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Potential for Grain Sorghum as a Trap and Nursery Crop for *Helicoverpa zea* and Its Natural Enemies and Dissemination of *Hear*NPV into Cotton

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Abstract: Experiments were conducted in 2020 and 2021 in College Station, TX; Stoneville, MS; and Blackville, SC, to evaluate the potential of grain sorghum to serve as a trap crop for *Helicoverpa zea* (Boddie), a nursery crop for natural enemies of *H. zea*, and a source of *Helicoverpa armigera* nucleopolyhedrovirus (*Hear*NPV) for *H. zea* management in cotton. The experiments consisted of three treatments, including cotton-only, non-treated cotton–sorghum, and *Hear*NPV-treated cotton–sorghum. Variables, including percent injury to fruiting forms, parasitized *H. zea* larvae, egg density, *H. zea* larval density, beneficial arthropod numbers, and *Hear*NPV prevalence, were compared between the treatments. Growing cotton in an intercropping system with grain sorghum did not result in a consistent increase in *H. zea* control and beneficial arthropod density relative to the cotton-only treatment. Additionally, our results did not show sufficient evidence that grain sorghum interplanted with cotton can serve as a source of *Hear*NPV that can favor *H. zea* control in cotton. However, we found that, if maintained in the cotton canopy, *Hear*NPV may favor some level of *H. zea* suppression in cotton. Based on our *Hear*NPV infection analyses using PCR, chrysopids, coccinellids, pentatomids, reduviids, formicids, anthocorids, and spiders appeared to be carrying *Hear*NPV. The virus was detected consistently in specimens of coccinellids, pentatomids, and reduviids across both years of the study. We suggest that further investigation on virus efficacy against *H. zea* in cotton using the sorghum–cotton system as well as the ability of grain sorghum to serve as a *H. zea* trap crop and source of *H. zea* natural enemies be considered in future studies.

Keywords: *Helicoverpa zea*; *Hear*NPV; IPM; biological insecticide; biological control

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1. Introduction

The introduction and widespread adoption of genetically modified corn, *Zea mays* L. (Poales: Poaceae) and upland cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae), producing *Bacillus thuringiensis* (*Bt*) (Bacillales: Bacillaceae) proteins has resulted in effective *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) control while causing marginal to no harm to non-target organisms [1,2]. However, with the occurrence of resistance in *H. zea* to one or more *Bt* proteins, remedial foliar insecticide applications are often required to prevent unacceptable injury in *Bt* cotton [3–10]. Due to widespread issues with pyrethroid resistance, insecticides containing chlorantraniliprole are the primary means for managing

H. zea in cotton in the U.S. [7,11,12]. Currently, reports of field-evolved resistance of lepidopteran pests to chlorantraniliprole are numerous [13]. To date, no chlorantraniliprole resistance has been reported for *H. zea*; however, because of the heavy reliance on this insecticide for *H. zea* management in cotton, grain sorghum, soybean, and other crops, there is concern that resistance may develop [14–17]. Thus, it is best to be proactive and develop additional management tactics targeting *H. zea* in cotton.

The adoption of intercropping, a type of sustainable agriculture practice, has demonstrated utility for insect pest management. Intercropping entails the simultaneous cultivation of two or more companion crop species in one field [18]. The companion crops may serve as repellents, trap crops, and/or natural enemy recruiters [19–22]. This ecosystem service provided by the intercropping system may promote insect pest suppression in the main crop, thus reducing or delaying the need for insecticide applications [23–26]. An intercropping system aimed at trap cropping involves cultivating a crop of interest simultaneously with another or several other crops that are more preferred by the pests of concern; this favors the diversion of the pest from the main crop. The adoption of this system has resulted in the successful management of multiple key pests in several economic crops, including *H. zea* in cotton [24,27–29].

Results from studies conducted in various regions of the world have demonstrated that grain sorghum, *Sorghum bicolor* L. Moench (Poales: Poaceae), may serve as an effective diversionary trap crop for *H. zea* and *Helicoverpa armigera* (*Hübner*) (Lepidoptera: Noctuidae) from cotton and as a source of *H. zea* natural enemies [24,30]. Thus, the implementation of an intercropping system of cotton with grain sorghum may divert *H. zea* from cotton to grain sorghum, while being an insectary for natural enemies that may disperse from the grain sorghum into the cotton for additional biological control of the pest [21].

