Research Article

Received: 14 June 2018 Revised: 1 October 2018 Accepted article published: 16 October 2018 Published online in Wiley Online Library: 18 December 2018

(wileyonlinelibrary.com) DOI 10.1002/ps.5234

First transgenic trait for control of plant bugs and thrips in cotton

Waseem Akbar,[a*] Anilkumar Gowda,[a] Jeffrey E Ahrens,[a] Jason W Stelzer,[a] Robert S Brown,[a] Scott L Bollman,[a] John T Greenplate,[a] Jeffrey Gore,[b] Angus L Catchot,[b] Gus Lorenz,[c] Scott D Stewart,[d] David L Kerns,[e†] Jeremy K Greene,[f] Michael D Toews,[g] David A Herbert,[h] Dominic D Reisig,[i] Gregory A Sword,[j] Peter C Ellsworth,[k] Larry D Godfrey[l] and Thomas L Clark[a]

Abstract

BACKGROUND: Plant bugs (*Lygus* spp.) and thrips (*Thrips* spp.) are two of the most economically important insect pest groups impacting cotton production in the USA today, but are not controlled by current transgenic cotton varieties. Thus, seed or foliar-applied chemical insecticides are typically required to protect cotton from these pest groups. Currently, these pests are resistant to several insecticides, resulting in fewer options for economically viable management. Previous publications documented the efficacy of transgenic cotton event MON 88702 against plant bugs and thrips in limited laboratory and field studies. Here, we report results from multi-location and multi-year field studies demonstrating efficacy provided by MON 88702 against various levels of these pests.

RESULTS: MON 88702 provided a significant reduction in numbers of *Lygus* nymphs and subsequent yield advantage. MON 88702 also had fewer thrips and minimal injury. The level of control demonstrated by this transgenic trait was significantly better compared with its non-transgenic near-isoline, DP393, receiving insecticides at current commercial rates.

CONCLUSION: The level of efficacy demonstrated here suggests that MON 88702, when incorporated into existing IPM programs, could become a valuable additional tool for management of *Lygus* and thrips in cotton agroecosystems experiencing challenges of resistance to existing chemical control strategies.
© 2018 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Keywords: *Lygus* spp.; *Frankliniella* spp.; Hemiptera; Thysanoptera; transgenic cotton; MON 88702; Cry51Aa2.834_16

1 INTRODUCTION

Bollgard® cotton (*Gossypium hirsutum* L.), which expresses the insecticidal Cry1Ac protein from *Bacillus thuringiensis* (*Bt*) (Berliner) targeting certain lepidopteran pests, was first introduced in the USA in 1996. Since then, additional transgenic traits targeting specific coleopteran and lepidopteran pests have been introduced in other major crops, including maize (*Zea mays L.*) and soybean (*Glycine max L*). Currently there are an estimated 78.8M ha of transgenic crops cultivated worldwide.[1] Numerous benefits of transgenic crops have been documented, including the reduction in use of chemical insecticides[2,3] and increased biological control activity of natural enemies of crop pests.[4–6]

Cultivation of *Bt*-cotton coupled with successful eradication of boll weevil (*Anthonomus grandis* Boheman) in the USA have contributed to appreciably fewer insecticide applications, but plant bugs (Hemiptera: Miridae) and thrips (Thysanoptera: Thripidae) have emerged as pests of significant importance.[7–11] The primary plant bug species present in cotton fields include tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois), western tarnished plant bug (*L. hesperus* Knight), cotton fleahopper (*Pseudatomoscelis seriatus* Reuter), clouded plant bug (*Neurocolpus nubilus* Say), and multiple species of stink bugs (Hemiptera: Pentatomidae). Among these, *L. lineolaris* comprises up to 94% of the plant bugs sampled

* Correspondence to: W Akbar, Bayer Crop Science, 700 Chesterfield Pkwy West, Chesterfield, MO, 63017, USA. E-mail: waseem.akbar@bayer.com

† Present address: Texas A& M University, College Station, TX

a Bayer Crop Science, Chesterfield, MO, USA

b The Department of Biochemistry, Molecular Biology, Entomology, & Plant Pathology, Mississippi State University, Stoneville, MS, USA

c Department of Entomology, University of Arkansas, Fayatville, NC, USA

d Department of Entomology and Plant Pathology, University of Tennessee, Jackson, TN, USA

e Department of Entomology, Louisiana State University, Winnsboro, LA, USA

f Department of Plant and Environmental Sciences, Clemson University, Clemson, SA, USA

g Department of Entomology, University of Georgia, Tifton, GA, USA

h Department of Entomology, Virginia Tech University, Suffolk, VA, USA

i Department of Entomology and Plant Pathology, North Carolina State University, Plymouth, NC, USA

j Department of Entomology, Texas A&M University, College Station, TX, USA

k Department of Entomology, University of Arizona, Maricopa, AZ, USA

l Department of Entomology and Nematology, University of California Davis, Davis, CA, USA

© 2018 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

in the midsouthern region[12] whereas the western USA is dominated by *L. hesperus*.[13] In recent years, the *Lygus* complex has been the top-ranked pest complex of cotton in the USA, requiring three spray applications per acre, which was the highest among all insect pests.[11] In 2015, *Lygus* caused the greatest crop losses among all cotton insect pests (238 507 lost bales), with 42% of cotton acres across the US Cotton Belt infested with *Lygus*. In the midsouthern region, which includes parts of Tennessee, Mississippi, Louisiana, Arkansas, and Missouri, 100% of the cotton acres were infested with *Lygus*, leading to an average cost of $42.28 per acre for five insecticidal sprays. The most severe losses were in the Delta region, where *Lygus* cost an average of $64.68 per acre to treat with six insecticide applications.[11] In some areas, growers have spent up to $100 per acre in foliar applications to achieve adequate *Lygus* control.[14] Most recently, increasing *Lygus* pressure has also been observed in the southeastern region. In North Carolina, *Lygus*-treated acres increased from 5% in 2009 to nearly 50% in 2015.

After the *Lygus* complex, thrips are the second most important pest of cotton in terms of economic losses.[11] Multiple species of thrips infest seedling cotton in the USA, including tobacco thrips (*Frankliniella fusca* Hinds), flower thrips (*F. tritici* Fitch), western flower thrips (*F. occidentalis* Pergande), onion thrips (*Thrips tabaci* Lindeman), and soybean thrips (*Neohydatothrips variabilis* Beach).[15] In extreme cases, thrips infestations can result in 30–50% of lint losses.[10] Insecticide applications at planting, including seed treatments or in-furrow granular or liquid sprays, have been widely adopted options for thrips control. Under heavier pressure, seed treatments can fail and foliar sprays are required to protect cotton.[16,17] In 2015, 81% of acres were infested with thrips across the Cotton Belt in the USA suffering an estimated loss of 147 602 bales. In the midsouthern USA, 88% of cotton acres were infested with thrips at an average treatment cost of $3.72 per acre. The Delta region was affected the most, with 100% of cotton acres infested and costing on average $6.48 per acre to growers for treatment.[11]

The emerging resistance issues to key chemistries in *Lygus* and thrips are rendering insecticides as a less sustainable management option for these pests. For example, *F. fusca* is developing resistance to the neonicotinoid insecticides,[18] the most commonly used materials for control of thrips in the mid-south and southeast, and susceptibility of *L. lineolaris* to pyrethroids[19] and acephate[20] has reduced, especially in the mid-south. Thus, there is an increasing need for new technologies to be incorporated into integrated pest management (IPM) systems for these important pests of cotton. Historically, efficacy of *Bt* proteins against hemipterans such as *Lygus* has been elusive. One hypothesis supporting limited *Bt* protein activity was thought to be due to extra-oral digestion enabled by proteolytic enzymes released during hemipteran feeding.[21] This hypothesis was later debunked by Brandt *et al.*[22] when they demonstrated that *Bt* crystal proteins, Cry1Ac and Cry2Ab, could be proteolytically processed in the *L. hesperus* digestive system while remaining intact; however, efficacy was not observed in this study.[22] Later, a 35 KDs *Bt* crystal protein belonging to the β pore-forming family of bacterial toxins and designated Cry51Aa2 (Accession GU570697)[23] was reported to negatively affect the survival and development of *L. hesperus* in diet bioassays; however, the first transgenic cotton plants expressing this protein did not provide significant protection from *Lygus* feeding.[24] Optimization of this protein informed in part by protein crystallography and modelling to identify limited amino-acid substitutions led to multiple variants with increased insecticidal activity against both *L. hesperus* and *L. lineolaris*.[25] Previously, one transgenic event expressing Cry51Aa2.834_16, designated MON 88702, was documented to show protection against *Lygus* and thrips in limited laboratory and field studies.[25,26] Here, we demonstrate results from additional field studies in developing this first-ever transgenic trait targeting *Lygus* spp. and thrips (mainly *Frankliniella* spp.) and discuss its potential fit and projected benefits as a new component of IPM in cotton agroecosystems.

