop loss estimated to be hundreds of millions of dollars (Head 1992; Yu et al. 2014). There are over 250

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species of aphids that are considered serious pests for agriculture (Blackman and Eastop 2000) and green peach aphids (GPAs; *Myzus persicae*) are key pests of many agriculturally important crops within this order. They are highly capable of transmitting more than 100 plant viruses, resulting in significant yield reductions (Blackman and Eastop 2000). Apart from the pest status, the rapidly developing chemical insecticide resistance has become a major problem in their control (Silva et al. 2012; Bass et al. 2014). *Bt* δ-endotoxins exhibit only low to moderate effects on aphid mortality and development (Faria et al. 2007; Lawo et al. 2009; Porcar et al. 2009; Li et al. 2011; Chougule et al. 2013). Transgenic approaches using lectin, proteinase inhibitor, plant derived R genes and RNAi control has been explored for aphid control with negligible to minimal success (Yu et al. 2014). However, so far, no transgenic approaches for host-plant resistance to aphids have been commercialized. Commercial breeding efforts have been hindered due to the limited availability of germplasm for plant resistance to aphids (Dogimont et al. 2010; Bhatia et al. 2012). Thus, there is a need for identification of new polypeptide toxins working through novel mechanisms for potential use in development of transgenic plants that are resistant to aphids.

Viruses of invertebrates are a potential source of insecticidal genes in the development of pest-resistant transgenic plants (Liu et al. 2006). *Chilo* iridescent virus or invertebrate iridescent virus 6 (CIV or IIV6) is a type species for genus *Iridovirus* and family *Iridoviridae*. CIV has been shown to induce mortality and deformity in cotton boll weevil (McLaughlin et al. 1972; Bilimoria 2001), significant reduction in cotton aphid (Bilimoria 2001) and citrus aphid (Hunter et al. 2001) populations, sub-lethal infections in scarab beetles (Jenkins et al. 2011) and covert infections in mosquito species (Marina et al. 2003). CIV virion protein extract (CVPE) induces apoptosis in spruce budworm and boll weevil cell lines which is dependent on JNK signaling pathway and apical caspase of the host cells (Bilimoria et al. 2001; Paul et al. 2007; Chitnis et al. 2008).

In our previous study, we showed that a full length iridovirus serine/threonine (s/t) kinase (ISTK) identified from CIV (ORF389L) and/or its truncated form (iridoptin) expressed in yeast induced apoptosis and/or host-protein shut off in insect cell lines (Chitnis et al. 2011). Iridoptin retains motifs for s/t kinase and an ATP-binding site, and Chitnis et al. (2011) demonstrated that kinase activity of this toxin is absolutely necessary for insecticidal activity, and that it presumably works through novel mechanism (Chitnis, Paul and Bilimoria, manuscript in preparation). Yeast-expressed and purified iridoptin induced mortality in green peach aphids (Becker, Ganapathy, and Bilimoria, unpublished) and cotton aphids (Parajulee and Bilimoria, unpublished).

These studies suggest an insecticidal role of ISTK and iridoptin, and the goal of this research was to assess the potential of transgenic plants expressing ISTK/iridoptin in aphid control. Expression of foreign genes in plants may be compromised due to mRNA instability and poor translational efficiency (Doran 2006; Jackson et al. 2014). This was overcome in the most successful *Bt* plants by codon optimization (Perlak et al. 1991). Many studies have indicated the necessity of redesigning genes of prokaryotic or non-plant eukaryotic origin for use in plant systems for proper expression (Perlak et al. 1990; Perlak et al. 1991; Sutton et al. 1992; Liu et al. 2003; Liénard et al. 2007; Wang et al. 2008; Wu et al. 2008; Webster et al. 2009; Sanahuja et al. 2011; Wu et al. 2011).

We hypothesized that viral ISTK/iridoptin nucleotide sequence requires codon optimization for robust expression in plants and ectopic expression of the optimized *ISTK* gene or iridoptin sub-fragment in plants will yield active and stable polypeptide in planta and this polypeptide will likely have aphicidal activity.

In this article, we present the development of transgenic *Arabidopsis* plants constitutively expressing the insecticidal iridoviral kinase, ISTK or their sub-fragment (iridoptin), and evaluate the aphicidal potential of these plants expressing the toxin by quantifying their effects on aphid growth and developmental duration, fecundity, and mortality. To the best of our knowledge, this is the first report of utilizing a novel- iridovirus-encoding kinase towards development of aphid-resistant transgenic plants.

Materials and methods

Codon-optimization and synthesis of the full-length *ISTK* gene sequence (1233 bp)

The native *ISTK* gene was optimized according to the plant codon usage (http://www.kazusa.or.jp/codon; *Arabidopsis thaliana* and *Nicotianna benthamiana*). The *ISTK* sequence was also modified to eliminate certain factors considered to be responsible for low mRNA stability and foreign-protein expression, and were proportionately replaced by their synonymous codons in the plant system. Splice sites were predicted using softberry FSPLICE (Find Splice Site in Genomic DNA) software accessible through http://www.softberry.com at 90 percent threshold and modified using the degenerate codons. Apart from the factors listed in Supplementary Table 1, localized AT rich regions in the viral gene sequences resembling plant introns were modified, consecutive strings of A + T or G + C nucleotides of 5 or more were avoided, G + C content in codon position 3 were preferred, pyrimidines (C, T) were used more frequently than purines (G, A) to mimic dicots. The final in-silico modified *ISTK* sequence *(oISTK)* was artificially synthesized by Geneart (Life Technologies), Germany.

Construction of expression vectors

The *ISTK* gene (native and optimized; ~ 1.2 kb) and *iridoptin* sub-fragment (native and optimized; ~ 0.9 kb) were each cloned into plant binary vector constructed with a dual 35S enhancer promoter sequence extracted from the PRG229 vector generously donated by Dr. Roberto Gaxiola (Arizona State University) and introduced into the pBI121 vector backbone in place of the CaMV 35S promoter (in-house constructed vector designated pD35S). Apart from the constitutive promoter for the gene of interest (GOI), the vector also harbored 2X/3X flag tag at the 3′end of the GOI, and a *Npt* II (Neomycin phosphotransferase II*;* kanamycin resistance gene) selection marker. Cloned vectors were used to transform bacterial strain *DH5α* through chemical transformation. Cloning and gene sequences were confirmed by PCR, DNA sequencing, and restriction analysis. The confirmed vector constructs were used to transform *Agrobacterium tumefaciens* strain GV3101 by freeze/thaw shock treatment (Weigel and Glazebrook 2006).

