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Choice and No-Choice Feeding Assays of Cotton Fleahoppers (*Pseudatomoscelis seriatus*) on Cotton Expressing the Mpp51Aa2 Protein

Brady P. Arthur [1], Charles P.-C. Suh [2], Benjamin M. McKnight [3], Megha N. Parajulee [4], Fei Yang [5] and David L. Kerns [1,*]

[1] Department of Entomology, Texas A&M University, College Station, TX 77843, USA
[2] USDA-ARS Southern Plains Agricultural Research Center, College Station, TX 77845, USA
[3] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843, USA
[4] AgriLife Research and Extension Center, Texas A&M University, Lubbock, TX 79403, USA
[5] Department of Entomology, University of Minnesota, St. Paul, MN 55108, USA
* Correspondence: david.kerns@ag.tamu.edu

Abstract: In Texas, the cotton fleahopper (*Pseudatomoscelis seriatus* (Reuter)) is considered a highly economically damaging pest of cotton (*Gossypium hirsutum* L.). Current control methods rely heavily on foliar chemical insecticides throughout the growing season. Considering the cost of insecticides and the critical timeliness of their application, chemical control methods are often not optimized to reduce potential yield losses. The Mpp51Aa2.834_16 gene in cotton (ThryvOn) has shown effectiveness against thrips and several piercing and sucking mirid insect pests, suggesting it has the potential to mitigate yield losses caused by the cotton fleahopper. Choice and no-choice caged feeding assays were conducted to assess the impact of cotton fleahoppers on ThryvOn cotton square retention under controlled laboratory conditions. In the choice assay, feeding by cotton fleahoppers significantly reduced square retention in the gene-lacking cotton to 46%, while the ThryvOn cotton retained 60% of the squares. In the no-choice assay, cotton fleahopper nymph feeding significantly reduced square retention in the cotton not expressing Mpp51Aa2 to 61%, whereas the ThryvOn cotton was unaffected. Based on the differences in square retention observed in both the choice and no-choice feeding assays, our findings indicate that the Mpp51Aa2 protein influences cotton fleahopper feeding preferences and the susceptibility of cotton plants to damage caused by cotton fleahoppers. Our study offers confirmation of the activity of ThryvOn on cotton fleahoppers observed in the field. The ThryvOn trait's activity towards cotton fleahoppers is consistent with that found for other mirid pests in cotton.

Keywords: Mpp51Aa2; ThryvOn; cotton fleahopper; *Psuedatomoscelis seriatus*; *Gossypium hirsutum*

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1. Introduction

The cotton fleahopper (*Pseudatomoscelis seriatus* (Reuter)) (Hemiptera: Miridae) has consistently ranked as one of the top insect pests of cotton in Texas. Infestations have become more common over recent years, with reported infestations occurring in 53% of the total cotton acres in 2012 which significantly expanded to 93% of the estimated cotton acres planted in 2022 in Texas [1,2]. This surge in infested acres has resulted in a substantial rise in bales lost, escalating from 25,098 in 2012 to 121,467 in 2022. This rise in the relative importance of cotton fleahoppers can be partially attributed to the widespread adoption of transgenic cultivars and the eradication of the boll weevil, *Anthonomus grandis* (Boheman), from most production areas in Texas. Eradication efforts and the widespread adoption of transgenic cotton expressing *Bacillus thuringiensis* (Berliner) (*Bt*) proteins have greatly reduced the need for insecticides to control these targeted insect species [3,4]. Nevertheless, insecticidal applications are heavily relied upon and are currently the most effective strategy for managing cotton fleahopper populations [5,6]. However, with the

increased cost of chemical applications from $6.24 to $12.90 per acre [1,2] over the past decade, there have been efforts to develop host plant resistance traits to reduce the impact of cotton fleahoppers. Although breeding for natural resistance mechanisms has shown promise, it remains cultivar-specific [7–9]. Despite efforts, the practical implementation of naturally occurring resistance towards cotton fleahoppers remains limited. This has led to emphasis on identifying transgenic traits with activity towards piercing-sucking pests.

Cotton fleahoppers, like other mirid pests, are sap-feeding insects with a modified proboscis and salivary glands that secrete lytic enzymes such as polygalacturonates, proteases, and other enzymes [10–12]. The pre-ingestion enzymatic digestion of plant cells and proteins is the primary cause of plant injury due to mirid feeding [13]. When the enzymatic degradation of cells occurs, plant sap is ingested by the cotton fleahopper [14]. The breakdown of plant cells by the saliva also triggers cotton's natural defense system to produce stress ethylene, ultimately leading to square abscission [15]. The activity of previously discovered Cry proteins has long been thought to have limited activity on hemipterans due to its low binding affinity in the midgut or the degradation of Cry proteins by pre-ingestion enzymes [16]. However, the discovery of the Mpp51Aa2 *Bt* protein from Bayer CropScience has shown potential for controlling hemipteran and thysanopteran pests in cotton [17,18]. Similar to other *Bt* proteins, the Mpp51Aa2 is a β pore-forming toxin that binds to the epithelial cells in the insect midgut, forming pores through the gut liner [17]. Unlike other currently available *Bt* proteins expressed by transgenic cotton cultivars, Jerga et al. [19] have demonstrated that Mpp51Aa2 has an affinity to bind to the epithelial cells in the midgut of piercing and sucking insect pests. Baum et al. [20] discovered that nymph mortality and nymph mass were both correlated to the concentration of Mpp51Aa2. As the expression of Mpp51Aa2 increased, nymph mortality increased and nymph mass decreased [20]. An artificial diet containing the Mpp51Aa2.834_16 variant of the protein determined lethal concentrations (LC_{50}) to be LC_{50} = 0.853 μg mg^{-1} for the tarnished plant bug *Lygus lineolaris* (Palisor de Beauvois) and LC_{50} = 0.3 μg mg^{-1} for the western tarnished plant bug *Lygus hesperus* (Knight) [17].