2 MATERIALS AND METHODS

2.1 Efficacy trials with *Lygus* spp.

2.1.1 *Field trials without Lygus-active insecticide sprays*

These trials were conducted over three years from 2012 to 2014 in the midsouthern (Stoneville, MS, Marianna, AR, Lonoke, AR, Winnsboro, LA) and southeastern (Rocky Mount, NC) regions for *L. lineolaris*, and in the southwestern (Maricopa, AZ, Five Points, CA) regions of the USA for *L. hesperus*. MON 88702 event in DP393 varietal background (henceforth MON 88702) and its non-transgenic near-isoline variety DP393, with similar agronomic properties (henceforth DP393), seeds were treated with Acceleron® (containing fungicides, the insecticide imidacloprid, and the nematacide thiodicarb) and planted in eight 9.14 m long rows with 96.5 cm row spacing. At each location, entries were replicated four times in a randomized complete block design. Standard local agronomic practices excluding insecticidal sprays to control hemipteran insects were adopted. Lepidopteran pests, when present, were managed following established recommendations in the area with insecticides in the diamide class that are not known to have activity against *Lygus*. Insect sampling was initiated when the majority of plants had at least three squaring nodes, with subsequent samplings at 7- to 10-day intervals for a total of six to eight samplings throughout the season.

2.1.2 *Field trials with Lygus-active insecticide sprays*

These trials were conducted in 2014 at Stoneville, MS, Marianna, AR, Jackson, TN, Winnsboro, LA, and Belvidere, NC, for *L. lineolaris* and in Maricopa, AZ, for *L. hesperus*. Seeds were treated with Acceleron® and planted in 9.14 m long rows with 96.5 cm row spacing. Trials were planted in a split plot design with un replicated main plot (treatment blocks named No Spray, Spray by Entry, and Spray by Negative). Sub-plots were eight rows of MON 88702 or DP393 with three replications in each treatment block. In the No Spray block, no insecticide to control *Lygus* was applied during the season. In the Spray by Entry block, insecticides were applied to an individual entry when the average of three reps of that particular entry reached conventional economic thresholds [ET]) of 3 *Lygus*/1.5 m for *L. lineolaris*[27] or 15 *Lygus*/100 sweeps for *L. hesperus*.[28] In the Spray by Negative block insecticides were applied to the entire block when the average of three reps of DP393 in that block reached conventional ET for *Lygus*. The numbers of insecticide sprays required and the class of insecticide applied varied across locations because of varying *Lygus* pressure. The insecticides applied belonged to different classes, including neonicotinoids, sulfaximines, flonicamid, pyrethroids, or tank mixes of sulfaximines and organophosphate, sulfaximines and carbamate, pyrethroids and neonicotinoids, organophosphate, and pyrethroids. In locations where multiple spray applications were made, insecticides of these classes were rotated as *Lygus* have developed resistance to some of these chemistries.[20,29–31] All insecticides were applied at rates recommended by the respective state extension department.

 © 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

2.1.3 *Sampling for* L. lineolaris

A drop-cloth method[27] was used to sample *L. lineolaris* that employed placing a drop cloth (76 × 91 cm) between two rows of cotton and vigorously shaking plants from both rows over the drop cloth (1.5 row-m per sample). *Lygus* were recorded as numbers of adults, large nymphs (fourth to fifth instar), and small nymphs (first to third instar). Two samplings were conducted at two different locations within the plot at each sampling time. The sampling was swapped between rows 2 and 3 in the first week and rows 6 and 7 the following week. No insect data were collected from two central rows (rows 4 and 5) as those were preserved for yield estimation. The insect counts from both samples were averaged for that plot as numbers per 1.5 row-m.

2.1.4 *Sampling for* L. hesperus

A sweep-net method[28,32] was used to sample *L. hesperus* that employed sweeping a net (38-cm diameter) through the top of the canopy for 20 sweeps and then counting the number of insects. From each plot, *L. hesperus* were recorded as adults, large nymphs (fourth to fifth instar), and small nymphs (first to third instar). Two runs of 10 sweeps each with a total of 20 sweeps were taken per plot; one subsample from either row 2 and 3 and the other from row 6 and 7, switching to the alternate row each week. The field-collected samples were placed in pre-labeled paper bags and brought back to the laboratory where insects were identified and enumerated.

2.1.5 *Seedcotton yield*

The two center rows (i.e. rows 4 and 5) were machine-harvested at the end of the growing season to estimate yield. Resulting seedcotton weights were first extrapolated to estimate pounds per acre and then converted to kg ha^{-1}.

2.2 Efficacy trials with thrips

Thrips trials were conducted in 2015 at 13 field sites located in Virginia (2), South Carolina (1), Georgia (1), Tennessee (2), Mississippi (4), Louisiana (2), and Arkansas (1). Two trials each (one early and one late planting date) were planted at locations in Suffolk VA, Jackson, TN, and Stoneville, MS. MON 88702 and DP393 with and without insecticide seed treatment and/or foliar insecticide spray application were tested in a randomized complete block design with four replications at each location. Seeds were treated at commercial rates with either Acceleron (containing fungicides, the insecticide imidacloprid, and the nematacide thiodicarb) or Acceleron without insecticide seed treatment (containing fungicides and the nematacide thiodicarb only). Planting dates varied among locations in early to mid-May, and agronomic practices were followed per standard recommendations for growing cotton in that area. For plots receiving foliar insecticide spray, a foliar application of Orthene (acephate @ 280 g ha^{-1} except at South Carolina location, where Radiant (spinetoram) @ 140 g ha^{-1}) was applied at the 1–2 true leaf stage after the first evaluation. Because thrips have a high reproductive rate and can quickly increase numbers on a suitable host, evaluation of injury and sampling for immatures were performed twice (1–2 and 3–4 true leaf stages) during this critical period for proper trait evaluation. Thrips injury was assessed using an injury rating scale of 0–5 where 0 is no damage, 1 is showing detectable visible injury to plant, 2 is showing minor injury (slight crinkling and/or silvery appearance) to the terminal bud and leaves, 3 is showing moderate injury (considerable crinkling and/or silvery appearance) to the terminal bud and leaves, 4 is showing severe injury (extensive crinkling and/or silvery appearance) to the terminal bud and leaves with some dead plants and aborted terminal buds, and 5 is showing severe stunting, reduced leaf area, terminal bud abortion of most plants, and plant death. Injury ratings were a composite of the overall appearance of plots based on individual plant appearance.

2.2.1 *Quantification and thrips species composition determination*

Thrips were also collected at the 1–2 and 3–4 true leaf stages from each location. Five or ten randomly selected plants from each plot were cut or pulled from the ground and placed into a glass or plastic jar (0.95 L) containing 50–70% ethyl or isopropyl alcohol. Jars were brought back to the laboratory and thrips were rinsed from the plants and/or jars onto filter paper. Numbers of immature and adult thrips were counted for each plot using dissecting microscopes. Adult thrips collected at each date of rating were identified to species level,[33] and percentage population of each species for each location determined.

2.3 Statistical analyses

Lygus populations were categorized as relative to the Cooperative Extension suggested ET for applying insecticide in each respective region. In the midsouthern and southeastern regions, the ET is 3 *Lygus* (any stage)/1.5 row-m using the drop-cloth method. In the Western region, the ET for *L. hesperus* is 15 total *Lygus* (including 4–8 nymphs)/100 sweeps using the sweep-net method. At each location populations were categorized as low, medium, or high pressure. Low pressure was characterized as *Lygus* counts being below the ET throughout the season. Medium pressure was characterized as *Lygus* counts being equal to or greater than one times the ET but less than three times the ET at any given time during the season. High pressure was characterized as counts being equal to or greater than three times the ET at any time during the growing season. Only the total numbers of nymphs (sum of small and large nymphs) present at the time of sampling and seedcotton yield were considered for efficacy evaluation. Total nymph numbers at each sampling time and yield were pooled across similar pressure categories and analyzed. Yield data were converted to kg ha^{-1} before analyses. The effect of transgenic trait (presence or absence) and insecticide spray (presence or absence and interaction of trait and spray in *Lygus*-active insecticide trials) on total nymph numbers and yield were evaluated using a generalized linear mixed model (PROC GLIMMIX[34]). Where the interaction between trait and spray was significant, the effect of the trait was analyzed separately within each spray regime in insecticide spray trials. Means were estimated using the LSMEANS statement and adjusted according to Tukey's HSD test ($\alpha = 0.05$).[34]

Due to variation of natural thrips pressure at different field sites, locations were categorized as low-, medium-, or high-pressure sites. Low, medium, and high natural thrips pressure was defined based on the greatest injury rating score recorded in any replication of the non-transgenic DP393 variety at that location. Low thrips pressure corresponded to an injury rating < 2, medium pressure corresponded to injury rating of ≥ 2 but < 4, and high pressure corresponded to an injury rating of ≥ 4. Thrips injury ratings and immature thrips counts were used for efficacy evaluation. Each of these parameters were analyzed separately for each time point across high- and medium-pressure locations. There were no low-pressure locations. The effects of transgenic trait (MON 88702 presence or absence), seed treatment (presence or absence), foliar spray (presence or absence), and all possible interactions were