Aphid rearing

GPAs were provided by Dr. Jyoti Shah (University of North Texas, Denton, TX). A GPA colony was then reared on an equal mixture of 2–3-week-old radish and mustard seedlings. Growth chamber conditions were maintained at 22 ± 2 °C with 14:10 h light:dark photoperiod and 50–60% relative humidity.

Plant transformation

Agrobacterium GV3101 clones containing the following plant binary vector constructs were used in the stable transformation of *Arabidopsis thaliana* (ecotype Columbia-0 or Col) plants: pD35S-*GUS*, pD35S-*oIRI*, and pD35S-*oISTK* using the modified floral dip method (Clough and Bent 1998). Mature T_1 seeds were harvested from the transformed plants. To select for putative stable and independent transformants (T_1 lines), the sterilized *Arabidopsis* seeds were plated on *Arabidopsis* seed germination media [1/2 Murashige Skoog (MS), 1% (w/v) Sucrose, 0.05% (w/v) MES, 0.8% (w/v) Agar, pH 5.8] containing 50 µg/mL kanamycin. To break dormancy, the sterilized seeds were kept at 4° C for 3 days before moving to room temperature (RT) under constant lighting. Selected transgenic T_1 lines were then moved to pots.

Analysis of transgenic plants by Polymerase Chain Reaction (PCR)

Genomic DNA was isolated from rosette leaves of 3–4-week-old putative transgenic lines (T_1) and PCR was carried out using GoTaq Green Master Mix (Promega). To ensure proper insertion of *oISTK* gene, forward primer was designed from the promoter region (5′ CTC GAG TGG CCA CCA TGG G 3′) and the reverse primer (5′ AAAA GAGCTCTCACTT GTC AT 3′) was gene sequence specific. For *oIRI* gene fragment, promoter-specific forward primer (5′ CTC GAG TGG CCA CCA TGG G 3′) and gene sequence-specific reverse primer (5′ AAAA GAGCTC TCACTT ATC GTC GTC 3′) were used. PCR conditions were set at 94 °C: 3 min; [94 °C: 45 s, 59.5 °C: 60 s, 72 °C: 90 s] 35 cycles; 72 °C: 5 min. Cloned pD35S-*oISTK* vector was used as a positive control for PCR amplification. Genomic DNA extracted from transgenic lines containing the *GUS* transgene was used as negative control. After PCR, samples were run on a 0.8% agarose gel, with 0.5 µg/mL ethidium bromide, for visualization of bands.

Screening T_1 lines using Western blot analysis

Arabidopsis rosette leaves (~ 10 mg; fresh weight) were grounded with 50 µL of protein extraction buffer [10 mM EDTA (pH 8.0), 0.1% (v/v) Triton X-100, 0.1% (w/v) sodium lauryl sarcosine, 40 mM sodium phosphate buffer, pH 7.0, 10 mM β-mercaptoethanol, 1 µg/mL leupeptin, 1 µg/mL pepstatin A, and 1 mM phenylmethylsulfonyl fluoride], and incubated on ice for 45 min to extract total proteins (TP). After incubation, samples were centrifuged at 14,000 g for 20 min at 4 °C to collect supernatant. Protein concentration was determined using the Bradford assay (Bradford 1976). TP were separated and resolved in a 10% SDS-PAGE and transferred onto a nitrocellulose membrane. After staining the blotted membrane with ponceau stain, it was blocked with blocking solution [5% (w/v) milk in Tris buffered saline- Tween 20 (TBS-T; 100 mM Tris–Cl, 154 mM NaCl; pH 7.5 with 0.1% Tween-20)] for 1 h (RT). Blocking was followed by primary antibody probing (Anti-flag antibody; Rockland, Cat # 600-401-383; 1:1000, with 2% milk-TBS-T) at 4 °C overnight and horse-radish peroxidase conjugated anti-rabbit secondary antibody (Novex, Cat # A16104; 1:5000; with 2% milk-TBS-T). Chemiluminescent substrate was used for detection.

Aphid bioassay

Several independent, transgenic T_1 lines, expressing ISTK/iridoptin at the protein level, were challenged with GPAs and investigated for their effects on survival, development, and reproductive rate of aphid populations via excised-leaf bioassays. Fully expanded, young leaves were detached from the selected 5 to 6-week-old transgenic lines and placed in tightly sealed sterile petri dishes (Fisher Scientific, Cat # 09-720-503) with moist adsorbant pads at the bottom. Each leaf was inoculated with five newly born GPA nymphs (< 12 h old). Five replicate plates were used for each transgenic line resulting in 25 aphid nymphs per treatment. Every 3 days, old leaves were replenished with new leaves from respective transgenic lines. Mortality and reproductive rate (number of new nymphs produced by the initial inoculum after developing into adults) were recorded every 2–3 days. The insect mortality rate was used to measure the level of insect resistance of each independent transgenic line. Because normal aphid development takes about 6–7 days for a new born aphid to become an adult, the first day of reproductive phase of these initial cohorts was used as a cut-off point to assess nymphal development to adulthood [between 7 and 9 days after treatment (DAT)]. The percentage developmental success was calculated based on the number of initial aphid inoculum in the cohort that developed into adulthood at the cut-off DAT. Transgenic lines transformed with the *GUS* construct were used as negative controls. Bioassays were conducted in a growth chamber with conditions as detailed in aphid rearing. Aphid mortalities were analyzed as percentages in each replicate, and fecundity (parthenogenetic reproduction) was measured as the mean of the total number of newborn nymphs produced in each replicate. After counting, the neonate nymphs were removed from the experimental dish to avoid the crowding effect. The entire experiment was conducted in three sequential runs, but each run had a GUS negative control and the transgenic line with the highest mortality (positive control; oIRI line 23) as control treatments to ensure consistency across the runs. The sequential experiments were necessitated by the experimental logistics and the availability of the resources. The period during which the experiment was carried out was kept narrow and chamber conditions were kept identical.

Statistical analysis

Aphid mortality and fecundity data were all analyzed using a one-way analysis of variance, with transgenic line as a source of variation (SAS Institute, 2010). Treatment means were separated by Tukey–Kramer HSD test at the $\alpha = 0.05$ level. Data for three GUS control runs, three oIRI 23 runs and two oISTK 11 runs were pooled to verify the consistency of the experiment. Upon the verification that the three sequential runs were consistent, all runs were pooled for the final analysis (at 8 or 9 DAT).