Field trials evaluating cotton expressing the Mpp51Aa2 protein have proved its efficacy towards hemipteran species in a field setting [21]. They reported a 23% reduction in adult tarnished plant bugs in cotton with the Mpp51Aa2 trait compared to an isoline that does not express the Mpp51Aa2 protein. However, there have been some conflicting conclusions as some field trials concluded that the thresholds for plant bugs in the field should not be altered [22], while others report that differences in population dynamics should be taken into consideration and that thresholds could be altered for cotton with the Mpp51Aa2 trait [23]. Disproportional numbers of small and large tarnished plant bug nymphs have been reported, where the numbers of large nymphs were statistically lower in cotton with the Mpp51Aa2 trait relative to the cotton lacking this trait. Subsequentially, large nymphs account for higher magnitudes of damage and, therefore, higher large nymph populations resulted in significantly lower yields at multiple levels of infestations [18].

Considering the similarities in biology and feeding strategy among the tarnished plant bug, western tarnished plant bug, and cotton fleahopper [24], it is possible that cotton expressing the Mpp51Aa2 protein may exhibit a similar activity on the cotton fleahopper. In a controlled cage study where sexually mature adult cotton fleahoppers were provided the choice to select and reproduce on cotton expressing Mpp51Aa2 or a gene-lacking isoline, no significant differences were observed in the numbers of small or large nymphs between the Mpp51Aa2 cotton and its gene-lacking isoline. However, when compared to the gene-lacking isoline, the Mpp51Aa2 cotton resulted in a three-fold reduction in subsequent adult numbers [25]. This reduction in adults ultimately resulted in a 1.7-fold decrease in the overall number of cotton fleahoppers (adults and nymphs combined) developing on the Mpp51Aa2 cotton [25].

The objective of the current study was to first determine if cotton expressing the Mpp51Aa2 trait affects the feeding preferences of cotton fleahoppers and then to evaluate

the susceptibility of cotton expressing the Mpp51Aa2 protein to cotton fleahopper feeding under controlled laboratory conditions.

2. Materials and Methods

2.1. Plant and Insect Sources

Choice and no-choice caged feeding assays were conducted in 2022 to determine the feeding preferences and magnitude of square damage caused by cotton fleahoppers. We employed two cotton cultivars in the assays, as follows: (1) Deltapine 2131 BG3TXF, which expresses the Mpp51Aa2 protein and is marketed as ThryvOn; and (2) Deltapine 2055 BG3XF, which lacks the Mpp51Aa2 protein and served as the gene-lacking control. The seeds for both cultivars were sourced from Bayer CropScience, St. Louis, MO, USA. The original seeds for both cotton cultivars were coated with a seed treatment containing imidacloprid (Acceleron Standard: Bayer CropScience, St. Louis, MO, USA). To eliminate the potential effects of an insecticide seed treatment, the seeds were washed to remove the seed treatment using the methods of Gassmann et al. [26]. Approximately 150 g of seed was placed in 1 L of deionized water with 4 mL of dish detergent (Dawn Ultra, Proctor & Gamble, Cincinnati, OH, USA) and agitated using a stirring plate and a magnetic stirring bar for 20 min. The seeds were removed from the solution and rinsed with deionized water, and the entire process was repeated to ensure all the visible seed treatment was removed. The washed seeds were then air-dried on paper towels for 24 h.

As Raszick et al. [27] showed, certain genotypic groups of cotton fleahoppers exhibited varied feeding preferences towards cotton; the cotton fleahoppers used in our assays were collected from a non-*Bt* cotton field that was squaring and naturally infested with these insects. Both adult and nymph cotton fleahoppers were aspirated from the terminal and flowers into a glass vial and placed in a growth chamber maintained at 28 ± 3 °C and 60% relative humidity with a photoperiod of 14:10 (L:D). They were allowed to feed on a diet of squaring cotton and fresh green beans (*Phaseolus vulgaris* L.) for 24 h prior to use in the assays to acclimate and ensure that healthy insects were utilized. At the time of infestation, later instar nymphs (fourth and fifth instar) were selected based on the relative size of the head capsule in relation to the thorax as well as the presence of early developing wing pads [12].

2.2. Choice Feeding Assay

The cotton plants were maintained in a controlled environment growth room with a photoperiod of 14:10 h (L:D) and held at approximately 28 to 31 °C. The plants were maintained with irrigation and fertilization as needed for optimum growth, as described by Yang et al. [28]. For the choice feeding assay, the plants were grown in 2 L pots at an initial density of two plants per pot. When the plants reached the first square stage, the pots were tinned to a density of one plant per pot, ensuring that the plants were uniform in size and growth stage. Two pots of each cultivar were placed in a square configuration towards the center of a BugDorm 3120 mesh cage (MegaView Science Co., Taichung, Taiwan), with the plants positioned approximately 12 cm apart to closely resemble field-scale spacing. All the cages were placed in the growth chamber room using the same environmental conditions as previously described. At the time of infestation, the cages were randomly assigned a treatment of infested or non-infested in a completely randomized design configuration in the growing room. There were six replicates of each combination of the main effects, as follows: ThryvOn/infested, gene-lacking/infested, ThryvOn/non-infested, and gene-lacking/non-infested. Because Mekala [29] showed that higher densities of feral cotton fleahoppers were needed when conducting choice feeding assays, twenty unsexed adult cotton fleahoppers were released (five cotton fleahoppers per plant) onto the terminals of the plants in each BugDorm. Given the limited mobility of cotton fleahopper nymphs between host plants in field conditions, nymphs were not utilized in the choice assay. Square retention was determined at seven and fourteen days after infestation by visually inspecting the whole plant and recording the number of abscised and present squares on

sympodial branches [30]. To evaluate square retention, surviving cotton fleahoppers were visually located and aspirated into a glass vial from individual cotton plants within the cages to prevent their escape during the evaluation. After repositioning the cages, these surviving cotton fleahoppers were placed back to their original positions on the cotton plants inside the cages.