Previous studies demonstrated that grain sorghum may also be an effective source for *Helicoverpa armigera* nucleopolyhedrovirus (*Hear*NPV) dissemination to cotton [24,30–32]. *Hear*NPV is a viral pesticide that is specific to Heliothines, including *H. zea* [33]. In the U.S., *Hear*NPV has demonstrated high efficacy for *H. zea* management in soybean, *Glycine max* (L.) Merr. (Fabales: Fabaceae) [34]. In soybean, *Hear*NPV is very persistent in the canopy [35], but in cotton, *Hea*rNPV persistence has not been sustained. This lack of persistence is thought to be primarily due to the high pH of the dew on cotton leaves, resulting in virus deactivation as the dew dries [36–38]. Initial *Hear*NPV infection of *H. zea* larvae in cotton is possible; however, it is unlikely an epizootic event will persist. Thus, the challenge of effectively integrating *Hear*NPV into cotton IPM is to devise a system where an epizootic nursery reservoir of *Hear*NPV can be initiated for persistent horizontal biotic and/or abiotic transmission into cotton.

This study aimed to evaluate the potential for utilizing grain sorghum as a trap crop for *H. zea* and a nursery crop for the natural enemies of *H. zea*. The study also assessed the potential for utilizing grain sorghum as a nursery crop for *Hear*NPV dissemination into the cotton canopy to manage *H. zea*.

2. Materials and Methods

2.1. Locations, Experimental Design, and Treatments

These experiments were performed at three distinct geographical and environmental locations that are representative of the southern U.S. Cotton Belt. The locations included College Station, TX (30.53741° N, 96.41988° W); Stoneville, MS (33.41253° N, 90.90761° W); and Blackville, SC (33.349397° N, 81.305996° W). Experiments were conducted over two years, with the first year (2020) serving as a proof-of-concept experiment and the second year (2021) serving as a validation experiment. The cotton used in these experiments was a non-Bt variety, DP 1822 XF (Bayer CropScience LP, St. Louis, MO, USA). The grain sorghum used consisted of equal blends of seed from six hybrids with different levels of maturity (Table 1, S&W Seed Company, Longmont, CO, USA). The seed was blended to extend the bloom period of the planted area to approximately 21 days to extend the attractiveness of the grain sorghum to ovipositing *H. zea*.

Table 1. Grain sorghum hybrids utilized.

Sorghum Hybrid	Minimum Days to 50% Bloom	Maximum Days to 50% Bloom
SP 78M30	72	76
SP 74M21	69	74
SP 68M57	66	71
SP 31A15	54	58
SP 43M80	58	62
251	50	54

2.2. Proof-of-Concept Experiment

This experiment was conducted in 2020 and consisted of three treatments at each location. The three fields were separated from one another by at least 0.5 km to avoid unintended spread of *Hear*NPV from one field to another. Two fields consisted of replicated (four each) alternating eight-rows-wide strips of grain sorghum or cotton (with a row spacing of 0.97–1.02 m) 61 m long. The third field consisted of a solid cotton block of 64 rows wide, with the same row spacing and length used in the interplanted fields. Each geographic location served as a field replicate. Grain sorghum was planted 7–10 days after planting cotton to closely time the expected first week of bloom of the cotton with the bloom of the earliest maturing grain sorghum hybrid.

All three fields and crops were grown using standard production practices, but they were not treated with insecticides that would harm *H. zea*. In one of the interplanted fields, the blooming grain sorghum was treated with *Hear*NPV (Heligen®, AgBiTech, Fort Worth, TX, USA) at 0.1 L/ha targeting the 1st and 2nd instar *H. zea* larvae. The treatment was applied by ground using a high-clearance sprayer calibrated to deliver a spray volume of 93.54 L/ha. The interplanted non-treated field served as a non-*Hear*NPV comparison. The cotton-only field served as a non-sorghum comparative treatment, allowing evaluation of the effectiveness of grain sorghum as a *H. zea* trap crop and natural enemy nursery. Pre-treatment data and samples were collected from all fields before the *Hear*NPV application and at 7, 14, and 21 days post-application.