Results

Native *ISTK/iridoptin* sequence analysis and optimization to better suit expression in plants

A wide range of factors that influence foreign gene expression in plants were considered in the in-silico modification of the native *ISTK* gene (1233 bp), resulting in a single-optimized gene sequence that could reach a detectable and improved expression level in plants. *Iridoptin* sequence is a sub-fragment of *ISTK* gene comprising of 1–909 bp. Nucleotide sequence analysis of the native *ISTK* gene revealed significant differences in codon usage between *ISTK* and plant genes, and presence of 23 potential mRNA degradation signals (ATTTA, ATTAA), five splice acceptor sites (AG) and five splice donor sites (GT) with reference to the plant system. The nucleotide sequence of the native *ISTK* gene was optimized by replacing the less preferred codon triplets with the respective highly preferred degenerate codons without altering the amino acid sequence. After codon optimization, the nucleotide sequence was again scanned and altered by modifying potential mRNA degradation signals, polyadenylation signals (AATAAA, AATAAT, AATTAA, and AACCAA), RNA polymerase II termination (polyA) signals (CAN[7–9]AGTNNA), splice acceptor sites (AG), splice donor sites (GT) and sites for common restriction enzymes that include *Bam* HI, *Sac* I, *Kpn* I, and *Not* I (Supplementary Table 1). Multiple negative cis-acting sites were successfully modified and eliminated wherever possible as mentioned in Supplementary Table 1. The sequence was simultaneously modified by increasing the frequency of codons with a C or G, in the third position, from ~ 16% to 58%. GC content was improved from ~ 26% in the native *ISTK* sequence to ~ 40% in the optimized sequence to prolong mRNA half-life. The *optimized ISTK (oISTK)* sequence contains 276 (out of 411) silent codon changes when compared to the respective native viral sequence. A value of 100 is set for a codon with the highest codon frequency and it was compared with less preferred codon triplets that are very likely to reduce the efficiency of expression using relative frequencies. About 60% of the total codons (~ 246 out of 411) of the *oISTK* gene showed a relative codon frequency between 91 and 100%. The codon adaptation index (or CAI) value describes how well the codons of this gene match with the codon usage of the host on a scale of 1. The *oISTK* sequence resulted in CAI of 0.86. Finally, after complete multi-parametric gene optimization, the native gene was 75% identical to the optimized sequence (Fig. 1a). In the optimization process, extreme care was taken to keep the protein sequence exactly the same while modifying only the nucleotides (Fig. 1b).

Construction of plant expression cassettes and creation of transforming *Agrobacteria*

A total of five plant expression vectors were developed, harboring the 1.2 kb native/optimized *ISTK*, 0.9 kb native/optimized *iridoptin* and native *GUS* nucleotide sequences in the backbone of pD35S plant-binary vector: 1. pD35S—*GUS*, 2. pD35S—*IRI*, 3. pD35S—o*IRI*, 4. pD35S—*ISTK*, 5. pD35S—o*ISTK* (Fig. 1c). The gene fragments were present under the control of the dual 35S promoter at the 5′ end and a 2X/3X/no flag tag at the 3′ end of the gene of interest. All constructs except the GUS construct contained a flag tag. The presence, size and orientation of respective gene inserts (GOI) in transformed *DH5α* cells were confirmed by PCR with gene-specific primers and primers for promoter and terminator sequence that flank the GOI; and also by restriction digestion analysis. *Agrobacterium tumefaciens* GV3101 clones transformed with these different vectors had the right size of inserts as confirmed by PCR. Sequencing of these PCR products was done to confirm orientation and the absence of any mutation in the gene inserts (data not shown).

Creation of T_1 generation transgenic *Arabidopsis* lines

A. tumefaciens GV3101 strains carrying the recombinant plant binary vectors with dual 35S enhancer promoter (pD35S-*oIRI*/ pD35s-*oISTK*/pD35S-*GUS*) were used to transform Arabidopsis plants (ecotype *Columbia-0*). In this study, lines transformed with GV3101 containing the *GUS* gene construct served as a negative control. Thirty to forty independent first generation (T_1) lines were identified by selection on kanamycin-containing media for each transformation.

Confirmation of integration of transfer DNA (T-DNA) into the *Arabidopsis* genome by PCR

PCR was carried out to demonstrate the integration of the right-sized gene insert into the plant genome in T_1 transgenic lines. PCR with genomic DNA, isolated from independent transgenic T_1 lines containing *oISTK*, using gene-specific/flanking region primers, yielded a band at the expected ~1200 bp region, and that of *oIRI* in ~900 bp region, when run on a 0.8% agarose gel (Fig. 2a, b). Cloned pD35S-*oISTK* vector was used as template for positive control and showed a ~ 1200 bp band. However, *GUS* negative control showed no band as expected (Fig. 2a).

Translation of *ISTK* and *iridoptin* in stable *Arabidopsis* transgenic lines

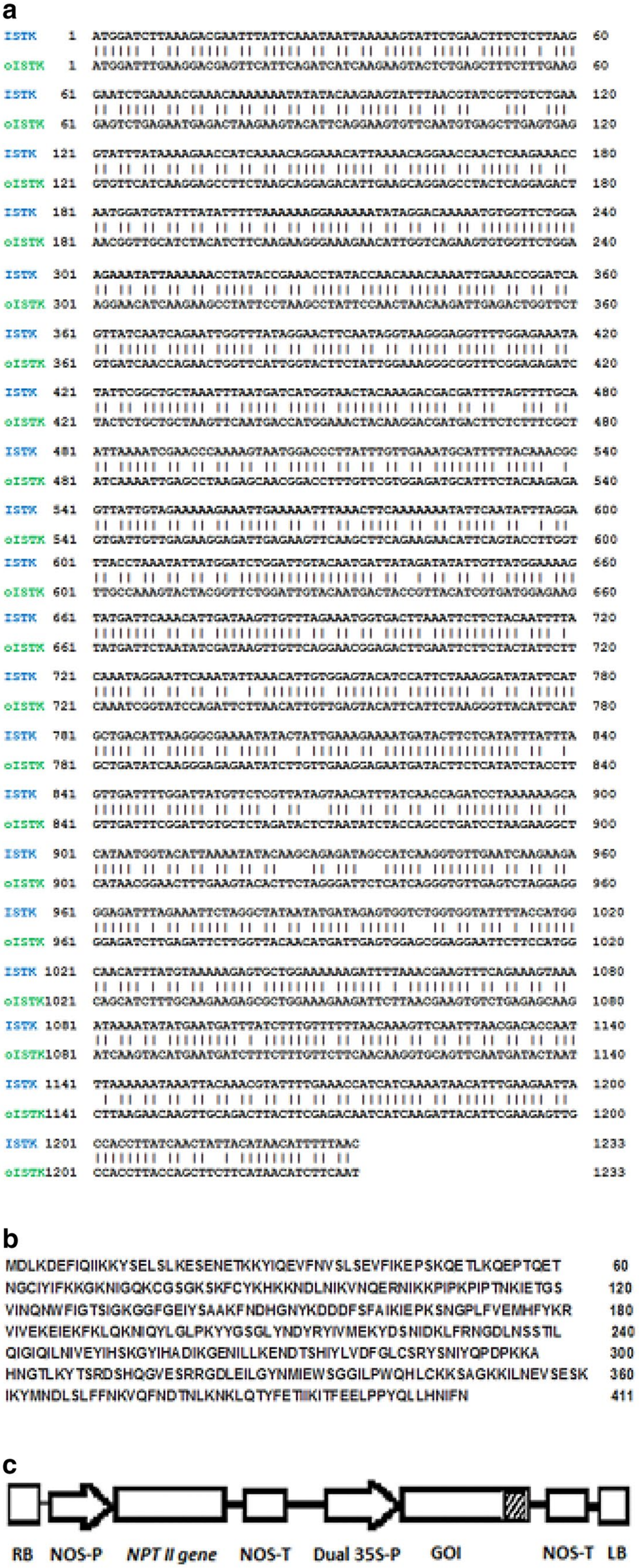
To facilitate the detection of transgene expression, a flag epitope tag was added to the 3′ end of *ISTK* and *iridoptin*. Optimization of the *ISTK/iridoptin* for expression in plant system resulted in detectable and significantly pronounced protein accumulation as compared to the corresponding native gene (which was undetectable) when transiently expressed in tobacco (Supplementary Fig. S1). This is the first evidence of detectable accumulation of the viral insecticidal polypeptides, ISTK and iridoptin, in the plant system.