2.3. No-Choice Feeding Assay

The plants for the no-choice feeding assay were grown in 1.9 L plastic jars at a density of two plants per jar. The plants were maintained to optimize growth in the same manner as described previously. Once the plants produced at least one square, approximately 30 days after planting, all the jars were thinned to a density of one plant per jar to ensure uniform plant size and growth stage. Jar lids with a 50 mm hole were then affixed to the top of the jars, with the plant stem growing through the center. The treatments were then assigned to the jars in a completely randomized design with five replicates of each treatment. During the first week of squaring, two cotton fleahoppers, either adults or fourth/fifth instar nymphs, were released onto the terminal of each plant. The combination of the main effect traits and an insect's life-stage resulted in the following treatments: ThryvOn/adult-infested, ThryvOn/nymph-infested, ThryvOn/non-infested, gene-lacking/adult-infested, gene-lacking/nymph-infested, and gene-lacking/non-infested. At the time of infestation, the enclosures were completed by attaching a 3.8 L jar with mesh screen sides atop the 1.9 L plastic jar containing the plant, as seen in Figure 1 (Uline, Pleasant Prairie, WI, USA). At the juncture of the two jars, a layer of modeling clay was used to prevent cotton fleahoppers from retreating down the stem of the plant and onto the soil. The caged plants were kept in the growth room in the same environmental conditions as previously described. Square retention was determined at seven and fourteen days after infestation in the same manner described for the choice test. When square retention was evaluated, the surviving cotton fleahoppers were visually located and aspirated off the plant into a glass vial until the cages were to be repositioned, and the remaining fleahoppers were reinfested onto the plants.

Figure 1. Cage assembly for the no-choice feeding assay. The plants were grown in a 1.9 L plastic jar, and, at the time of infestation, a 3.8 L plastic jar with mesh sides was affixed to the top of the 1.9 L jar with a layer of modeling clay used between the juncture of the jars to prevent the cotton fleahoppers from retreating down the stem to the soil.

2.4. Statistical Analyses

To adjust for natural square shedding, the square retention ratings were corrected using Abbott's formula [31], where the square retention of the non-infested treatment of each cultivar served as the control for the corresponding cultivar in both the choice and no-choice assays. The square retention comparisons for both assays were analyzed utilizing Graphpad Prism 9.3.0 [32]. The statistical differences among the treatments for the choice feeding assays were determined using a two-way ANOVA with trait and infestation as the main effects. In the no-choice feeding assay, the square retention comparisons between life stages were conducted using ANOVA. The treatment differences were delineated using the two-stage linear step-up Benjamini, Krieger, and Yekutieli procedure to account for false

discovery at a Q value of 0.05 [33]. The square retention differences between the cultivars by life stages were completed with an ANOVA, followed by a paired *t*-test at an $\alpha = 0.05$.

3. Results

3.1. Choice Feeding Assay

At seven days after infestation with adult cotton fleahoppers, both the trait ($p < 0.0001$) and infestation ($p < 0.0001$) effects were significant on square retention. There was also a significant interaction between trait and infestation ($p < 0.0001$). The square retention of the gene-lacking/infested combination was 29.86%, which was significantly lower than that of the ThryvOn/infested, ThryvOn/non-infested, and gene-lacking/non-infested (Figure 2) combinations. The square retention of the ThryvOn/infested combination was 94.91% and was not significantly different than that of the ThryvOn/non-infested or gene-lacking/non-infested ones. The square retention at fourteen days after infestation also exhibited significant main effects of trait ($p = 0.047$), infestation ($p < 0.0001$), and their interaction ($p = 0.047$) (Figure 3). The square retention of the gene-lacking/infested combination was 45.83%, while the ThryvOn/infested group showed a significantly higher square retention, at 59.64%. The square retention values of both the gene-lacking/infested and ThryvOn/infested combinations were significantly lower than those of the gene-lacking/non-infested and ThryvOn/non-infested cotton.

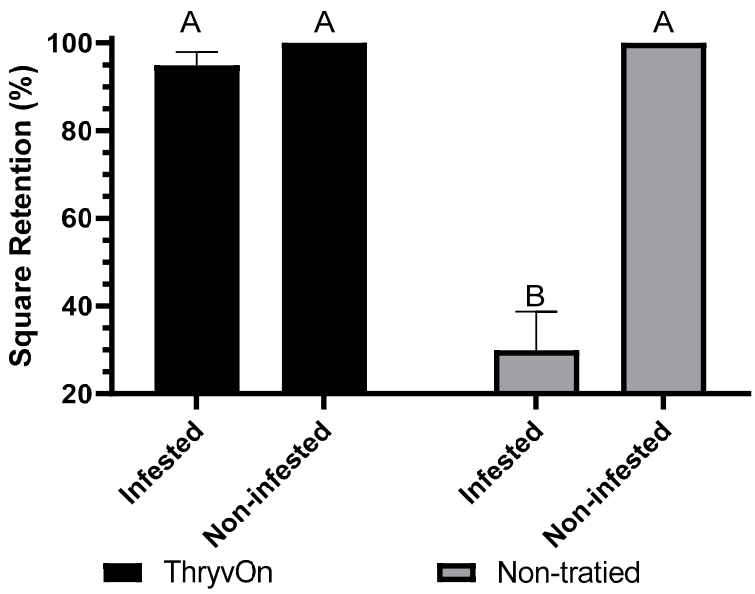

Figure 2. Choice feeding assay square retention percentages (mean ± SEM) on ThryvOn and gene-lacking cotton seven days after infestation with cotton fleahopper adults. There was a significant interaction between cotton trait and infestation treatment ($p < 0.0001$). Different letters indicate treatments that are significantly different (Benjamini, Krieger, and Yekutieli $Q = 0.05$).

3.2. No-Choice Feeding Assay

The square retention of the gene-lacking/non-infested cotton was significantly higher than that of the gene-lacking/nymph-infested ($p = 0.033$) and gene-lacking/adult-infested cotton ($p = 0.007$) (Figure 4B). The gene-lacking/nymph-infested and gene-lacking/adult-infested combinations were not significantly different from each other ($p = 0.479$). The square retention of the ThryvOn/non-infested cotton was statistically similar to that of the ThryvOn/nymph-infested ($p = 0.252$) and ThryvOn/adult-infested ($p = 0.737$) cotton (Figure 4A). Likewise, the square retention of the ThryvOn/nymph-infested combination was not significantly different from that of the ThryvOn/adult-infested ($p = 0.479$) treatment. When comparing square retention among different life stages within traits, the ThryvOn/adult-infested group (96.55%) showed a significantly higher square retention

than the gene-lacking/adult-infested one (77.45%) (p = 0.0484) (Figure 5A). Additionally, there were significant differences in square retention between the ThryvOn/nymph-infested (93.33%) and gene-lacking/nymph-infested cotton (66.00%) (p = 0.0194) (Figure 5B).