Beneficial arthropods and *H. zea* larvae were sampled from the grain sorghum using the beat-bucket method [39]. Four locations within each replicate were sampled. At each location, 25 heads were sampled (100 heads total per replicate) by bending the sorghum panicle into a 2.5-gallon bucket and vigorously shaking it against the bucket walls to dislodge *H. zea* larvae and beneficial arthropods. Samples were collected into 1-gallon plastic bags and returned to the laboratory for counting. The number of *H. zea* larvae was recorded and sized as small (1st and 2nd instar) or large (3rd, 4th, and 5th instar). Beneficial arthropods were identified into families and counted. Samples of *H. zea* and beneficial arthropods (pooled by family) were stored at −80 °C until they were evaluated for *Hear*NPV infection utilizing polymerase chain reaction (PCR). An additional beat-bucket sample of *H. zea* larvae from 100 sorghum heads was collected from each sorghum replicate. When available, ≥3rd instar *H. zea* larvae from this sample were collected into 29 mL Solo condiment cups (Dart Container Corporation, Mason, MI, USA) containing laboratory-based meridic diet (WARD'S Stonefly Heliothis diet, Rochester, NY, USA). Collected larvae were transported to the laboratory and held for parasitoid emergence and identification.

Cotton within the cotton–sorghum interplanting and cotton-only fields was sampled using three methods: visual sampling, beat-bucket sampling, and drop-cloth sampling. The visual sampling method was primarily aimed at detecting eggs and injured fruiting forms, and the drop-cloth method was used to collect *H. zea* larvae used to determine *Hear*NPV infection and parasitism rates of *H. zea*. For the visual sampling method, each replicated strip was sampled by inspecting 25 individual plants using the method described by Calvin et al. [15]. For each plant, the terminal was inspected for evidence of *H. zea* feeding and the presence of *H. zea* larvae. Four (two small from the upper [top five nodes] canopy and two larger and lower) squares were sampled from each plant for evidence of injury and the presence of *H. zea* larvae. Four (two small [approximately 1 cm in diameter] with bloom

tags [dried/attached blossoms] and two larger [approximately 2.0–2.5 cm in diameter] without bloom tags) bolls were sampled on each plant for injury and larvae. Injury to squares and bolls was recorded as positive only when the outer tissue was penetrated, when the fruit-feeding injury would result in square abortion, or when the carpel wall of the boll was penetrated. The size of each *H. zea* larvae for all sampling was recorded as small or large. Additionally, when inspecting the various plant structures, the number of Heliothine eggs was recorded for each plant.

Predators within the cotton plots were sampled using a beat bucket as described by Knutson et al. [40]. A 5-gallon bucket was held at a 45° angle to the ground and the sample plants were grasped near the base and quickly bent into the bucket. Ten beat-bucket samples per replicated cotton strip were taken, with three plants sampled per beat bucket. The plants were rapidly beaten against the inside of the bucket 12–16 times for 3–4 s, and then were removed from the bucket. The leaves and fruiting forms that remained in the bucket and the dislodged predators were collected in 1-gallon plastic bags and transported to the laboratory for identification and counting. Leaves and fruiting forms dislodged were examined for predators. Additionally, four drop-cloth samples were collected per replicated strip of cotton. Black drop-cloths of 0.97 m long by 0.76 m wide were utilized. Approximately 1.5 m of cotton was vigorously shaken, causing *H. zea* to dislodge and drop onto the drop-cloth. Dislodged fruits and leaves were examined for the presence of *H. zea* larvae. The $\geq$ 3rd instar *H. zea* larvae from one-half of the larvae collected from each replicated strip were collected into 29 mL Solo condiment cups containing laboratory-based meridic diet; these larvae were transported to the laboratory and allowed to develop to estimate parasitism. The other half of each sample and the collected predators were pooled and stored at −80 °C. These samples were then analyzed to estimate *Hear*NPV presence using polymerase chain reaction (PCR). When the number of *H. zea* larvae collected in a sample was low, all larvae collected were submitted for PCR analysis. Throughout the sampling period, precautions were taken to minimize anthropogenic dispersal of *Hear*NPV. Samples were taken in the untreated field first, and then in the *Hear*NPV treated field, starting from the furthest to the closest transect to the sorghum block at each date. Additionally, sampling tools were dedicated and labeled for each treatment.