© 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

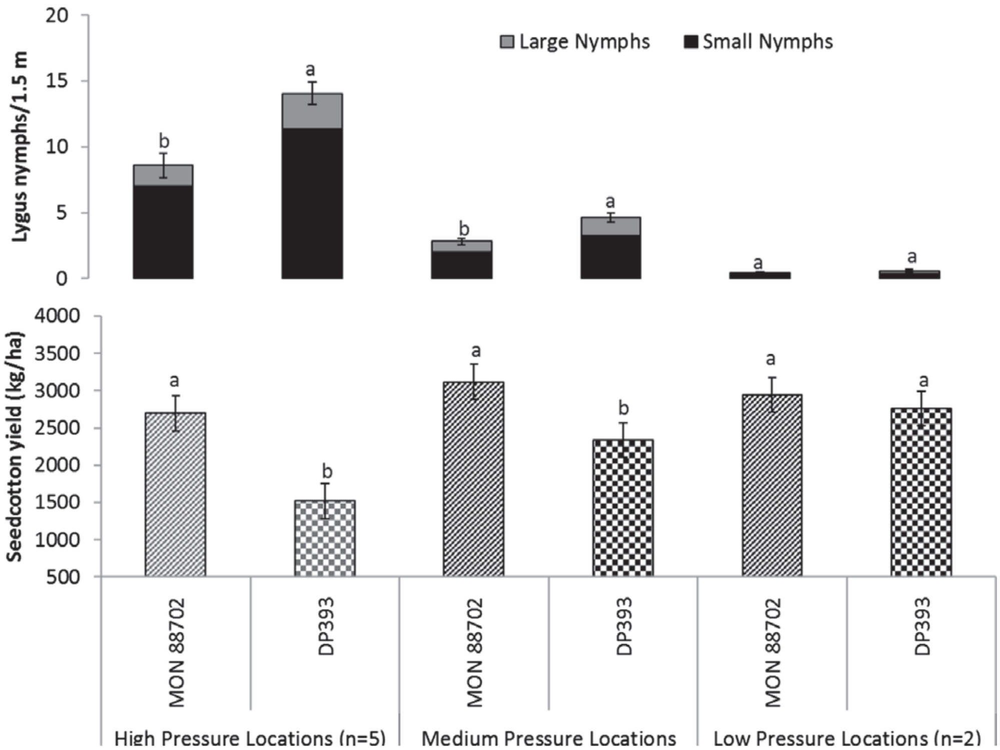

Figure 1. *Lygus lineolaris* nymph counts and seedcotton yield in trials with no *Lygus*-active insecticides at Stoneville, MS, Marianna, AR, Lonoke, AR, Winnsboro, LA, and Rocky Mount, NC, during 2012–2014. Means followed by different letters *within each pressure category* are significantly different from each other at $P \leq 0.05$ (LSMEANS test). Data shown as mean ± SEM. Standard errors for nymphs are for total numbers of nymphs.

evaluated for statistical significance using PROC GLIMMIX. Means were estimated using the LSMEANS statement and adjusted according to Tukey's HSD test ($\alpha = 0.05$).[34] *Lygus* spp. and thrips adults were excluded from analyses as they are mobile and their presence in a plot at time of sampling did not confirm their development on that plant and could confound assessment of efficacy.

3 RESULTS

3.1 *Lygus* efficacy trials without *Lygus*-active insecticide sprays

3.1.1 Efficacy against L. lineolaris *at low-pressure locations*

Two locations (Winnsboro, LA in 2012 and Lonoke, AR in 2014) met the criteria of low *Lygus* pressure. Across these locations, differences for nymph counts or seedcotton yield were not statistically significant between MON 88702 and DP393 (Fig. 1).

3.1.2 Efficacy against L. lineolaris *at medium-pressure locations*

Five locations (Lonoke, AR in 2012, Marianna, AR and Winnsboro, LA in 2013, and Marianna, AR and Winnsboro, LA in 2014) met the criteria of medium *Lygus* pressure. Across these locations, a 1.6-fold (39%) reduction in total nymph counts was recorded on MON 88702 compared with DP393 ($F = 12.37$; d.f. = 1, 4; $P = 0.025$). The trait also had a significant effect on seedcotton yield ($F = 28.82$; d.f. = 1, 4; $P = 0.006$) with a 1.3-fold (780 kg ha^{-1}; 33%) increase in yield from MON 88702 compared with DP393 (Fig. 1).

3.1.3 Efficacy against L. lineolaris *at high-pressure locations*

Five locations (Stoneville, MS, Marianna, AR in 2012, Stoneville, MS in 2013, Stoneville, MS, and Rocky Mount, NC in 2014) met the criteria of high *Lygus* pressure. The greatest differences between MON 88702 and DP393 were recorded at these high-pressure locations. The trait had a significant effect on total nymph counts ($F = 15.88$; d.f. = 1, 4; $P = 0.016$), where 1.6-fold (39%) fewer *Lygus* nymphs were recorded on MON 88702. Yield was also significantly affected by the trait in these locations ($F = 85.51$; d.f. = 1, 4; $P = 0.016$), with MON 88702 yielding 1.8-fold (1176 kg ha^{-1}; 77%) higher than DP393 (Fig. 1).

3.1.4 Efficacy against L. hesperus *at low-pressure locations*

One location (Maricopa, AZ in 2013) met the criteria of low *Lygus* pressure. Under these conditions, differences for nymph counts or seedcotton yield were not significant between MON 88702 and DP393 (Fig. 2).

3.1.5 Efficacy against L. hesperus *at medium-pressure locations*

Three locations (Maricopa, AZ in 2012, and Five Points, CA in 2012 and 2013) met the criteria of medium *Lygus* pressure. Total nymph counts were not significantly affected by the trait across these locations, but yield was significantly affected by trait ($F = 28.02$; d.f. = 1, 2; $P = 0.0339$), with 1.3-fold (998 kg ha^{-1}; 34%) higher yield from MON 88702 compared with DP393 (Fig. 2).

3.2 *Lygus* efficacy trials with *Lygus*-active insecticide sprays

3.2.1 Efficacy against L. lineolaris *at medium-pressure locations*

One location (Winnsboro, LA) met the criteria of medium *Lygus* pressure (Fig. 3). Interaction between trait and insecticide spray had a significant effect on total nymph counts ($F = 5.25$; d.f. = 2, 12; $P = 0.023$) but trait did not show a significant effect in the Spray by Entry and Spray by Negative blocks when each spray regime was analyzed separately. Interaction of trait and spray did

 © 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

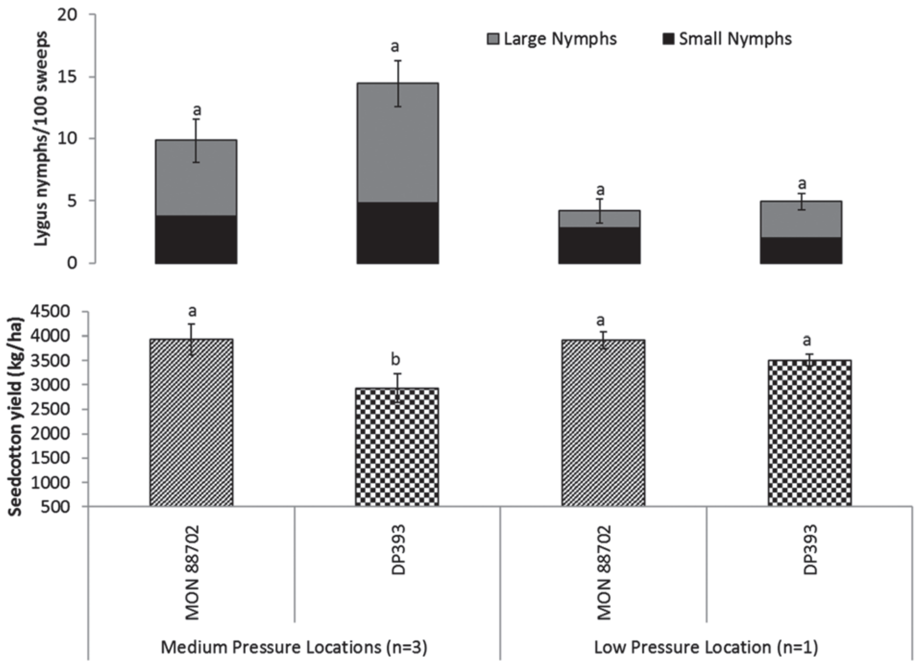

Figure 2. *Lygus hesperus* nymph counts and seedcotton yield in trials with no *Lygus*-active insecticides at Maricopa, AZ and Five Points, CA, during 2012–2014. Means followed by different letters *within each pressure category* are significantly different from each other at $P \leq 0.05$ (LSMEANS test). Data shown as mean ± SEM. Standard errors for nymphs are for total numbers of nymphs.