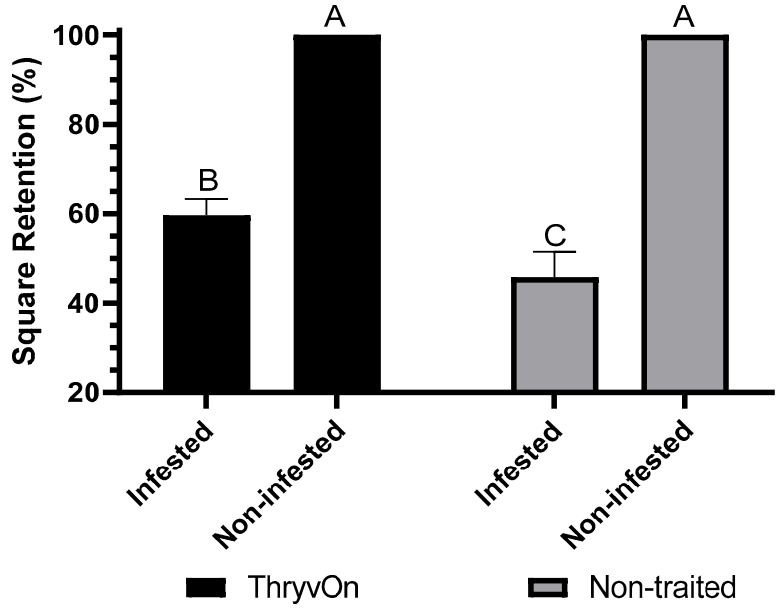

Figure 3. Choice feeding assay square retention percentages (mean ± SEM) on ThryvOn and gene-lacking cotton fourteen days after infestation with cotton fleahopper adults. There was a significant interaction between cotton trait and infestation (p = 0.047). Different letters indicate treatments that are significantly different (Benjamini, Krieger, and Yekutieli Q = 0.05).

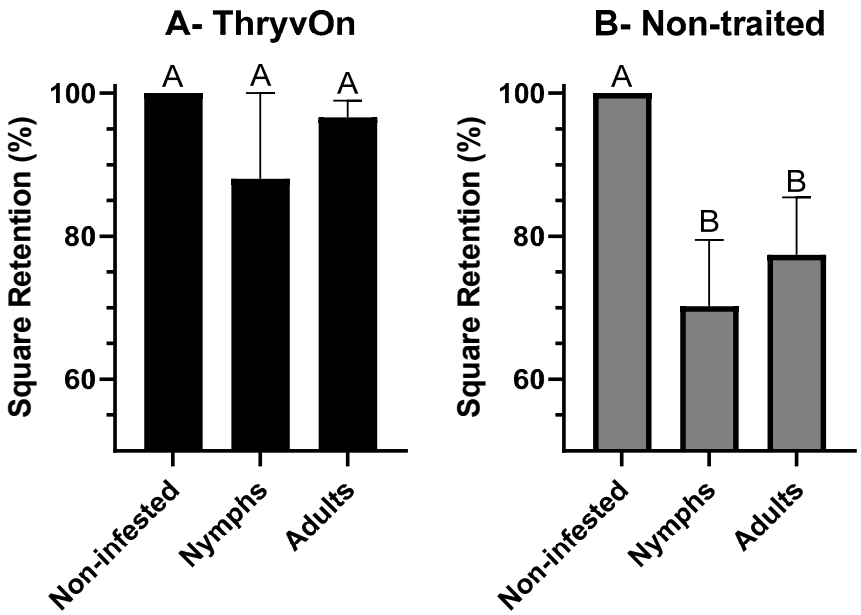

Figure 4. (**A**,**B**): No-choice assay square retention percentages (mean ± SEM) on ThryvOn and gene-lacking cotton seven days after infestation with cotton fleahopper adults or nymphs. Different letters indicate treatments that are significantly different (Benjamini, Krieger, and Yekutieli Q = 0.05).

Fourteen days after infestation, square retention on the gene-lacking/nymph-infested treatment was 60.80%, which was significantly lower ($p \leq 0.0099$) than that of the gene-lacking/adult-infested (85.40%) and gene-lacking/non-infested (100%) ones (p = 0.0005) (Figure 6B). There were no significant differences in square retention between the gene-lacking/non-infested and gene-lacking/adult-infested cotton (p = 0.0518). Similarly, the

square retention of the ThryvOn/non-infested cotton was statistically similar to that of the ThryvOn/adult-infested (p = 0.2509) and ThryvOn/nymph-infested cotton (p = 0.0713) (Figure 6A). Unlike the square retention seven days after infestation, square retention after fourteen days of infestation did not show significant differences among the life stages between the two cultivars (Figure 7A,B). The square retention of the ThryvOn/adult-infested cotton (88.17%) was similar to that of the gene-lacking/adult-infested (85.34%) cotton (p = 0.7645). The ThryvOn/nymph-infested cotton's square retention (76.08%) was not statistically different (p = 0.3144) but was numerically higher than that of the gene-lacking/nymph-infested cultivar (60.75%).