Western blot analyses were carried out to demonstrate the expression and accumulation of ISTK and iridoptin at the polypeptide level in the transgenic T_1 *Arabidopsis* lines. Total proteins extracted from the 20–30 PCR-positive lines of each of the *oISTK* and *oIRI* transformants were tested for the presence of respective polypeptide accumulation. ISTK and iridoptin polypeptides were accumulated at the predicted molecular weight region (ISTK: 50 kDa; iridoptin: 37 kDa region) (Fig. 3a, b). Flag tags (2X/3X) add approximately 2–3 kDa to the actual polypeptide size. GUS lines served as a negative control and detected no band as expected (Fig. 3a, b). Immuno-precipitated ISTK and iridoptin from the transgenic plant protein extracts were identified using MALDI-TOF analysis (Supplementary Figs. S2 and S3). Hence, this serves as a confirmation of ectopic expression of ISTK and iridoptin in the plant cells after extensive optimization of the gene sequence to suit expression in plant system. The Western blot analysis also helped in the identification of ISTK/iridoptin expressing T_1 lines to carry out further bioassays.

Based on more Western blot analyses (data not shown) of samples from leaves of different lines, the transgenic T_1 lines expressing iridoptin used in the bioassays were placed in one of the three following categories with respect to iridoptin detection levels in total proteins: (1) oIRI lines 6, 8 and 13 were included in the "high expresser" group in which the iridoptin polypeptide levels were detectable when 50 µg total proteins (TP) were loaded in SDS-PAGE followed by Western blot analysis; (2) the "average expresser" category include lines that had detectable levels of iridoptin when 130 µg of TP were loaded in the gel (Lines 14, 20 and 27) and not with 50 µg of TP; 3) Lines that had detectable levels of iridoptin when more than 130 µg TP were loaded were categorized in "low expresser" group (Lines 5, 16, 23, 26, 28, 30) (Table 1).

Among the several ISTK-expressing T_1 lines, oISTK 31 was classified as high expresser and the remaining lines (except oISTK 35—data not available) were considered average/low expressers. Nevertheless, reliable information was not available to distinguish ISTK-expressing lines between low versus average expresser as in the case of oIRI lines.

Fig. 1 DNA sequences of native and optimized *ISTK/iridoptin*, amino acid sequence of ISTK/iridoptin, and the recombinant expression vector construct containing *ISTK/iridoptin*. **a** Alignment of the full-length native (*ISTK/iridoptin*) and the plant codon-optimized *ISTK* (*oISTK/oIRI*) showing positions of nucleotide replacement (*ISTK:* 1–1233 bp; *iridoptin:* 1–909 bp). **b** Amino acid sequence of ISTK (1–411 aminoacid; iridoptin: 1–303 aminoacid) (Chitnis et al. 2011). **c** Schematic diagram of the transformation vector, pD35S containing the gene of interest (GOI). The GOI, such as *oIRI, oISTK,* or *GUS*, is under the control of the dual 35S promoter (Dual 35S-P) with 2X/3X flag tag (▨) or without flag tag at the 3′end of the gene insert. The *GUS* gene construct lacks the flag tag at the 3′ end of the gene. RB and LB, right and left borders of the T-DNA region. NOS-P and NOS-T, NOS promoter and terminator sequence. *NPT II* (kanamycin resistance) gene is the selective marker



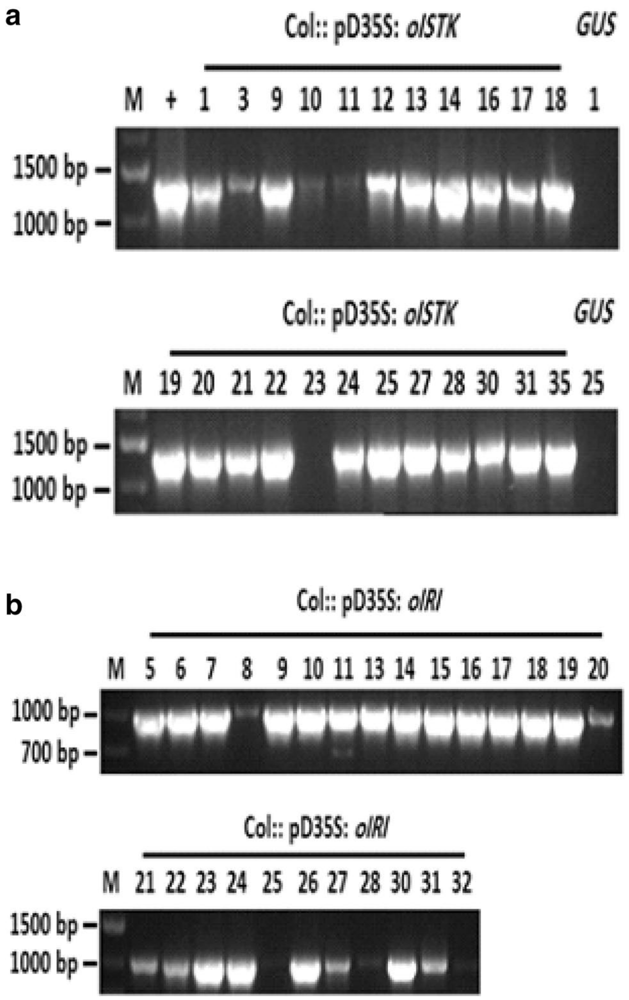


Fig. 2 PCR analysis of putative transgenic T$_1$ *Arabidopsis* lines transformed with the *oISTK* or *oIRI* construct. **a** Analysis of *oISTK* transgenic plants. Lanes: M, DNA Marker; +, PCR with the recombinant vector pD35-*oISTK* as template; GUS 1 and GUS 25, PCR with transgenic Arabidopsis lines (T$_1$) transformed with the pD35S-*GUS* vector; Col:: pD35S: *oISTK* (1–35), PCR with several independent putative transgenic *Arabidopsis* lines transformed with the pD35S-*oISTK* binary vector. **b** Analysis of *oIRI* transgenic plants. Col:: pD35S: *oIRI* (5–32), PCR with several independent putative transgenic *Arabidopsis* lines transformed with the pD35S-*oIRI* binary vector