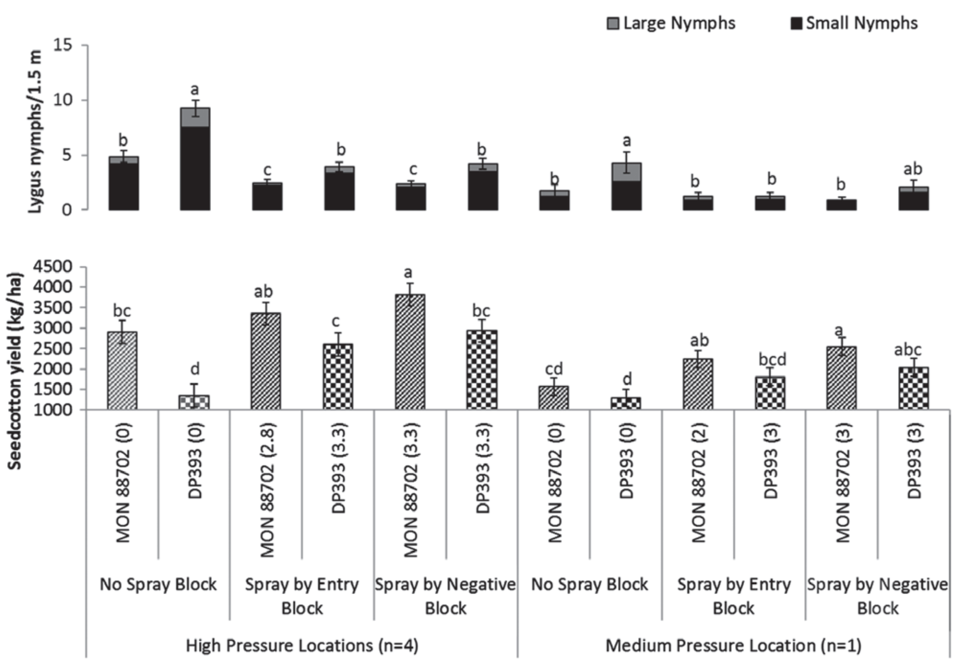

Figure 3. *Lygus lineolaris* nymph counts and seedcotton yield in trials with *Lygus*-active insecticides at Stoneville, MS, Marianna, AR, Jackson, TN, Winnsboro, LA, and Belvidere, NC, during 2014 (numbers in parentheses next to MON 88702 and DP393 indicate the numbers of insecticide applications). Means followed by different letters *within each pressure category* are significantly different from each other at $P \leq 0.05$ (LSMEANS test). Data shown as mean ± SEM. Standard errors for nymphs are for total numbers of nymphs.

not show a significant effect on yield, but a significant increase in yield by spraying with insecticide ($F = 10.80$; d.f. = 2, 4; $P = 0.024$) and presence of trait ($F = 6.95$; d.f. = 1, 6; $P = 0.039$) was recorded.

In the No Spray block, a 2.5-fold (60%) reduction in total nymph counts was recorded on MON 88702 compared with DP393; however, the yield advantage of MON 88702 was not significant in this block. In the Spray by Entry block, two sprays were applied on MON 88702 whereas DP393 required three sprays. An average of 3.0 spray applications were made on MON 88702 and DP393 in the Spray by Negative block. Significant differences were not detected for either total nymph counts or yield between MON 88702 and DP393 in these blocks (Fig. 3).

3.2.2 *Efficacy against* L. lineolaris *at high-pressure locations*

Four locations (Belvidere, NC, Jackson, TN, Marianna, AR, and Stoneville, MS) met the criteria of high *Lygus* pressure.

 © 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

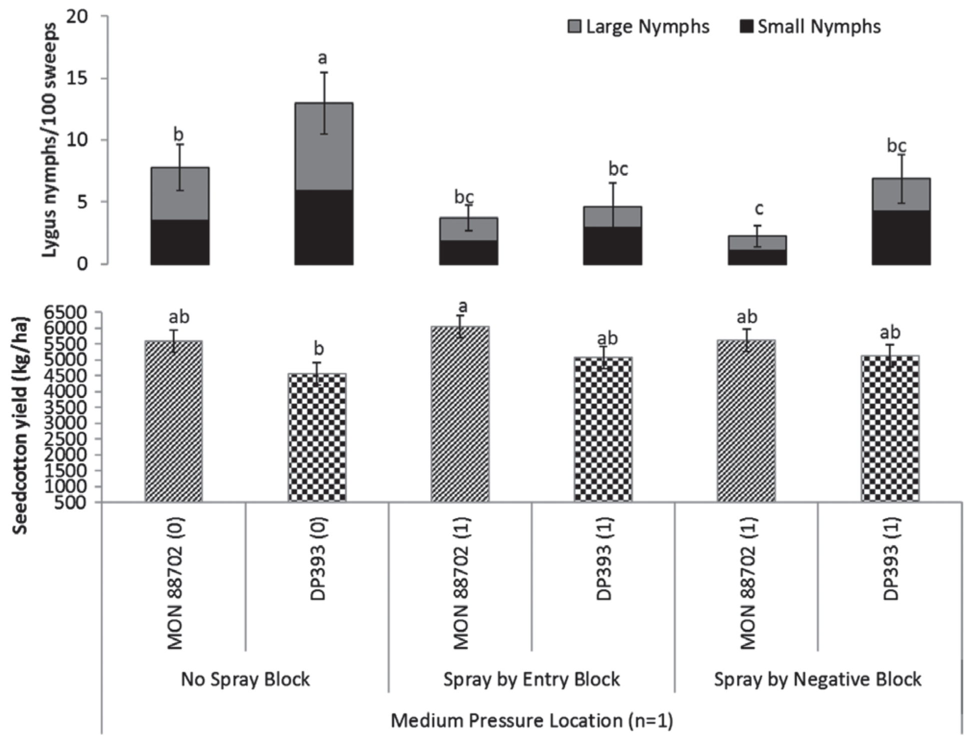

Figure 4. *Lygus hesperus* nymph counts and seedcotton yield in trial with *Lygus*-active insecticides at Maricopa, AZ, during 2014 (numbers in parentheses next to MON 88702 and DP393 indicate the numbers of insecticide applications). Means followed by different letters are significantly different from each other at $P \leq 0.05$ (LSMEANS test). Data shown as mean ± SEM. Standard errors for nymphs are for total numbers of nymphs.

Across these locations, interaction between trait and insecticide spray had a significant effect on total nymph counts ($F = 9.08$; d.f. = 2, 6; $P = 0.015$) but trait did not show a significant effect in the Spray by Entry and Spray by Negative blocks when each spray regime was analyzed separately. Yield analyses also showed significant effects of trait and spray interaction ($F = 6.31$; d.f. = 2, 6; $P = 0.033$) (Fig. 3). Trait also showed a significant effect on yield in the Spray by Negative block ($F = 12.41$; d.f. = 1, 3; $P = 0.039$) but not in the Spray by Entry block when each spray regime was analyzed separately.

In the No Spray block, a 1.9-fold (48%) reduction in total nymph counts was recorded on MON 88702 compared with DP393. A 2.1-fold (1550 kg ha^{-1}; 115%) yield advantage was also recorded for MON 88702 in this block. In the Spray by Entry block, MON 88702 received 2.8 sprays with a 1.6-fold (38%) reduction in total nymph counts compared with DP393, which received 3.3 sprays. A 1.3-fold (749 kg ha^{-1}; 29%) yield advantage was recorded for MON 88702 in this block. In the Spray by Negative block, where an average of 3.3 sprays was applied on both MON 88702 and DP393, a 1.8-fold (43%) reduction in total nymph counts and a 1.3-fold (882 kg ha^{-1}; 30%) yield advantage were recorded for MON 88702. MON 88702 had as few nymphs in the No Spray block as DP393 had in blocks where it received 3.3 spray applications for *Lygus* control. Also, the yield of MON 88702 in the No Spray block was as high as DP393 receiving 3.3 sprays (Fig. 3).

3.2.3 *Efficacy against* L. hesperus *at medium-pressure location*

Maricopa, AZ was a medium-pressure location (Fig. 4). The interaction between trait and spray did not have a significant effect on total nymph counts and yield at this location. Only trait showed a significant effect on total nymph counts ($F = 8.58$; d.f. = 1, 8; $P = 0.019$) and yield ($F = 8.36$; d.f. = 1, 6; $P = 0.028$).

In the No Spray block, a 1.7-fold (40%) reduction in total nymph counts was recorded for MON 88702 compared with DP393; however, the yield advantage of MON 88702 was not significant in this block. In the Spray by Entry and Spray by Negative blocks, both MON 88702 and DP393 received one insecticide application each and significant differences were not detected for either total nymph counts or yield between MON 88702 and DP393 in these blocks (Fig. 4).