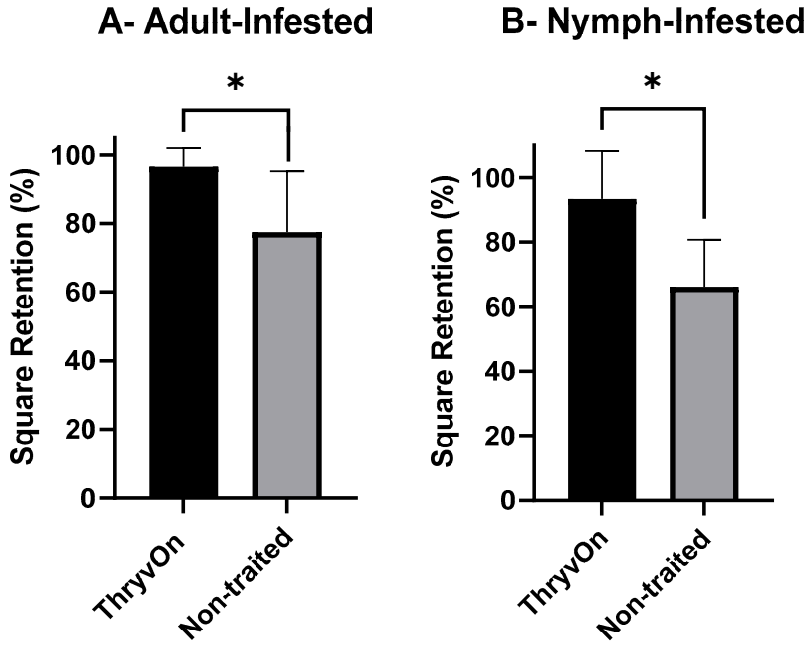

Figure 5. (**A**,**B**): No-choice assay square retention (mean ± SEM) on ThryvOn and gene-lacking cotton seven days after infestation with cotton fleahopper adults or nymphs: adult-infested (**A**) and nymph-infested cotton (**B**). Asterisks indicate treatments that are significantly different (ANOVA, Student's $p < 0.05$).

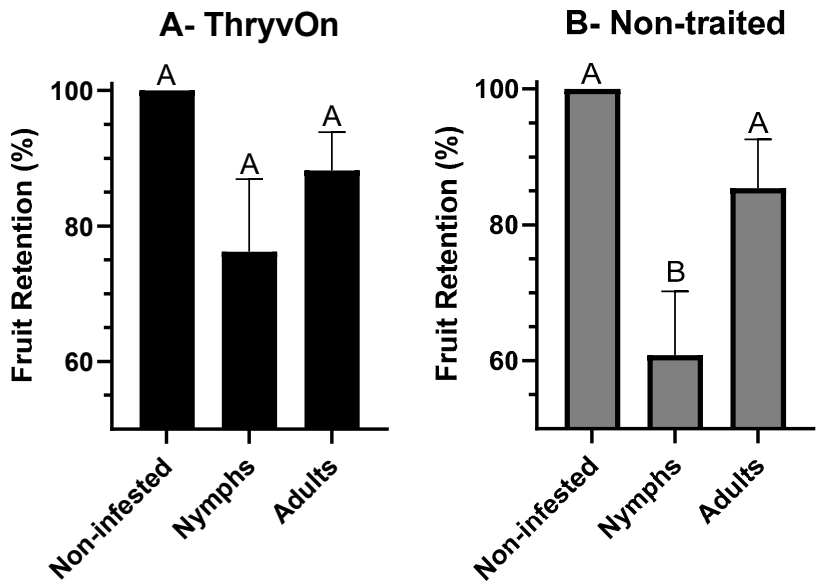

Figure 6. (**A**,**B**): No-choice assay square retention percentages (mean ± SEM) on ThryvOn and gene-lacking cotton fourteen days after infestation with cotton fleahopper adults or nymphs. Different letters indicate treatments that are significantly different (Benjamini, Krieger, and Yekutieli Q = 0.05).

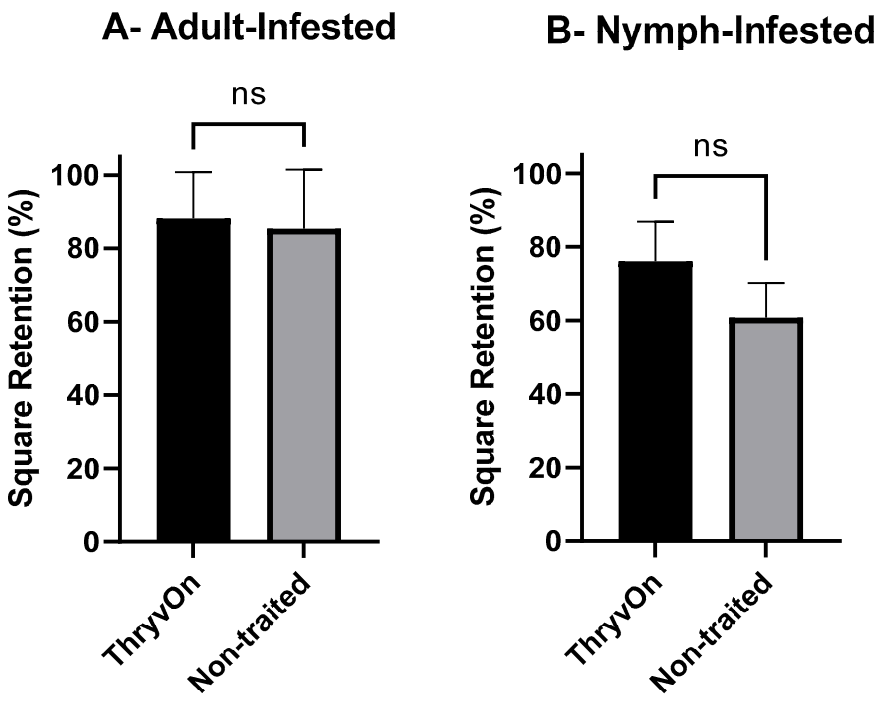

Figure 7. (**A**,**B**): No-choice assay square retention percentages (mean ± SEM) on ThryvOn and gene-lacking cotton fourteen days after infestation with cotton fleahopper adults or nymphs: adult-infested (**A**) and nymph-infested (**B**) cotton. "ns" denotes treatments are not significantly different.