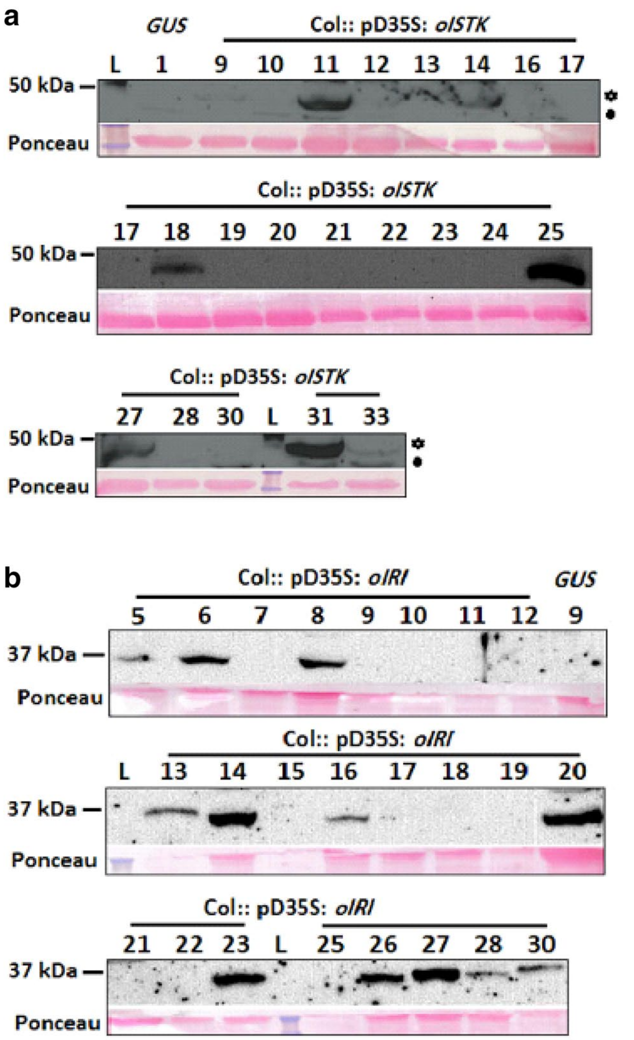


Fig. 3 Western blot analyses of T$_1$ transgenic *Arabidopsis* (*oISTK* or *oIRI*) lines using anti-flag antibody. **a** Analysis of *oISTK* transgenic plants. L, protein molecular weight markers; GUS, total protein extracts from transgenic line containing the *GUS* gene construct; Col:: pD35S: *oISTK* (1–33), total protein extracts from different T$_1$ transgenic lines containing *oISTK* gene construct. Star (✱), ISTK band detected at 50 kDa region. Ponceau, blot stained with Ponceau stain shows unequal loading in these lanes. Dot (●), non-specific band detected in all samples including GUS controls. **b** Analysis of *oIRI* transgenic plants. Col:: pD35S: *oIRI* (5–30), total protein extracts from different T$_1$ transgenic lines containing *oIRI* gene construct

Aphid resistance in iridoptin-expressing transgenic T$_1$ lines

Five similar-aged leaves from each of the different transgenic lines, together with *GUS*-containing lines (control) were challenged with less than 15 h old-GPA nymphs to investigate the effect of plant-expressed iridoptin on aphid survival, development, and fecundity/reproductive potential.

In the first run, several T$_1$ iridoptin-expressing lines (oIRI 20, oIRI 23, oIRI 26, oIRI 13) were examined. Aphid survival and fecundity were investigated over a period of 21 days after treatment (DAT). Line oIRI 23 showed significant reduction in the inoculated aphid population with an average of 92% mortality in less than 2 DAT and showed 100% mortality on 4 DAT. The oIRI 20 line showed over 65% mortality on 2 DAT and 96% mortality by 9 DAT, a period during which introduced aphids could have developed

Table 1 Summary of expression pattern and aphicidal activities of different T_1—oIRI/oISTK lines

Based on expression level	Col:: pD35S: *oIRI* (T_1) lines
High expressers	6(L), 8 (L), 13 (L)
Average expressers	14 (N), 20 (H), 27 (L)
Low expressers	5 (ND), 16 (ND), 23 (H), 26 (H), 28 (ND), 30 (ND)
Based on expression level	Col:: pD35S: *oISTK* (T_1) lines
High expressers	31 (L)
Rest (average and low expressers)	11 (H), 18 (H), 35 (H), 27 (N), 25 (L)

Labelled as *H* high insecticidal activity, *L* low insecticidal activity, *N* no insecticidal activity, *ND* not determined

into adults and undergone active reproduction. Lines oIRI 26 and 13 steadily reduced the aphid survival, with 30–50% mortality on 9 DAT (data not shown). Mortality rates were compared at 9, 13 and 21 DAT among the iridoptin-expressing lines evaluated (Fig. 4a). A significant increase in GPA mortality was observed on iridoptin-expressing leaves from lines 20, 23, and 26 compared with that in GUS control, for all 3 days analyzed. GUS control showed 0% mortality until 13 DAT and an average of 28% mortality by the end of the assay period which could be partly due to aging of the adult aphids. Data collection in line oIRI 13 was discontinued after 13 DAT due to lack of availability of leaf material to test. At the end of the assay period (21 DAT), remaining oIRI lines (20, 23 and 26) showed 100% mortality of inoculated aphids compared to only 28% mortality in GUS control. While being considered as a high expresser, oIRI 13 appeared to not perform as well as the low and average expressers (oIRI 20, 23 and 26) at 13 DAT. A direct correlation between the expression levels of iridoptin and the aphicidal activity was not observed.

Normally, a GPA takes about 6–7 days for a neonate aphid to become an adult. Once it becomes an adult, it starts parthenogenetic reproduction. Hence, 7–9 DAT (beginning of the aphid reproductive period) was used as a cut-off point to assess the nymphal development to adulthood. Because aphids were introduced into the treatment very early in their life cycle, effects of iridoptin on aphid's growth and development were also observed. At 9 DAT, there was a notable delay in the development of surviving, iridoptin-fed aphids, whereas 100% GUS control-fed aphids developed to adulthood (Fig. 4b).