3.3 Thrips efficacy trials

3.3.1 *Efficacy against thrips at medium-pressure locations*

Five locations (Blackville, SC, Sidon, MS, St Joseph, LA, and both early and late planted trials at Stoneville, MS) met the criteria of medium thrips pressure (Fig. 5). At the 1–2 leaf stage, trait showed a significant effect on thrips injury ($F = 21.53$; d.f. = 1, 4; $P = 0.009$). The presence of seed treatment did not show additional reduction in injury on DP393 or MON 88702 when there was low pressure during the first rating (Fig. 5(A)). Counts of immature thrips were also affected by the trait ($F = 11.96$; d.f. = 1, 4; $P = 0.026$). DP393 without seed treatment had the highest thrips counts and MON 88702 with seed treatment had the lowest thrips counts. In the presence of seed treatment, numbers were reduced significantly on DP393 compared with DP393 without seed treatment. Thrips numbers were very low on MON 88702 with and without insecticide seed treatment (Fig. 5(B)).

By the 3–4 leaf stage, injury increased to medium-pressure levels on DP393, whereas there was no increase in injury on MON 88702 (Fig. 5(C)). Three-way interactions of trait, seed treatment, and foliar spray were not significant. Two-way interactions of trait and seed treatment showed significant effect on thrips injury ($F = 17.24$; d.f. = 1, 4; $P = 0.014$). DP393 without seed treatment or foliar spray had the greatest injury while MON 88702 in the

 © 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

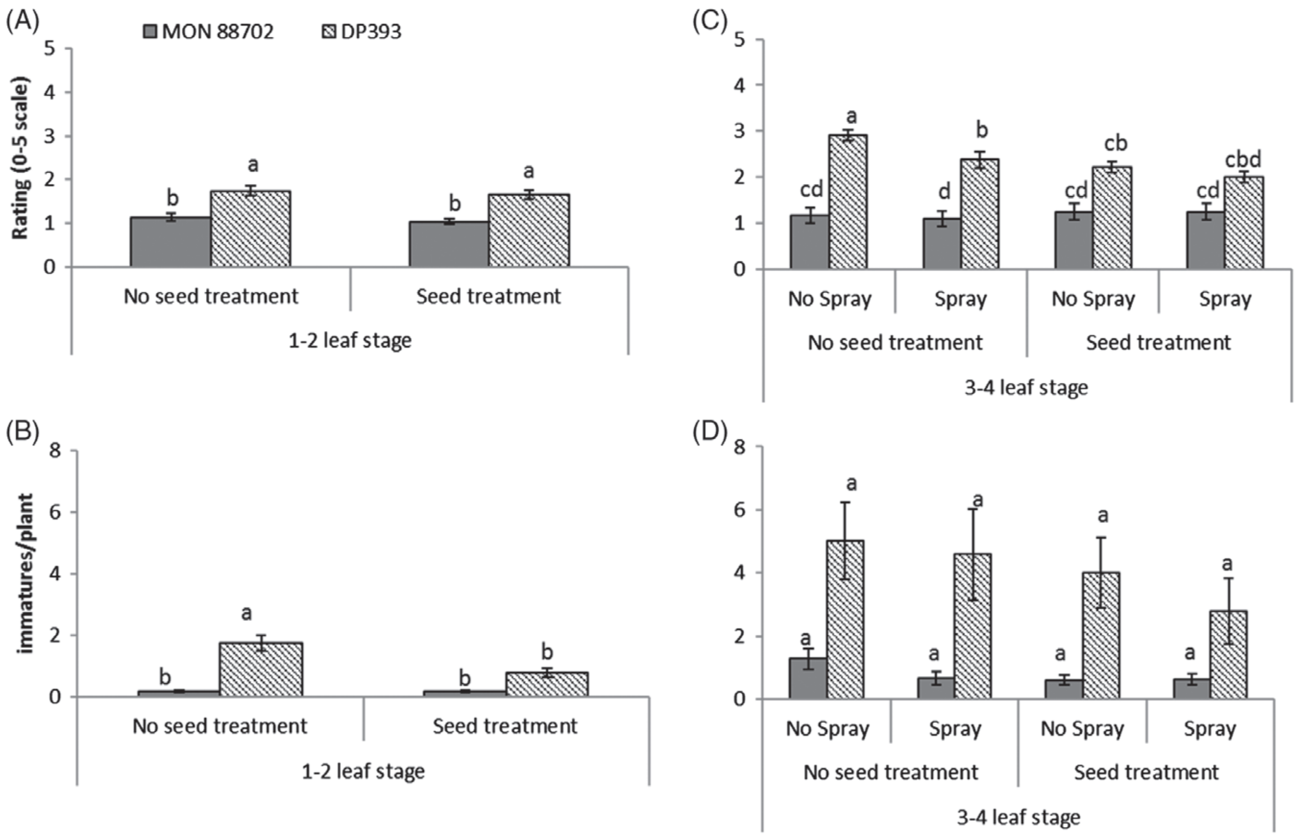

Figure 5. Ratings of cotton injury from thrips (A,C) and counts of immature thrips (B,D) across five medium-pressure locations in Blackville, SC, Sidon, MS, St Joseph, LA, and Stoneville, MS, during 2015. Means followed by different letters in each graph are significantly different from each other at $P \leq 0.05$ (LSMEANS test). Data shown as mean ± SEM.

presence of foliar spray had the least injury. The presence of seed treatment, foliar spray, or both significantly reduced injury on DP393, whereas injury on MON 88702 even without these additional treatments remained as low as DP393 that received both seed treatment and foliar spray (Fig. 5(C)). No two-way or three-way interaction affected immature counts. Counts of immature thrips were highly variable on DP393 across locations, and analysis did not detect significant differences across treatments or treatment combinations; however, numerical differences were obvious, with the highest on DP393 without seed treatment or foliar spray and the lowest on MON 88702 in the presence of seed treatment. A trend for reduction in counts on both DP393 and MON 88702 was recorded in the presence of seed treatment and/or foliar spray (Fig. 5(D)).

3.3.2 *Efficacy against thrips at high-pressure locations*

Eight locations (Starkville, MS, Tifton, GA, Winnsboro, LA, Marianna, AR, and both early and late planted trials at Jackson, TN and Suffolk, VA) met the criteria of high thrips pressure (Fig. 6). At the 1–2 leaf stage, trait and seed treatment interaction showed significant effect on thrips injury ($F = 12.25$; d.f. = 1, 7; $P = 0.009$). The greatest injury was recorded on DP393 without seed treatment and the least on MON 88702 with seed treatment (Fig. 6(A)). The presence of seed treatment benefited both DP393 and MON 88702, as injury was significantly reduced on both in its presence. MON 88702 without seed treatment had significantly lower injury than DP393 with seed treatment (Fig. 6(A)). Abundance of immature thrips was affected by trait at the 1–2 leaf stage ($F = 6.15$; d.f. = 1, 7; $P = 0.042$). DP393 without seed treatment had the highest counts of immature thrips and MON 88702 with a seed treatment had the lowest counts (Fig. 6(B)). There was a trend for lower thrips counts on both DP393 and MON 88702 in the presence of seed treatment. MON 88702 without seed treatment had thrips counts as low as DP393 with seed treatment **(**Fig. 6(B)).

By the 3–4 leaf stage, injury on DP393 had increased, but no increase in injury was recorded on MON 88702 (Fig. 6(C)). Three-way interactions of trait, seed treatment, and foliar spray were not significant. Injury was significantly affected by two-way interaction of trait and seed treatment ($F = 9.47$; d.f. = 1, 7; $P = 0.0178$) and trait and foliar spray ($F = 9.58$; d.f. = 1, 7; $P = 0.0174$). DP393 without seed treatment or foliar spray had the greatest injury, and MON 88702 in the presence of seed treatment plus foliar spray had the least injury recorded (Fig. 6(C)). DP393 benefited from the presence of seed treatment, the foliar spray, or both, and a trend for lower injury was also recorded for MON 88702 with these additional treatments. Under this high pressure, MON 88702 without seed treatment or foliar spray had significantly lower injury than DP393 receiving both seed treatment and foliar spray (Fig. 6(C)). No two-way or three-way interaction affected immature counts. Counts of immature thrips were significantly affected by trait ($F = 7.01$; d.f. = 1, 7; $P = 0.0330$) at the 3–4 leaf stage. MON 88702 without seed treatment and foliar spray had as few thrips as DP393 with both seed treatment and foliar spray (Fig. 6(D)). Application of foliar spray on MON 88702 showed a trend for a decrease in counts, but counts were significantly reduced when seed treatment or a combination of seed treatment plus foliar spray were present (Fig. 6(D)).

© 2018 The Authors.

Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

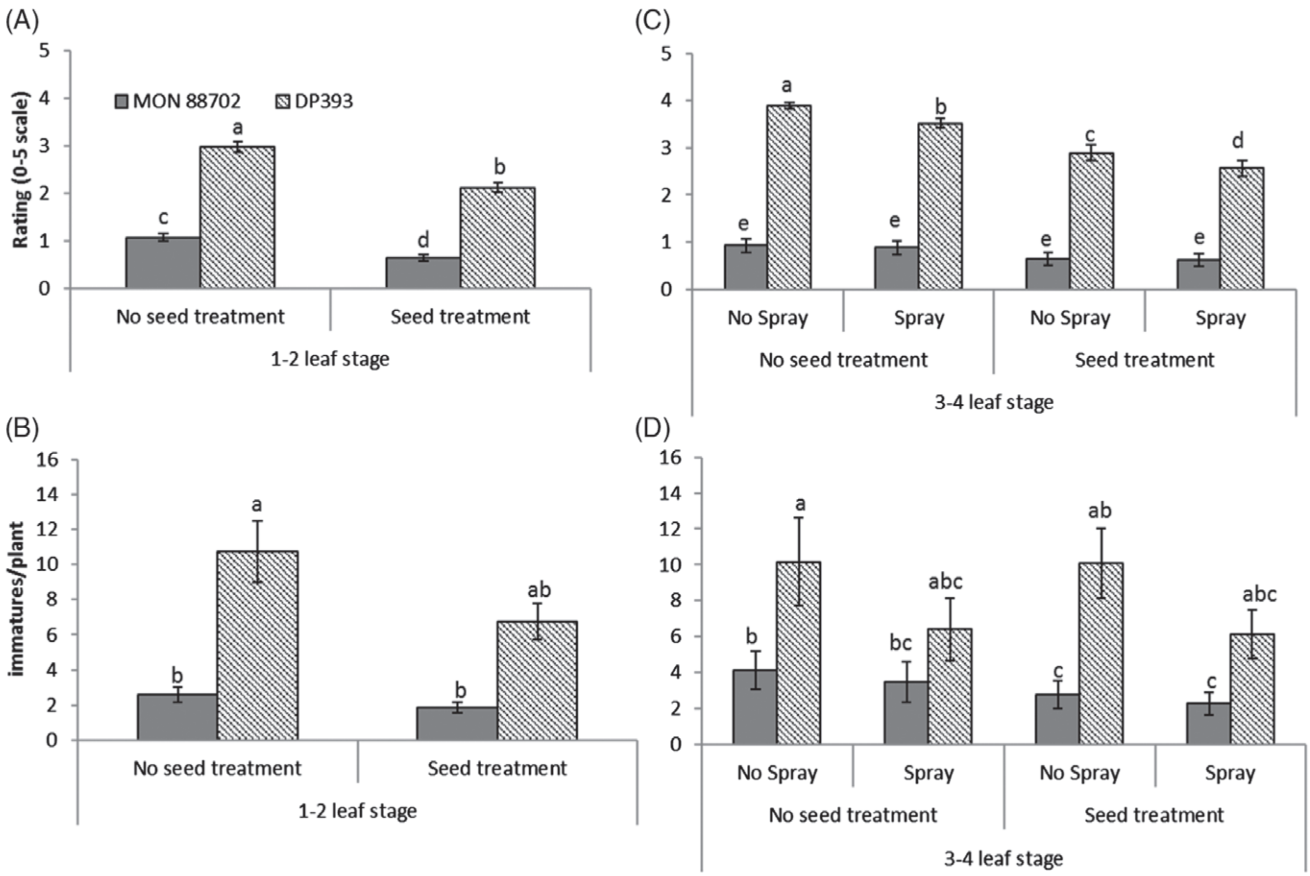

Figure 6. Ratings of cotton injury from thrips (A,C) and counts of immature thrips (B,D) across eight high-pressure locations in Starkville, MS, Tifton, GA, Winnsboro, LA, Marianna, AR, Jackson, TN, and Suffolk, VA, during 2015. Means followed by different letters in each graph are significantly different from each other at $P \leq 0.05$ (LSMEANS test). Data shown as mean ± SEM.

3.3.3 *Thrips species composition in 2015 thrips trials*

In most locations with medium pressure of thrips, more *F. fusca* were present than other species, except for first collections at St Joseph, LA (dominated by *F. occidentalis*), and both collections at Blackville, SC (dominated by *F. tritici*). Across four locations with medium pressure of thrips on first collection, a total of 160 adults were identified, and *F. tritici* was the most abundant species, followed by *F. fusca* and *F. occidentalis*. For the second collection, a total of 216 adults were identified, which were dominated by *F. fusca* followed by *F. tritici* and *F. occidentalis* (Table 1).

At eight locations with high pressure of thrips, *F. fusca* was always the most abundant species. Across these locations, 758 and 1394 adults were identified from the first and second collections, respectively. The most abundant species was *F. fusca* followed by *F. tritici* at both collection times (Table 1).

4 DISCUSSION

These studies describe field efficacy of a *Bt* protein against two important insect pest complexes of cotton, *Lygus* spp. and mainly *Frankliniella* spp., that are not the targets for current commercial transgenic traits. These studies were conducted across the Cotton Belt of the USA at sites representing varying degrees of natural insect pressure, and with or without insecticide treatments to demonstrate the potential fit of this new trait in current management strategies.

4.1 MON 88702 provides *Lygus* protection

Significant reductions in *Lygus* numbers due to MON 88702 have previously been documented in cage studies with high densities of *Lygus*,[25,26] but yield data were not recorded in those studies. Populations of *Lygus* during the season are considered significant factors affecting yield;[35] however, not all life stages of *Lygus* are equally damaging. Like stink bugs,[36] larger *Lygus* nymphs are considered more damaging to cotton than small nymphs.[37] Therefore, we differentiated *Lygus* nymphs into small (first to third instar) and large (fourth to fifth instar) in these studies. MON 88702 always had fewer large nymphs than the DP393 negative control (Figs 1–4), but high counts of small nymphs were also recorded on MON 88702. This result was expected, as the insecticidal *Bt* proteins usually require a longer interval than the conventional insecticides to cause lethal effects.[38] It is highly probable that most of the small nymphs recovered in these trials were not actively feeding at the time of sampling and were not impacting yield negatively. Also, continued oviposition by immigrating adults likely contributed to the abundance of smaller nymphs on MON 88702. Overall, the *Lygus* counts and yield data reported here, although in small-plot test scenarios, show a proportional advantage of this *Bt* trait. MON 88702 demonstrated the highest reduction in counts of *L. lineolaris* and yield advantages (77% and 115%; Figs 1 and 3) over DP393 under high pressure. With *L. lineolaris* counts decreasing to medium pressure levels, the yield advantage over DP393 was also lowered (33%, 21%) and it further decreased to 7% under low pressure (Figs 1 and 3). For *L. hesperus*, high pressure was not achieved

 © 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 1. Thrips species composition in cotton at various trial locations (Suffolk, VA, Tifton, GA, Blackville, SC, Winnsboro, LA, St Joseph, LA, Starkville, MS, Sidon, MS, Stoneville, MS, Jackson, TN, Marianna, AR) in 2015

				Species composition (%)				
Thrips Pressure	# locations	Collection stage	Total adults	*F. fusca*	*F. tritici*	*F. occidentalis*	*N. variabilis*	Other thrips
Medium	5	1–2 leaf	160	28.12	40.00	21.88	10.00	0.00
	5	3–4 leaf	216	59.72	18.52	11.11	10.19	0.46
High	8	1–2 leaf	758	77.70	13.59	5.54	2.90	0.26
	8	3–4 leaf	1394	80.27	11.91	5.67	1.72	0.43

at either location but MON 88702 demonstrated yield advantages (34%, 23% under medium pressure and 12% under low pressure; Figs 2 and 4).

4.2 MON 88702 can supplement existing insecticide-based management strategies for *Lygus*

Currently, insecticide sprays are the primary management tactic for *Lygus,* and often multiple applications throughout the season are required. Sometimes, especially under high *Lygus* pressure in the midsouthern USA, even weekly insecticide applications do not provide adequate control.[35] *Lygus* have developed insecticide resistance to multiple classes of insecticides[20] and managing *Lygus* with a rotation of chemistries is becoming a challenge. In the Spray by Entry block in the insecticide trials, a spray regime was followed that would normally be practiced by many growers following the conventional thresholds established in their areas. Though *Lygus* numbers reached above conventional threshold on MON 88702, it required fewer sprays and produced higher yields, especially under high pressure from *L. lineolaris*. Significant interactions between spray and trait indicated that MON 88702 also benefited from insecticide sprays, as indicated by yield responses in the Spray by Entry and Spray by Negative blocks. Interestingly, the yield of MON 88702 without any spray treatments (No Spray block) was comparable to when it received 2.8 sprays (Spray by Entry block) and to DP393 receiving 3.3 spray applications (Spray by Entry and Spray by Negative blocks), suggesting a reduced need for insecticides and thus potential adjustments of spray threshold recommendations for MON 88702. The same was true for trials under medium pressures, where MON 88702 with 2 sprays (Spray by Entry block) compared well with 3 sprays on DP393. Though application of 2 or 3 sprays on MON 88702 significantly increased yield, seedcotton yield of MON 88702 without sprays was comparable to DP393 receiving 3 sprays (Fig. 3). Trends for increased yield were also recorded for *L. hesperus*, though data were limited, with only one location under medium pressure (Fig. 4).