4. Discussion

In this study, both cotton cultivars, with or without the Mpp51Aa2 protein, also expressed lepidopteran-active *Bt* traits Cry1Ac, Cry2Ab2, and Vip3a19. However, these *Bt* proteins are presumed to have a negligible influence on our results, as previous research has documented that Cry1Ac, Cry2Ab, and Vip3Aa are only active on lepidopteran pests but are not effective on mirids like cotton fleahoppers [16,20]. Given that the cultivars used in this study were nearly genetically identical, aside from the presence of the Mpp51Aa2 protein, any differences in feeding preference and square retention can be attributed to the presence of the Mpp51Aa2 protein in the ThryvOn cultivar. Previous work by Knutson et al. [34] showcased discernible differences in cotton fleahopper feeding preferences between cultivars through choice and no-choice feeding assays. While their study was able to identify naturally derived genes which confer tolerance to cotton fleahopper feeding, there were no plant-incorporated *Bt* proteins evaluated [34]. Utilizing similar methods, we found no significant damage to the ThryvOn cultivar compared to the non-infested control one week after the infestation. However, noticeable differences in square retention were observed in the ThryvOn cultivar after fourteen days, although still statistically higher than the square retention of the -infested/gene-lacking cultivar. This could potentially be explained by the high levels of infestation used in this study. As shown by Arthur et al. [35], cotton expressing the Mpp51Aa2 protein suffered a significant square loss when exposed to high populations of cotton fleahoppers. Previous research has consistently concluded that ThryvOn cotton can deter the feeding of various insect pests of cotton, including tobacco thrips (*Frankliniella fusca* Hinds), western flower thrips (*Frankliniella occidentalis* Pergande), onion thrips (*Thrips tabaci* Lindeman), tarnished plant bugs, western tarnished plant bugs, and cotton fleahoppers [18,36,37]. In this choice feeding assay the feeding preference of cotton fleahoppers would have been difficult to monitor as the insects could move from plant to plant. However, the differences in the square retention observed between the ThryvOn and gene-lacking cultivars would suggest a feeding preference effect towards the gene-lacking cotton consistent with the previous literature. Graham et al. [36] demonstrated that the presence of the Mpp51Aa2 protein in an artificial diet

had no significant impact on the feeding of early instar tarnished plant bug nymphs; however, adult tarnished plant bugs exhibited a preference for diet packs lacking the Mpp51Aa2 protein. In addition to the preference for a non-Bt artificial diet, the adults also favored non-Bt excised squares and oviposited significantly more eggs in the non-Bt diet packs. Bachman et al. [25] observed a substantial 19-fold decrease in tarnished plant bug large nymphs on cotton expressing Cry51Aa2 relative to a gene-lacking variety. Although square retention or evidence of feeding was not reported, Bachman et al. [25] and Asiimwe et al. [37] did observe significantly higher numbers of adult cotton fleahoppers on gene-lacking cotton relative to those found in the cotton expressing the Mpp51Aa2 protein. Cotton fleahoppers have a wide host range [14,38] so, if other more preferable hosts are available, the feeding deterrence effect brought on by ThryvOn cotton could potentially delay the onset of infestation.

Adult and nymph feeding damage on the ThryvOn cotton appeared to be negligible in the no-choice assay. These findings align with the observations by Arthur et al. [35], where significantly higher square retention was found in ThryvOn cotton compared to a gene-lacking cultivar among diverse cotton fleahopper population densities across multiple years in the field. These results suggest that, even under forced conditions, cotton fleahoppers did not cause significant damage to ThryvOn cultivars. In contrast to the ThryvOn cultivar, the gene-lacking cultivar exhibited differences both seven and fourteen days after infestation among both fleahopper life stages. A comparison of square retention between the ThryvOn and gene-lacking cultivars by cotton fleahopper life stages seven days after infestation indicated higher levels of square abscission caused by both adult and nymph cotton fleahoppers in the gene-lacking cotton. However, the square retention differences between the cultivars by cotton fleahopper life stage had equilibrated by fourteen days after infestation. Field trials assessing the efficacy of ThryvOn cotton have reported similar results that square retention was not significantly reduced by the feeding of tarnished plant bugs [21]. Additionally, they reported that damage from tarnished plant bugs in cotton with the Mpp51Aa2 trait exhibited higher square retention than the gene-lacking isoline. When integrated into a pest management system using foliar insecticides, cotton with the Mpp51Aa2 trait required fewer insecticide applications compared to a gene-lacking cultivar [22]. Similarly, Graham et al. [36] reported significantly lower numbers of adult tarnished plant bugs as well as less damage to flowers and bolls in cotton with the Mpp51Aa2 trait compared to an Mpp51Aa2 gene-lacking cultivar.

Our results also indicate there was a higher magnitude of damage occurring from nymph feeding in comparison to adults. This is likely due to the biological characteristics of the nymphs; they are unable to easily move from one host plant to another, and their objective is to feed and develop. Meanwhile, adults can move from one host plant to another quickly and are also focused on reproduction. These findings align with reports that late instar nymphs of western tarnished plant bugs have a more substantial impact on square damage [39]. Studies have shown disproportional numbers of small and large tarnished plant bug nymphs on cotton cultivars, with statistically lower numbers of late instar nymphs in Mpp51Aa2 cotton relative to Mpp51Aa2 gene-lacking cotton [22,23]. The prevalence of large nymphs corresponds to higher damage levels and reduced yields across varying infestation levels [18]. Arthur et al. [35] also reported a 2.6:1 ratio of small to large cotton fleahopper nymphs in ThryvOn cotton compared to a 1.1:1 ratio in a gene-lacking cultivar, along with increased fruit retention in the ThryvOn cultivar. Our results suggest a potential rationale for the differences in square retention percentages observed between the ThryvOn and gene-lacking cultivars: large nymphs tend to cause greater damage to cotton compared to adults. Consequently, the higher population of large nymphs in the gene-lacking cultivar likely leads to increased levels of fruit abscission.

5. Conclusions

In summary, the discovery of the Mpp51Aa2 protein has broadened the spectrum of insects controlled by transgenic cotton cultivars, effectively targeting not only chewing

insect pests but also sap-feeding insects like the cotton fleahopper. The results of our study confirm the observations of field trials where consistent benefits of improved square retention were noted in ThryvOn cotton despite the presence of mirid pests [8,21,22]. Confirmation of the activity of ThryvOn cotton in a controlled environment increases the confidence that the implementation of ThryvOn cotton into an insect pest management strategy for cotton fleahoppers could offer valuable square protection by deterring insect feeding. As concluded from other studies, the feeding deterrence of ThryvOn cotton in a field setting could reduce the necessity for foliar insecticides, offering flexibility in the timing of applications for optimal efficacy [21,23,35]. Moreover, the increased square retention observed in ThryvOn cotton, even under high infestation levels, could alleviate much of the time and labor required to optimally manage cotton fleahoppers in cotton production.