2.3. Validation Experiment

In 2021, the validation experiment was conducted similarly to the proof-of-concept experiment, but instead of the grain sorghum being interplanted with the cotton, it was planted on the edge of the field to simulate a practical means of implementation for growers. At each location, three approximately 2.0 ha blocks of cotton were utilized, with the blocks being separated from one another by at least 0.5 km. Two of the fields were bordered on the predominantly upwind side, with 8–12 rows of grain sorghum blended with 6 varied maturity hybrids (Table 1). Sorghum was planted 7–10 days after planting cotton to synchronize the bloom of the earliest maturing sorghum with the first week of bloom of the cotton. Planting the sorghum upwind from the cotton minimized the potential for herbicide drift from the cotton into the sorghum and maximized the potential for arthropods and *Hear*NPV dispersal from the sorghum into the cotton. Each geographic location served as a field replicate. Both crops were grown using standard production practices but were not treated with insecticides that would harm *H. zea*. The blooming sorghum in one of the cotton–sorghum fields was treated with *Hear*NPV (Heligen®, AgBiTech, Fort Worth, TX, USA) at a rate of 0.1 L/ha, targeting the 1st and 2nd instar larvae. The treatment was applied using a high-clearance sprayer calibrated to deliver a spray volume of 93.54 L/ha. The untreated field bordered with sorghum served as a non-*Hear*NPV comparison. The cotton-only field served as a non-sorghum treatment to evaluate the effectiveness of grain sorghum as a *H. zea* trap crop and natural enemy nursery. Pre-treatment data and samples were collected from all fields before the *Hear*NPV application and at 7, 14, and 21 days post-application.

Sorghum was sampled as described in the proof-of-concept experiment. Four locations, with 25 sorghum heads per location, were sampled within the sorghum. As previously described, *H. zea* larvae and beneficial arthropod density were determined for each sample date. In both the cotton-only and cotton bordered by sorghum fields, the cotton was sampled based on replicated transects originating from the sorghum planting or the edge of the predominant upwind edge for the cotton-only planting. Each field was divided into equally spaced grids and the transects were divided into 4 equally spaced transects along those grids (Figure 1). Data were collected along each transect at 7.6, 15.2, 30.5, 61.0, and 91.4 m. At each transect location, 10 plants were visually sampled, and 5 beat-bucket and 2 drop-cloth samples were taken as previously described. As in the proof-of-concept experiment, percent injury to fruiting forms, eggs, *H. zea* larvae, predators, percent parasitism of larvae, and *Hear*NPV infection were determined for each sample transect distance by replicate and sample date. Data were collected and samples were processed as previously described in the proof-of-concept experiment. Precautions, as described previously, were taken to minimize anthropogenic dispersal of *Hear*NPV.

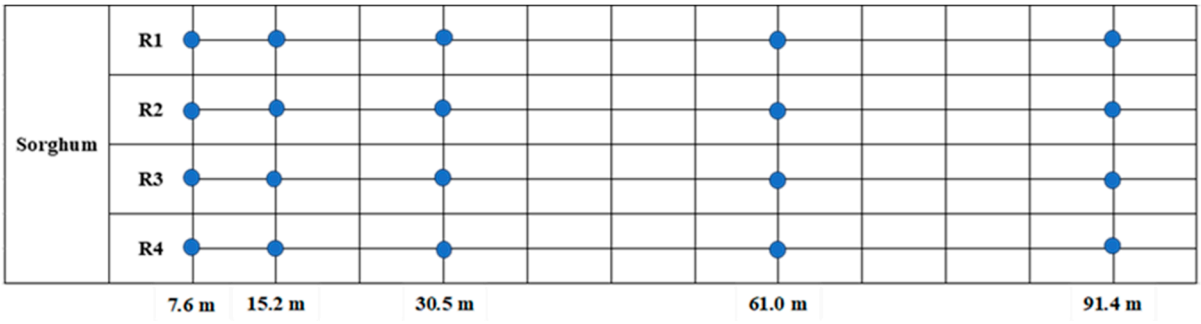

Figure 1. Distribution of transect locations across cotton fields. R1 to R4 = transect replicates 1–4. The dots represent the transect locations along each transect replicate.