4.3 MON 88702 provides thrips protection

Cotton maturity is impacted by several factors, including planting date, varietal maturity, nitrogen rate, irrigation management, and early season pest management of which thrips are the most important.[14] Unlike other regional pests of cotton, thrips are a widespread annual pest throughout much of the Cotton Belt in the USA.[11] There are several species of thrips infesting cotton, and among them *F. fusca* is the most abundant species at most southern locations as documented in this and previous studies.[39,40] Damage to cotton is caused by direct feeding of adult and larval thrips. Adult thrips disperse into cotton as wild hosts near cotton fields start senescing. The mere presence of an adult on a cotton plant does not necessarily suggest the need for treatment. The best indicators of a need for thrips control in the midsouthern and southeastern regions are the presence of immature thrips along with injury symptoms on newly emerging leaves up to the first fifth true leaf.[10] Usually, signs of plant injury and at least one immature thrips per plant are thresholds for thrips treatment in most southern cotton-growing states.[10] Our research focused on these two parameters during the most critical period in cotton plant phenology for susceptibility to thrips. We recorded a marked increase in both injury and/or immature numbers on DP393 from the 1–2 to the 3–4 true leaf stages, indicating continued build-up of thrips populations in our trial fields (Figs 5 and 6). However, plots with MON 88702 were nearly free of injury symptoms, suggesting thrips protection by the trait.

4.4 MON 88702 can supplement existing insecticide-based management strategies for thrips

Currently, insecticide seed treatments are the most convenient and adopted option for thrips control and are considered an integral component of cotton IPM programs across many regions of the Cotton Belt. The neonicotinoid insecticides, such as thiamethoxam and imidacloprid, have been the primary seed treatment tools for thrips control even with resistance to this class of insecticide.[18] When the incidence of thrips infestation is high, and seed treatments do not provide sufficient control, growers usually apply at least one supplemental foliar spray. We also included these standard practices in our studies reported here. Though DP393 with insecticide seed treatment had fewer immature thrips at first sampling in locations with medium pressure, this advantage did not translate into lower injury on DP393 (Fig. 5). The benefit of insecticide seed treatment in terms of fewer immatures and corresponding reduction in injury on both DP393 and MON 88702 was more obvious at locations with high pressure from thrips during the 1–2 true leaf stage (Fig. 6). By the 3–4 true leaf stage, injury ratings and numbers of immatures on DP393 remained above conventional thresholds, despite receiving a foliar spray application, but injury on MON 88702 remained minimal. The addition of a seed treatment and a foliar spray on MON 88702 showed a trend for decreased numbers of thrips and injury, suggesting this trait can also benefit from these additional treatments. At locations with high pressure from thrips, immature thrips were present on MON 88702 during the first sampling, and their numbers increased by the second sampling, but that did not result in a corresponding increase in injury on MON 88702. These observations are not surprising as the immatures observed are most likely the result of a continuous influx of adults into test fields, deposition of eggs, and larval hatch. The lack of increase in corresponding injury strengthens our previous understanding that transgenic traits take a relatively longer time to cause mortality, and those immatures are not likely to be causing feeding injury.[38]

© 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

MON 88702 demonstrated less thrips injury when compared with DP393 receiving the standard commercial insecticide treatments. Under medium thrips pressure, DP393 required at least a seed treatment or a combination of both seed treatment and foliar spray to decrease injury to levels recorded on MON 88702 without seed treatment or foliar spray. Under high pressure, even without these additional treatments, MON 88702 exhibited significantly lower injury than DP393 receiving both seed treatment and foliar spray. These data support the notion that MON 88702 can become a valuable management tool along with existing chemical-based strategies during critical stages of cotton phenology, especially in areas facing considerable thrips problems.

4.5 Potential impact of MON 88702 for cotton IPM

For much of the Cotton Belt, ensuring early maturity is key for economic production of cotton as delays in maturity caused by thrips and weather issues render young plants stressed and more vulnerable to plant bugs and other pest problems for a longer period, thus leading to increased yield losses. Because chemical insecticides are currently the only option available, more insecticide sprays are required to manage plant bugs in areas with thrips problems. The early season protection demonstrated by MON 88702 can help maintain normal healthy cotton development, which ultimately could benefit protection from *Lygus* spp. damage and further reduce the need for insecticides. Though imidacloprid treatment of DP393 seed showed benefit at most locations in our studies, resistance to this class of insecticides is reported in these locations in *F. fusca*.[18,41] Without addition of newer seed treatments in current management options, growers must resort to previously abandoned in-furrow sprays of broad-spectrum insecticides or foliar insecticide use patterns, both which can negatively affect beneficial insect species in cotton agroecosystems.[42]

Though additional large-scale studies are warranted, the data presented here suggest that the ET, a determinant for insecticide application, could possibly change for both *Lygus* and thrips on MON 88702 and further lessen the need for foliar sprays. This reduction in use of chemical insecticides can preserve the biological control activity of beneficial insects in cotton fields[4,43–45] and thus decrease the chances of secondary pest outbreaks, such as spider mites, which have been shown to flare with more sprays.[27] Because *Bt*-based traits have a different mode of action to chemical insecticides, cross-resistance is not likely to occur[46] and the introduction of a trait accompanied by proper management will be valuable in dealing with the incidence of resistance in these pests. MON 88702 will be stacked with other transgenic traits providing protection against lepidopteran pests and tolerance to herbicides for implementing an effective system approach in cotton IPM. Additional studies will determine strategies to manage resistance to MON 88702 and other transgenic traits in all target insect pests of the final stack product.

CONCLUSIONS

Using the criteria of season-long nymph counts and yield for *Lygus*, and leaf injury and counts of immature thrips during the 1–4 true leaf stages, a clear advantage of MON 88702, especially under high infestation levels of these pests, was demonstrated. Counts of *Lygus* (especially large nymphs) were lower on MON 88702, which resulted in significant yield protection and advantages. Unsprayed MON 88702 yielded as high as its non-transgenic near isoline DP393 receiving multiple applications of insecticide sprays. Plots of MON 88702 were also nearly free of thrips injury and below conventional ET during the most critical time in cotton phenology. Untreated MON 88702 outperformed non-transgenic DP393 that received the commercial standard of seed treatment plus one foliar spray targeting thrips. Such a level of efficacy suggests that MON 88702, when incorporated into existing IPM programs, could become an important tool for management of *Lygus* and thrips in cotton agroecosystems, especially where the success of IPM programs is becoming a challenge with existing chemical control strategies. The development of a transgenic trait with field-demonstrated efficacy for hemipteran and thysanopteran pests of cotton is a breakthrough which can open avenues for the development of such traits for similar pests in other cropping systems as well.

ACKNOWLEDGEMENTS

We are grateful to many teams in Bayer Crop Science for assistance with cotton transformation, nursery seed production, field testing, statistical analyses, and manuscript reviews. Special thanks to Dan Pitts and Eric Blinka for their help in coordinating many of the field trials.

COMPETING FINANCIAL INTEREST

Jeffrey Gore, Angus Catchot, Scott Stewart, Gus Lorenz, David Kerns, Jeremy Greene, Michael Toews, Ames Herbert, Dominic Reisig, Gregory Sword, Peter Ellsworth, and Larry Godfrey have no competing financial interests. The remaining authors are currently employed by Bayer Crop Science and declare competing financial interests; further, Robert Brown, Thomas Clark, and John Greenplate (late) were previously employed by Monsanto Company, now acquired by Bayer Crop Science.

REFERENCES

1 James C, *Global Status of Commercialized Biotech/GM Crops: 2014*. ISAAA Brief No. 49. ISAAA Briefs, Ithaca, NY (2014).
2 Benbrook CM, Impacts of genetically engineered crops on pesticide use in the US – the first sixteen years. *Environ Sci Eur* **24**:24 (2012).
3 Cattaneo MG, Yafuso C, Schmidt C, Huang C-y, Rahman M, Olson C *et al.*, Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. *Proc Natl Acad Sci U S A* **103**:7571–7576 (2006).
4 Tian J-C, Yao J, Long L-P, Romeis J and Shelton AM, Bt crops benefit natural enemies to control non-target pests. *Sci Rep* **5**:16636 (2015).
5 Romeis J, Meissle M and Bigler F, Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat Biotechnol* **24**:63–71 (2006).
6 Ellsworth PC, Fournier A, Frisvold G and Naranjo SE, in *Chronicling the socio-economic impact of integrating biological control, technology, and knowledge over 25 years of IPM in Arizona*, In Proceedings of the 5th International Symposium on Biological Control of Arthropods, ed. by Mason PG and Vincent C, Langkawi, Malaysia, September 11–15, 2017, CABI, Wallingford, UK pp. 214–216 (2017).
7 Greene J, Turnipseed S, Sullivan M and May O, Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. *J Econ Entomol* **94**:403–409 (2001).
8 Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H *et al.*, Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* **328**:1151–1154 (2010).
9 Wu K, Li W, Feng H and Guo Y, Seasonal abundance of the mirids, *Lygus lucorum* and *Adelphocoris* spp.(Hemiptera: Miridae) on Bt cotton in northern China. *Crop Prot* **21**:997–1002 (2002).
10 Cook D, Herbert A, Akin DS and Reed J, Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States. *J Int Pest Manage* **2**:B1–B9 (2011).