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References

1. Cook, D.R.; Threet, M.; Huff, K. 2022 Cotton Insect Losses Estimates. In Proceedings of the Beltwide Cotton Conference, New Orleans, LA, USA, 10–12 January 2023.
2. Williams, M.R. Cotton Insect Losses 2012. In Proceedings of the Beltwide Cotton Conference, San Antonio, TX, USA, 4–7 January 2013; pp. 546–586.
3. González, J.C.S.; Kerns, D.L.; Head, G.P.; Yang, F. Status of Cry1Ac and Cry2Ab2 resistance in field populations of Helicoverpa zea in Texas, USA. *Insect Sci.* **2021**, *29*, 487–495. [CrossRef] [PubMed]
4. Lu, Y.; Wu, K.; Jiang, Y.; Xia, B.; Li, P.; Feng, H.; Wyckhuys, K.A.; Guo, Y. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* **2010**, *328*, 1151–1154. [CrossRef] [PubMed]
5. Parker, R.D.; Knutson, A.; Allen, E.; Biles, S.; Kerns, D.L.; Jungman, M.J. *Managing Cotton Insects in the Southern, Eastern and Blackland Areas of Texas*; Texas A&M University: College Station, TX, USA, 2008.
6. Vyavhare, S.S.; Reed, B. Control of Cotton Fleahopper in Cotton Using Foliar Applied Insecticides, 2019. *Arthropod Manag. Tests* **2020**, *45*, tsaa090. [CrossRef]
7. McLoud, L.A.; Hague, S.; Knutson, A.; Wayne Smith, C.; Brewer, M. Cotton Square Morphology Offers New Insights into Host Plant Resistance to Cotton Fleahopper (Hemiptera: Miridae) in Upland Cotton. *J. Econ. Entomol.* **2015**, *109*, 392–398. [CrossRef] [PubMed]
8. Knutson, A.; Isaacs, S.; Campos, C.; Campos, M.; Smith, C. Resistance to Cotton Fleahopper Feeding in Primitive and Converted Race Stocks of Cotton, Gossypium hirsutum. *J. Cotton Sci.* **2014**, *18*, 385–392.
9. Walker, J.K.; Niles, G.A.; Gannaway, J.R.; Robinson, J.V.; Cowan, C.B.; Lukefahr, M.J. Cotton Fleahopper Damage to Cotton Genotypes. *J. Econ. Entomol.* **1974**, *67*, 537–542. [CrossRef]
10. Celorio-Mancera, M.d.l.P.; Carl Greve, L.; Teuber, L.R.; Labavitch, J.M. Identification of endo-and exo-polygalacturonase activity in Lygus hesperus (Knight) salivary glands. *Arch. Insect Biochem. Physiol. Publ. Collab. Entomol. Soc. Am.* **2009**, *70*, 122–135. [CrossRef]
11. Zhu, Y.C.; Zeng, F.; 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.* **2003**, *33*, 889–899. [CrossRef]
12. Brett, C.H. *The Cotton Flea Hopper in Oklahoma*; Oklahoma Agricultural Experiment Station: Stillwater OK, USA, 1946.