2.4. HearNPV Infection Analysis

*Hear*NPV infection of *H. zea* larvae was determined using methods described by Black et al. (2019). For each sample, *Hear*NPV occlusion bodies were purified and extracted, and the DNA was subsequently separated and extracted utilizing a DNA extraction kit (DNeasy Blood and Tissue Kit: Qiagen, Germantown, MD, USA). Extracted DNA was amplified with HearNPV polyhedrin-specific primers HzSpolh-2F (5′-CCCTACTTTGGGCAAAACC-3′) and HzSpolh-2R (5′-TCGGTTTGGTTGGTCGCATA-3′) (IDT, Coralville, IA) using a Veriti™ 96-Well Thermal Cycler (Applied Biosystem, Foster City, CA, USA). A volume of 50 µL of PCR mixture was used and consisted of 1 µL extracted DNA sample, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM each primer, 1× GoTaq Flexi Buffer, and 1.25 U of GoTaq DNA polymerase (Promega, Madison, WI, USA). To confirm the effective amplification of the target gene, a positive control and a negative control consisting of *Hear*NPV and deionized water, respectively, were included in each individual thermocycler run. Once amplified, samples were visualized using a 4200 TapeStation with D1000 ScreenTape Assay (Agilent Technologies, Inc., Waldbronn, Germany) for *Hear*NPV confirmation. *Hear*NPV presence was confirmed when a band was present at 400 base pairs (bp). For the *Hear*NPV-positive samples, PCR products were sequenced (Eurofins, Louisville, KY, USA) to confirm the *Hear*NPV polyhedron sequence.

2.5. Statistical Analyses

For the proof-of-concept experiment, the percent injury to fruiting forms, percent parasitized larvae, percent *H. zea* eggs, percent large larvae, percent small larvae, and number of beneficial arthropods were compared between treatments using a multiple Student's *t*-test [41]. For the validation experiment, the percent injury to fruiting forms, number of beneficial arthropods, percent large larvae, and percent small larvae were

compared between treatments and between distances within the treatment using a multiple Student's *t*-test [41]. To compare the virus detection frequency between treatments, the Kruskal–Wallis test [41] was performed. The percent injury to fruiting forms was calculated based on 100 fruiting forms; the percent parasitized larvae was determined based on the proportion of larvae that were parasitized; and the percent *H. zea* eggs, percent small larvae, and percent large larvae were calculated based on the number of eggs, small larvae, and large larvae per 100 plants.

3. Results

3.1. Proof-of-Concept Experiment

Comparing Damaged Fruit Incidence, *H. zea* Larvae, Eggs, Beneficial Arthropod Density, and Parasitized Larvae Occurrence between Cotton-Only, Non-Treated Cotton–Sorghum, and *Hear*NPV-Treated Cotton–Sorghum.

When the cotton-only treatment was compared with the non-treated cotton–sorghum for *H. zea* parameters, no significant differences were detected for the percent injury to fruiting forms (t = 1.42, df = 76.806, p = 0.1591), percentage of eggs (t = 1.48, df = 64.723, p = 0.1435), percentage of small larvae (t = 0.89, df = 86, p = 0.3781), or percentage of large larvae (t = 0.86, df = 75.8, p = 0.3942). Additionally, there were no differences detected for the number of beneficial arthropods (t = −1.07, df = 86, p = 0.288). However, significant differences were detected in the percentage of parasitized *H. zea* larvae, with cotton-only exhibiting a greater incidence of parasitized larvae (Figure 2f; t = 2.03, df = 43, p = 0.0484).