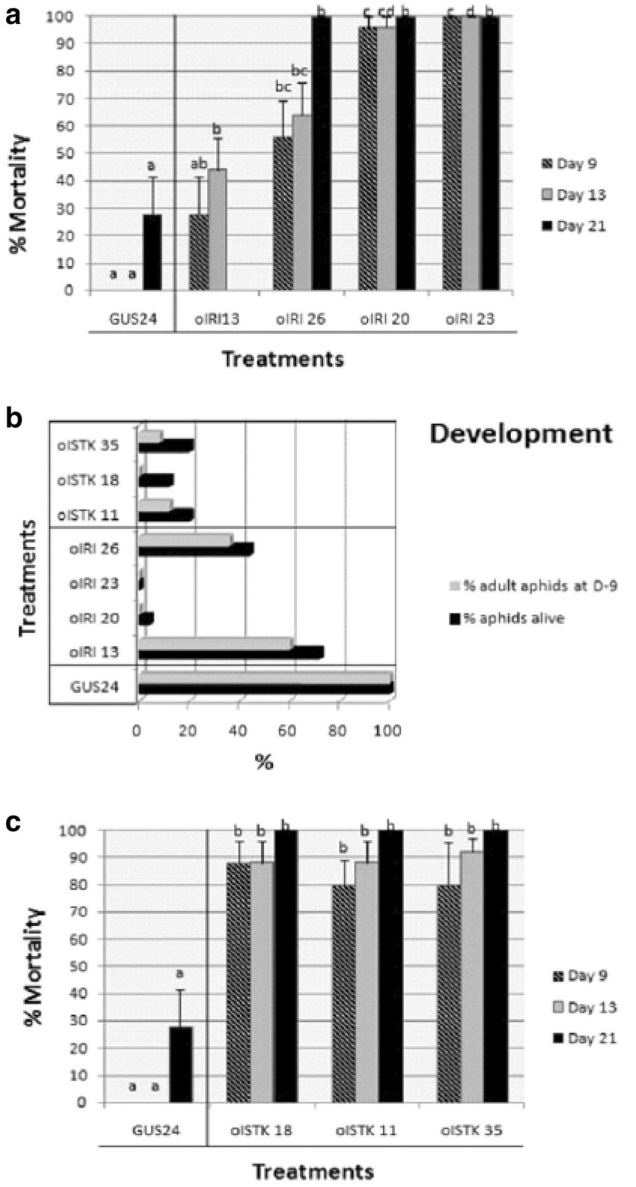


Fig. 4 Analyses of transgenic plants for their aphicidal activities. **a** and **c** Mean percentage mortality of green peach aphids in excised-leaf bioassay on iridoptin/ISTK-expressing transgenic T_1 lines at 9, 13 and 21 DAT. Note: no data available for oIRI 13 line at 21 DAT. Different letters represent significant difference between treatments in each day ($p < 0.05$); Points and bars represent means ± SE. **b** Comparison chart that summarizes the pattern of developmental delay that was observed in the growth of aphids, from nymphal to adult stage, that fed on different *iridoptin/ISTK*-expressing leaves, when compared to GUS control. Red, GUS control; Grey, oIRI lines; Blue, oISTK lines

Total fecundity of 86.6 ± 9.35 nymphs per replicate was observed in GUS control at 21 DAT. However, the fecundity was significantly reduced in all oIRI lines evaluated in this run (oIRI 20: 0.4 ± 0.4; oIRI 23: 0; oIRI 26: 12.6 ± 5.04 nymphs per replicate). The low/negligible fecundity on these iridoptin-fed aphids can be attributed to both increased mortality and developmental inhibition of mother aphids. Among the three oIRI lines, oIRI 26 resulted in reproduction rate of 0.51 ± 0.11 nymphs per aphid per day compared to no reproduction on oIRI 20 and oIRI 23; the GUS control resulted in 2.26 ± 0.54 nymphs per aphid per day.

Because oIRI 23 line showed high mortality against GPA, it was then considered as a positive control to identify more iridoptin-expressing lines with aphicidal activity. In the second run of the experiment, evaluated at 8 DAT, an average expresser oIRI 14 showed no mortality (Supplementary Fig. S4a) or reproductive defects (data not shown) on GPA and was similar to the GUS control.

In the third run, line oIRI 27, another average expresser, exhibited moderate effects on aphid mortality (32 ± 10.19%, 8 DAT) compared to 0% mortality in GUS negative control and 100% mortality in the oIRI 23 line (positive control) (Supplementary Fig. S4b). However, significant difference in fecundity at 8 DAT between GUS and oIRI 27 was observed (GUS: 42 ± 5.26; oIRI 27: 7.6 ± 2.1 nymphs per replicate). A simultaneous analysis of development of aphids fed on oIRI 27 leaves showed a substantial delay in the development of aphids to adult stage. At 7 DAT, when 100% of aphids feeding on GUS control leaves developed into adults, < 50% of the surviving aphids had developed to adult stage in oIRI 27 leaves (Supplementary Fig. S4c); not all surviving aphids developed to adulthood even at 9 DAT (Supplementary Fig. S4d). Interestingly, high expressers such as oIRI 6 and 8 had no significant mortality or fecundity effects against GPA, further confirming a lack of direct correlation to the expression levels of iridoptin in the plants (data not shown).

The transgenic line oIRI 23 appeared to consistently perform superior across all lines evaluated and showed 100% mortality in less than 4 DAT in all three consecutive trials conducted within a period of 30 days. These lines also showed 100% mortality when GPA adults were directly exposed to the leaves in an excised-leaf bioassay (data not shown).

This study clearly demonstrated the inhibitory effect of iridoptin-expressing leaves on GPA survival, development, and fecundity. However, a direct relationship between expression levels and the inhibitory effects was not observed, for reasons that are currently not well understood (Table 1).