13. Shackel, K.A.; de la Paz Celorio-Mancera, M.; Ahmadi, H.; Greve, L.C.; Teuber, L.R.; Backus, E.A.; Labavitch, J.M. Micro-injection of Lygus salivary gland proteins to simulate feeding damage in alfalfa and cotton flowers. *Arch. Insect. Biochem. Physiol.* **2005**, *58*, 69–83. [CrossRef]
14. Esquivel, J.F.; Esquivel, S.V. Identification of Cotton Fleahopper (Hemiptera: Miridae) Host Plants in Central Texas and Compendium of Reported Hosts in the United States. *Environ. Entomol.* **2009**, *38*, 766–780. [CrossRef]
15. Martin, W.R., Jr.; Morgan, P.W.; Sterling, W.L.; Meola, R.W. Stimulation of Ethylene Production in Cotton by Salivary Enzymes of the Cotton Fleahopper (Heteroptera: Miridae). *Environ. Entomol.* **1988**, *17*, 930–935. [CrossRef]
16. Brandt, S.L.; Coudron, T.A.; Habibi, J.; Brown, G.R.; Ilagan, O.M.; Wagner, R.M.; Wright, M.K.; Backus, E.A.; Huesing, J.E. Interaction of two Bacillus thuringiensis delta-endotoxins with the digestive system of Lygus hesperus. *Curr. Microbiol.* **2004**, *48*, 1–9. [CrossRef] [PubMed]
17. Gowda, A.; Rydel, T.J.; Wollacott, A.M.; Brown, R.S.; Akbar, W.; Clark, T.L.; Flasinski, S.; Nageotte, J.R.; Read, A.C.; Shi, X.; et al. A transgenic approach for controlling Lygus in cotton. *Nat. Commun.* **2016**, *7*, 12213. [CrossRef] [PubMed]
18. Akbar, W.; Gowda, A.; Ahrens, J.E.; Stelzer, J.W.; Brown, R.S.; Bollman, S.L.; Greenplate, J.T.; Gore, J.; Catchot, A.L.; Lorenz, G.; et al. First transgenic trait for control of plant bugs and thrips in cotton. *Pest Manag. Sci.* **2019**, *75*, 867–877. [CrossRef] [PubMed]
19. Jerga, A.; Chen, D.; Zhang, C.; Fu, J.; Kouadio, J.-L.K.; Wang, Y.; Duff, S.M.; Howard, J.E.; Rydel, T.J.; Evdokimov, A.G. Mechanistic insights into the first Lygus-active β-pore forming protein. *Arch. Biochem. Biophys.* **2016**, *600*, 1–11. [CrossRef] [PubMed]
20. Baum, J.A.; Sukuru, U.R.; Penn, S.R.; Meyer, S.E.; Subbarao, S.; Shi, X.; Flasinski, S.; Heck, G.R.; Brown, R.S.; Clark, T.L. Cotton Plants Expressing a Hemipteran-Active Bacillus thuringiensis Crystal Protein Impact the Development and Survival of Lygus hesperus (Hemiptera: Miridae) Nymphs. *J. Econ. Entomol.* **2012**, *105*, 616–624. [CrossRef] [PubMed]
21. Graham, S.H.; Stewart, S.D. Field Study Investigating Cry51Aa2.834_16 in Cotton for Control of Thrips (Thysanoptera: Thripidae) and Tarnished Plant Bugs (Hemiptera: Miridae). *J. Econ. Entomol.* **2018**, *111*, 2717–2726. [CrossRef]
22. Corbin, J.C.; Towles, T.B.; Crow, W.D.; Catchot, A.L.; Cook, D.R.; Dodds, D.M.; Gore, J. Evaluation of Current Tarnished Plant Bug (Hemiptera: Miridae) Thresholds in Transgenic MON 88702 Cotton Expressing the Bt Cry51Aa2.834_16 Trait. *J. Econ. Entomol.* **2020**, *113*, 1816–1822. [CrossRef]
23. Whitfield, A. *Evaluation of Thresholds, Control, and Behavioral Responses of Tobacco Thrips, Frankliniella fusca (Hitch), and Tarnished Plant Bugs, Lygus lineolaris (Beauvoris), in ThryvOn Cotton*; University of Arkansas: Fayetteville, AR, USA, 2023.
24. Miles, P.W. The Saliva of Hemiptera. In *Advances in Insect Physiology*; Treherne, J.E., Berridge, M.J., Wigglesworth, V.B., Eds.; Academic Press: Cambridge, MA, USA, 1972; Volume 9, pp. 183–255.
25. Bachman, P.M.; Ahmad, A.; Ahrens, J.E.; Akbar, W.; Baum, J.A.; Brown, S.; Clark, T.L.; Fridley, J.M.; Gowda, A.; Greenplate, J.T.; et al. Characterization of the Activity Spectrum of MON 88702 and the Plant-Incorporated Protectant Cry51Aa2.834_16. *PLoS ONE* **2017**, *12*, e0169409. [CrossRef]
26. Gassmann, A.J.; Petzold-Maxwell, J.L.; Keweshan, R.S.; Dunbar, M.W. Field-Evolved Resistance to Bt Maize by Western Corn Rootworm. *PLoS ONE* **2011**, *6*, e22629. [CrossRef]
27. Raszick, T.J.; Suh, C.P.-C.; Dickens, C.M.; Sword, G.A. Genome-wide markers reveal temporal instability of local population genetic structure in the cotton fleahopper, Pseudatomoscelis seriatus (Hemiptera: Miridae). *Pest Manag. Sci.* **2020**, *76*, 324–332. [CrossRef] [PubMed]
28. Yang, F.; Kerns, D.L.; Brown, S.; Kurtz, R.; Dennehy, T.; Braxton, B.; Head, G.; Huang, F. Performance and cross-crop resistance of Cry1F-maize selected Spodoptera frugiperda on transgenic Bt cotton: Implications for resistance management. *Sci. Rep.* **2016**, *6*, 28059. [CrossRef] [PubMed]
29. Mekala, D.K. *Screening Upland Cotton for Resistance to Cotton Fleahopper (Heteroptera: Miridae)*; Texas A&M University: College Station, TX, USA, 2004.
30. Ritchie, G.L.; Bednarz, C.W.; Jost, P.H.; Brown, S.M. *Cotton Growth and Development*; University of Georgia: Athens, GA, USA, 2007.
31. Abbott, W.S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol* **1925**, *18*, 265–267. [CrossRef]
32. GraphPad Prism. *GraphPad Prism for Windows 64-Bit, 9.3.0*; GraphPad: Boston, MA, USA, 2021.
33. Benjamini, Y.; Krieger, A.M.; Yekutieli, D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* **2006**, *93*, 491–507. [CrossRef]
34. Knutson, A.E.; Mekala, K.D.; Smith, C.W.; Campos, C. Tolerance to Feeding Damage by Cotton Fleahopper (Hemiptera: Miridae) Among Genotypes Representing Adapted Germplasm Pools of United States Upland Cotton. *J. Econ. Entomol.* **2013**, *106*, 1045–1052. [CrossRef] [PubMed]
35. Arthur, B.P.; Suh, C.P.; McKnight, B.M.; Parajulee, M.N.; Yang, F.; Kerns, D.L. Field Evaluation of Cotton Expressing Mpp51Aa2 as a Management Tool for Cotton Fleahoppers, Pseudatomoscelis seriatus (Reuter). *Toxins* **2023**, *15*, 644. [CrossRef] [PubMed]
36. Graham, S.H.; Musser, F.M.; Jacobson, A.L.; Chitturi, A.; Catchot, B.; Stewart, S.D. Behavioral Responses of Thrips (Thysanoptera: Thripidae) and Tarnished Plant Bug (Hemiptera: Miridae) to a New Bt Toxin, Cry51Aa2.834_16 in Cotton. *J. Econ. Entomol.* **2019**, *112*, 1695–1704. [CrossRef]
37. Asiimwe, P.; Brown, C.R.; Ellsworth, P.C.; Reisig, D.D.; Bertho, L.; Jiang, C.; Schapaugh, A.; Head, G.; Burzio, L. Transgenic cotton expressing Mpp51Aa2 does not adversely impact beneficial non-target hemiptera in the field. *Crop Prot.* **2023**, *173*, 106384. [CrossRef]

38. Beerwinkle, K.R.; Marshall, H.F. Cotton Fleahopper (heteroptera:Miridae) Responses to Volatiles from Selected Host Plants . *J. Cotton Sci.* **1999**, *3*, 153–159.
39. Cooper, W.R.; Spurgeon, D.W. Feeding injury to cotton caused by Lygus hesperus (Hemiptera: Miridae) nymphs and prereproductive adults. *Environ. Entomol* **2013**, *42*, 967–972. [CrossRef]