 © 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

11 Williams M, Cotton insect losses-2015, in *Proceedings of the Beltwide Cotton Conferences*. National Cotton Council, New Orleans, LA, pp. 507–525 (2016).
12 Musser F, Stewart S, Bagwell R, Lorenz G, Catchot A, Burris E *et al.*, Comparison of direct and indirect sampling methods for tarnished plant bug (Hemiptera: Miridae) in flowering cotton. *J Econ Entomol* **100**:1916–1923 (2007).
13 5.Ellsworth PC and Barkley V, Cost-effective Lygus management in Arizona cotton, in *Cotton, A College of Agriculture and Life Sciences Report*, ed. by Silvertooth JC. University of Arizona, College of Agriculture and Life Sciences, Tucson, AZ, pp. 299–307 (2001).
14 Gore J, Catchot A, Cook D and Musser F, *Best Management Practices for Tarnished Plant Bug in Cotton*. (2015). [Online]. Available: http://www.mississippi-crops.com/wp-content/uploads/2015/03/Best-Management-Practices-for-Tarnished-Plant-Bug-in-Cotton.pdf [15 May 2017].
15 Leigh T, Roach S, Watson T, King E, Phillips J and Coleman R, Biology and ecology of important insect and mite pests of cotton, in *Cotton Insects and Mites: Characterization and Management*, ed. By Edgar GK, Jacob RP and Randy JC. The Cotton Foundation, Memphis, TN pp. 17–85 (1996).
16 Kerns DL and Cattaneo MG, *Suggested Insecticides for Managing Cotton Insects in the Lower Rio Grande Valley 2009*. Texas AgriLife Extension Service, Texas A&M, College Station, TX (2009).
17 Stewart S, Patrick R and McClure A, *2010 Insect Control Recommendations for Field Crops, Cotton, Soybeans, Field Corn, Sorghum, Wheat, and Pasture*. University of Tennessee Extension Service Knoxville, TN, pp. 3–16 (2010).
18 Huseth AS, Chappell TM, Langdon K, Morsello SC, Martin S, Greene JK *et al.*, *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. *Pest Manag Sci* **72**:1934–1945 (2016).
19 Snodgrass GL, Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Mirida) in cotton in the Mississippi Delta. *J Econ Entomol* **89**:783–790 (1996).
20 Snodgrass G, Gore J, Abel C and Jackson R, Acephate resistance in populations of the tarnished plant bug (Heteroptera: Miridae) from the Mississippi River Delta. *J Econ Entomol* **102**:699–707 (2009).
21 Zhu YC, Zeng F and Oppert B, Molecular cloning of trypsin-like cDNAs and comparison of proteinase activities in the salivary glands and gut of the tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae). *Insect Biochem Mol Biol* **33**:889–899 (2003).
22 Brandt SL, Coudron TA, Habibi J, Brown GR, Ilagan OM, Wagner RM *et al.*, Interaction of two *Bacillus thuringiensis* d-endotoxins with the digestive system of *Lygus hesperus*. *Curr Microbiol* **48**:1–9 (2004).
23 Crickmore N, Zeigler D, Feitelson J, Schnepf E, Van Rie J, Lereclus D *et al.*, Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol Mol Biol Rev* **62**:807–813 (1998).
24 Baum JA, Sukuru UR, Penn SR, Meyer SE, Subbarao S, Shi X *et al.*, Cotton plants expressing a hemipteran-active *Bacillus thuringiensis* crystal protein impact the development and survival of *Lygus hesperus* (Hemiptera: Miridae) nymphs. *J Econ Entomol* **105**:616–624 (2012).
25 Gowda A, Rydel TJ, Wollacott AM, Brown RS, Akbar W, Clark TL *et al.*, A transgenic approach for controlling Lygus in cotton. *Nat Commun* **7**:12213 (2016).
26 Bachman PM, Ahmad A, Ahrens JE, Akbar W, Baum JA, Brown S *et al.*, Characterization of the activity spectrum of MON 88702 and the plant-incorporated protectant Cry51Aa2. 834_16. *PLoS One* **12**:e0169409 (2017).
27 Musser FR, Catchot AL, Stewart SD, Bagwell RD, Lorenz GM, Tindall KV *et al.*, Tarnished plant bug (Hemiptera: Miridae) thresholds and sampling comparisons for flowering cotton in the midsouthern United States. *J Econ Entomol* **102**:1827–1836 (2009).
28 Ellsworth PC, Lygus control decision aids for Arizona cotton, in *Cotton*, ed. by Silvertooth JC. University of Arizona, College of Agriculture, Tucson, AZ, pp. 269–280 (2000).
29 Snodgrass GL and Scott WP, A discriminating-dose bioassay for detecting pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations. *Southwest Entomol* **24**:301–307 (1999).
30 Snodgrass GL and Scott WP, Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations during a three-year period in the Delta of Arkansas, Louisiana, and Mississippi. *J Econ Entomol* **93**:441–446 (2000).
31 Snodgrass GL and Scott WP, Tolerance to acephate in tarnished plant bug (Heteroptera: Miridae) populations in the Mississippi River Delta. *Southwest Entomol* **27**:191–199 (2002).
32 Ellsworth PC, Brown L, *Anatomy of a Cotton Sweep*. (2012). [Online]. Available: http://cals.arizona.edu/apmc/docs/SweepsAnatomyv2c.pdf [4 June 2018].
33 Reed JT, Allen C, Bagwell R, Cook D, Burris E, Freeman B *et al.*, *A key to the thrips (Thysanoptera: Thripidae) on seedling cotton in the Mid-Southern United States*. Office of Agricultural Communications, Division of Agriculture, Forestry, and Veterinary Medicine at Mississippi State University, Mississippi State MS (2006).
34 SAS Institute, *SAS/STAT9.2 User's Guide*, 2nd edn. SAS Institute, Cary, NC (2008).
35 Musser FR, Lorenz GM, Stewart SD, Bagwell RD, Leonard BR, Catchot AL *et al.*, Tarnished plant bug (Hemiptera: Miridae) thresholds for cotton before bloom in the midsouth of the United States. *J Econ Entomol* **102**:2109–2115 (2009).
36 Greene JK, Turnipseed SG, Sullivan MJ and Herzog GA, Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. *J Econ Entomol* **92**:941–944 (1999).
37 Cooper W and Spurgeon D, Feeding injury to cotton caused by *Lygus hesperus* (Hemiptera: Miridae) nymphs and prereproductive adults. *Environ Entomol* **42**:967–972 (2013).
38 Ali M and Luttrell R, Response estimates for assessing Heliothine susceptibility to Bt toxins. *J Econ Entomol* **102**:1935–1947 (2009).
39 Stewart S, Akin D, Reed J, Bacheler J, Catchot A, Cook D *et al.*, Survey of thrips species infesting cotton across the Southern US Cotton Belt. *J Cotton Sci* **17**:1–7 (2013).
40 Wang H, Kennedy G, Reay-Jones FPF, Reisig DR, Toews MD, Roberts PM *et al.*, Molecular identification of thrips species infesting cotton in the southeastern USA. *J Econ Entomol* **111**:892–898 (2018).
41 Ames Herbert GK, *New Survey Shows High Level and Widespread Resistance of Thrips to Neonicotinoid Insecticides*. Virginia Tech, Blacksburg, VA [Online]. Available: https://blogs.ext.vt.edu/ag-pest-advisory/files/2015/02/NeonicThripsResistance.pdf [20 September 2018].
42 Mansfield S, Dillon ML and Whitehouse MEA, Are arthropod communities in cotton really disrupted? An assessment of insecticide regimes and evaluation of the beneficial disruption index. *Agric Ecosyst Environ* **113**:326–335 (2006).
43 Head G, Moar W, Eubanks M, Freeman B, Ruberson J, Hagerty A *et al.*, A multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. *Environ Entomol* **34**:1257–1266 (2005).
44 Naranjo SE, Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget arthropod natural enemies. *Environ Entomol* **34**:1193–1210 (2005).
45 Whitehouse MA, Wilson L and Fitt G, A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. *Environ Entomol* **34**:1224–1241 (2005).
46 Anilkumar KJ, Rodrigo-Simón A, Ferré J, Pusztai-Carey M, Sivasupramaniam S and Moar WJ, Production and characterization of *Bacillus thuringiensis* Cry1Ac-resistant cotton bollworm *Helicoverpa zea* (Boddie). *Appl Environ Microbiol* **74**:462–469 (2008).

 © 2018 The Authors.
Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.