Aphid resistance in ISTK-expressing transgenic T_1 lines

Excised leaf bioassays with *oISTK*-expressing lines were conducted simultaneously with *iridoptin*-expressing lines as described above. *Experiment 1*: leaves from three oISTK lines (11, 18 and 35) showed significant reduction in the initial inoculum population when compared to the GUS control. At 9 DAT, the aphid mortality had increased to as high as 80–90% in these treatments, with 0% mortality in the GUS control (Fig. 4c). At 21 DAT, all oISTK treatments showed 100% mortality compared to 28% mortality in the GUS control (Fig. 4c). This demonstrates the complete inhibition of aphid survival in these *oISTK*-expressing leaves. Aphid fecundity was also significantly affected by these oISTK lines. Reproduction was noted at 9 DAT and steadily increased to a total of 86.6 ± 9.3 nymphs per replicate in GUS control at 21 DAT, whereas ISTK treatments resulted in a total of 4.8 ± 3.24 (oISTK 11), 0.8 ± 0.58 (oISTK 18) and 1.4 ± 0.97 (oISTK 35) nymphs per replicate. These data clearly demonstrated a significant inhibition of population growth when exposed to ISTK-expressing leaves. The reduction in fecundity could be attributed to the mortality effects on initial inoculum and slower development of surviving initial inoculum to the adult stage when fed on *ISTK*-expressing leaves (Fig. 4b).

Experiment 2 In the presence of a positive control (oIRI 23 line) and a negative control (GUS 9), additional oISTK lines were examined for aphicidal effects. The transgenic line oISTK 27 had no significant effect on GPA survival and was similar to the GUS control (Supplementary Fig. S4a). *Experiment 3:* oISTK lines 25 and 31 showed minimal/no mortality (Supplementary Fig. S4b) but significant decrease in fecundity of adult aphids (data not shown). There was a corresponding delay in the development of initial inoculum to the adult stage, when exposed to these lines (Supplementary Figs.S4c and S4d). The reproductive potential of aphids was 2.81, 0.32, and 1.37 nymphs/aphid/day for GUS, oISTK 31, and oISTK 25, respectively.

As observed in *iridoptin*-expressing T_1 lines, negative effects on aphid survival, development and fecundity were also observed when fed on *ISTK*-expressing leaves. However, they were not directly proportional to the expression levels of the respective toxins in plants (Table 1).

Comparison of effects of ISTK and iridoptin-expressing leaves on aphid survival and fecundity at 8 or 9 DAT

Three different effects on GPAs were observed when exposed to these different iridoptin/ISTK expressing transgenic lines. Lines oIRI 20, 23, 26 and oISTK 11, 18, 35 significantly increased mortalities and decreased fecundity of GPAs when compared to the GUS control (Fig. 5a, b). This could lead to a significant reduction in the population growth. Lines oIRI 13, oIRI 27, and oISTK 31 had no significant effects on aphid survival (Fig. 5a) but showed significant reduction in fecundity (Fig. 5b). However, lines oIRI 14, oISTK 25, and oISTK 27 had similar effects as GUS control on both aphid survival and fecundity (Fig. 5a, b).

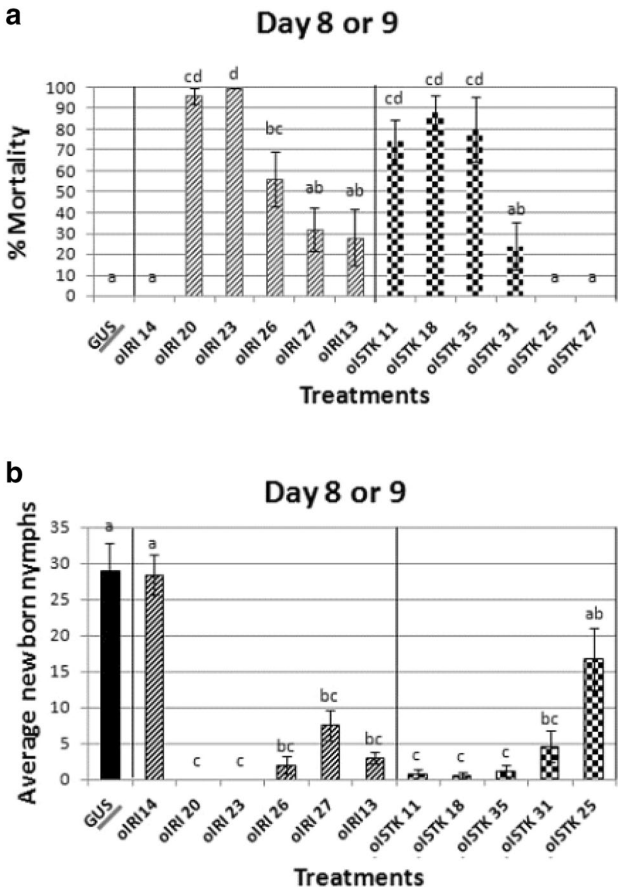


Fig. 5 Comparison chart combining multiple runs evaluating the effects of different iridoptin/ISTK-expressing T_1 lines on mortality (**a**) and fecundity (**b**) per replicate. GUS line was used as the negative control on 8 or 9 DAT. Data of the line oIRI23 resulted from the three independent runs and the data of oISTK 11 were from two independent runs. Data for rest of the lines were from a single run of the experiment

Discussion

Development of transgenic plants expressing foreign insecticidal genes would act as a valuable tool for generating plants toxic to the insect pests. Expression of the native, un-optimized *iridoptin* gene fragment in stable transgenic *Arabidopsis* lines showed expression at the RNA level, but not at the protein level, suggesting poor or no translation of this foreign mRNA in the plant system. A gene with the sequence adapted for an insect-virus may not have the appropriate coding sequence for efficient expression in plants. Efforts were taken to optimize *ISTK* and *iridoptin* DNA sequences to better suit expression in plants. Various factors were considered simultaneously during the gene optimization process and modified to achieve the detectable expression. However, it cannot be assured that the algorithm followed, in this gene optimization process, has achieved an optimal combination of sequence variables to result in the best possible-expression level.

These optimized constructs under the control of dual 35S promoter were introduced into *Arabidopsis* via the *Agrobacterium*-mediated floral dip method. Putative T_1 transgenic lines were obtained by screening the T_1 seeds on kanamycin selection media. PCR analyses were carried out to confirm the presence of the right sized gene inserts. Further screening of these PCR positive lines was carried out using Western blot analysis and transgenic lines (T_1) expressing the expected 50 kDa ISTK/37 kDa iridoptin at the polypeptide level were identified. Both optimized constructs (*oISTK/oIRI*) showed detectable levels of accumulation of respective polypeptides suggesting that the codon optimization of these genes were successful. Out of a large population of stable, transgenic T_1 plants screened for the expression of ISTK/iridoptin, ~ 45% of plants showed expression at the polypeptide level. The expression levels varied from barely detectable to strong bands in the expected molecular weight region using anti-flag antibody. These T_1 transgenic plants were comparable to wild-type morphology and growth, when grown on potted soil in growth chambers. Two different strong constitutive promoters, i.e., CaMV 35S and dual 35S promoters, were used to test expression of optimized *ISTK* and *iridoptin* constructs. However, there was no significant difference in the protein expression levels among them (data not shown).

The purpose of this study was to determine the possible utility of this novel, iridovirus-insecticidal kinase in insect control using transgenic technology. Here, as a first step, it has been demonstrated that it is possible to ectopically produce CIV's ISTK and a truncated form, iridoptin, in plants. Excised-leaf bioassays using T_1 transgenic plants expressing ISTK/iridoptin were used to determine the plant expressed toxin's effect on survival and reproductive potential of green peach aphids. Several transgenic T_1 lines expressing ISTK/iridoptin were shown to induce high mortality (60–100%) in as little as 4–8 days following exposure, accompanied by reduced fecundity of the surviving aphids. Developmental inhibition of surviving aphids was also observed in many of those lines. Increased pre-reproductive time clearly indicated retardation of aphid development. However, irrespective of the toxin expression levels, several lines showed very low or no inhibitory effects on GPAs. Lack of correlation between expression levels and respective insecticidal activities complicated comparisons between the different transgenic lines. However, the optimization strategy used in this study appears to have resulted in desirable levels of expression in stable transgenic *Arabidopsis* plants to show significant aphid mortality and suppression. In-planta expression of ISTK/iridoptin providing strong inhibitory effects on the survival and fecundity of GPAs was established in stable transgenic *Arabidopsis* plants. Based on these observations, it is likely not required to obtain higher accumulation of these toxins in plants. An accurate estimation of the concentration range required for an adequate control of GPAs in these transgenic plants is warranted.

Bioassay experiments showed that surviving aphids that fed on the treatment leaves (expressing ISTK/iridoptin) frequently avoided feeding and were found to roam around in the petri plates. This was not observed in the GUS-expressing control leaves. More detailed studies would help to elucidate the mode of action of ISTK and iridoptin at the organismal level and the apparent avoidance behavior. The most studied *Bt* (Cry) toxins are shown to result in feeding inhibition due to paralysis of insect gut and mouth parts (Aronson et al. 1986; Deist et al. 2014). A few other observations were made during the bioassays: Some aphids continuously feeding on ISTK/iridoptin-expressing leaves had extremely small body sizes which ultimately succumbed to death compared to the normal body size in GUS control-feeding aphids. Also, some deformed and paralyzed aphids were found in the treatments. The encounters of such aphids were not significantly high but were only observed in aphids feeding on ISTK/iridoptin-expressing leaves.

The frequency at which the aphid-resistant lines were recovered was low (~3 out of 20–23 transgenic plants (T_1) screened, i.e., 13–15%). The analysis performed in this study did not show a direct correlation between the expression levels of the toxin and the insecticidal activity. In fact, some of the high expression lines showed low or no inhibitory effects on aphid survival and growth. Previous studies in our laboratory suggested that kinase activity of iridoptin is absolutely necessary for its insecticidal effects (Chitnis et al. 2011). The effects of plant-expressed ISTK and iridoptin on aphids, or any target-insect, might be dependent on the availability of these active forms of the toxins in transgenic plants, rather than dependence on just the levels of accumulation. Dose-independent effects of toxins in feeding-insect antibiosis have been reported with *Bt*-toxin expressing canola plants against a lepidopteran pest (Stewart et al. 1996). Another study over-expressing *Mi-1.2* gene in tomato conferred developmentally regulated resistance in potato aphids. Here, irrespective of the use of constitutive promoter, aphid resistance was developmentally regulated with resistance observed in the mature, flowering stage plants but lacked resistance in the seedling stage. Constitutive expression showed 3–eightfold increase in *Mi-1.2* mRNA accumulation levels when compared to that of native promoter of the gene. However, there was no significant difference in aphid numbers between these two types of plants (with native promoter versus constitutive expression) at the flowering stage (Goggin et al. 2004). A study on Bollgard cotton showed no correlation between bollworm survival and Cry1A(c) expression levels in different plant parts (Gore et al. 2001). Inconsistencies have been observed in the expression patterns of reporter genes under the control of constitutive CaMV35S-like promoters in different cell types (Yang and Christou 1990). Plethora of information is available on such dose-independent effects of insecticidal toxins against insect pests in transgenic plants (Chen et al. 2005; Jiang et al. 2006; Sadeghi et al. 2007).

Numerous approaches have been undertaken by researchers to improve different plant traits using plant transformation with foreign genes. However, the biggest concern is the unsolicited effects of integration of foreign gene in the host plant genome and subsequent expression effects (Tagashira et al. 2005; Li et al. 2006). In this study, observations showed reproductive defects such as lack of seeds in some aphid-resistant T_1 lines and expression silencing in the second generation plants of few aphid-resistant lines (data not shown). More careful and detailed studies on the ability of inheritance of stable expression and insecticidal activity of ISTK and iridoptin in these transgenic plants are warranted. Use of phloem-specific promoters or wound-inducible promoters, or a chimeric version with both, might be a more optimal and useful approach as this study aims at sap-sucking pests like aphids and hence would avoid any unwanted effects of constitutive, over-expression in plants (Smigocki et al. 1993; Duan et al. 1996; Rao et al. 1998; Godard et al. 2007; Sadeghi et al. 2007; Tiwari et al. 2011; Dutt et al. 2012; Will and Vicinskas 2013; Chandrasekhar et al. 2014). This approach would also reduce the metabolic cost of over-expression of foreign genes. Hence, further evaluation of ISTK/iridoptin with these promoters is warranted to expand the knowledge base on *ISTK, iridoptin*, and similar toxin genes for insect control.

This is the first report of utilizing a novel-iridoviral kinase towards development of aphid-resistant transgenic plants. The current study has generated proof-of-concept data that will facilitate the use of the optimized *ISTK/iridoptin* to successfully generate stable transgenic lines of agronomic importance. The analysis of exploiting the applicability of this viral kinase in aphid suppression in plants, although preliminary, will create a new niche for identification and exploration of similar toxins, as aphid control agents are of potential integrated pest management interest. This could be a torch bearer for rapid progress in utilization and screening of similar viral insecticidal genes, as PIPs, that can be isolated from the reservoir of invertebrate iridoviruses. Such insect-iridoviruses appear to be an untapped resource for identification of more potential insecticidal toxins for an extremely wide range of insect pests (Williams et al. 2005; Ince et al. 2018). Hence, this study would serve as a pioneer in the use of CIV for an effective and sustainable insect pest control in agriculture.

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Author contributions SG, SLB and HZ conceptualized the study and planned the transgenic plant related work. SG and MNP planned and designed the insect and bio-assay studies. SG, HZ, MNP and SLB secured the funding for this project. SG performed the experiments. SG, MSF, MNP and HZ interpreted the results and prepared the manuscript.

Compliance with ethical standards

Conflict of interest The authors of this manuscript declare no conflicts of interest. The funding sponsors had no role in the design and performance of the experiments, or in the decision to publish the results.

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