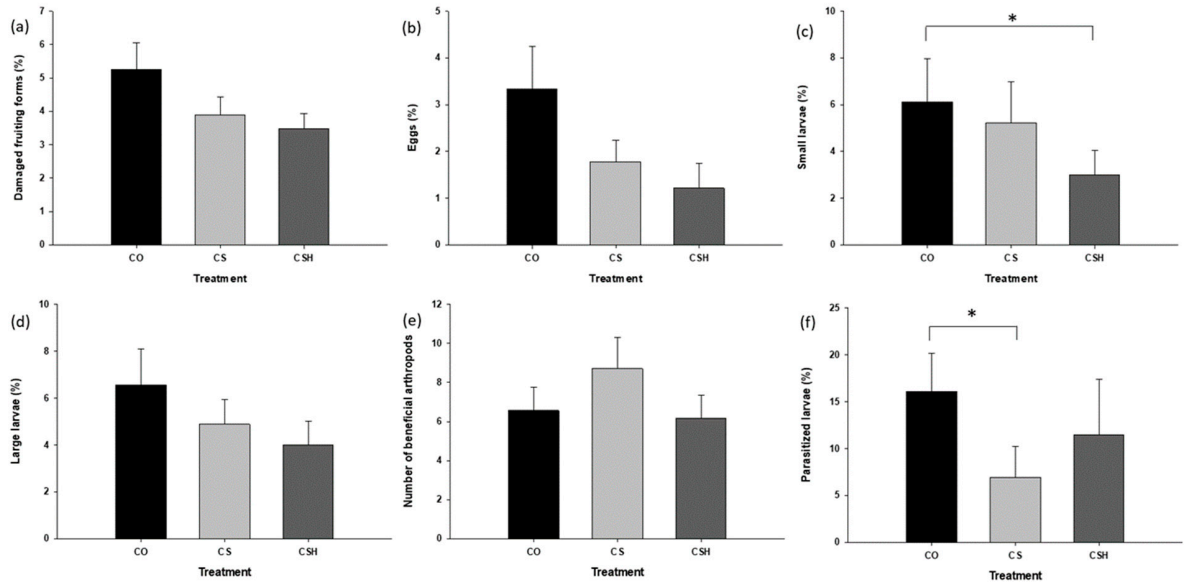

Figure 2. Means (±SE) for percentage of injured fruiting forms (**a**), eggs (**b**), percentage of small larvae (**c**), percentage of large larvae (**d**), number of beneficial arthropods (**e**), and percentage parasitized larvae (**f**) as affected by grain sorghum and *Hear*NPV in 2020. CO = cotton only, CS = cotton intercropped with grain sorghum, and CSH = cotton intercropped with grain sorghum treated with *Hear*NPV. The asterisks indicate the comparisons were significantly different ($p \leq 0.05$).

When the cotton-only treatment was compared with the *Hear*NPV-treated cotton–sorghum for *H. zea* parameters, no significant differences were detected in the percentage of injured fruiting forms (t = 1.88, df = 69.031, p = 0.0642), percentage of eggs (t = 1.45, df = 78.588, p = 0.1521), or the percentage of large larvae (t = 1.39, df = 73.512, p = 0.1676). There were also no differences detected in the number of beneficial arthropods (t = 0.23, df = 86, p = 0.8165) or the percentage of parasitized *H. zea* larvae (t = 0.82, df = 37, p = 0.4179). Significant differences were detected for the percentage of small *H. zea* larvae (t = 2.18, df = 63.927, p = 0.0328), with cotton-only exhibiting a greater incidence (Figure 2c).

The non-treated cotton–sorghum did not differ from the *Hear*NPV-treated cotton–sorghum in the percent injury to fruiting forms (t = 0.49, df = 86, p = 0.6278), percentage of eggs (t = 0.13, df = 77.868, p = 0.8944), percentage of small larvae (t = 1.27, df = 69.662, p = 0.2072), percentage of large larvae (t = 0.66, df = 86, p = 0.509), number of beneficial arthropods (t = 1.28, df = 78.158, p = 0.2057), or percentage of parasitized larvae (t = −0.77, df = 40, p = 0.4449).

3.2. Validation Experiment

3.2.1. Comparing Damaged Fruit Incidence, *H. zea* Larvae, and Beneficial Arthropod Density between Cotton-Only, Non-Treated Cotton–Sorghum, and *Hear*NPV-Treated Cotton–Sorghum

For this experiment, the percentages of eggs and parasitized larvae were not evaluated due to the incompleteness of the data for these variables. Significant differences between the cotton-only and non-treated cotton–sorghum were not observed for the percent injury to fruiting forms by *H. zea* (t = −0.56, df = 390.42, p = 0.5792), percentage of small larvae (t = −0.92, df = 398, p = 0.3585), percentage of large larvae (t = 1.53, df = 398, p = 0.1261), or the number of beneficial arthropods (t = 1.08, df = 332.67, p = 0.2826).

The cotton-only plots had significantly fewer injured fruiting forms (Figure 3a; t = −2.76, df = 398, p = 0.006) and small larvae (Figure 3b; t = −3.01, df = 361.55, p = 0.0028) than cotton from the *Hear*NPV-treated cotton–sorghum plots. However, the *Hear*NPV-treated cotton–sorghum plots resulted in a greater number of beneficial arthropods in the cotton (Figure 3d; t = −2.04, df = 396.6, p = 0.0416). There was no significant difference between the two treatments for the percentage of large larvae (t = −0.25, df = 398, p = 0.8024).

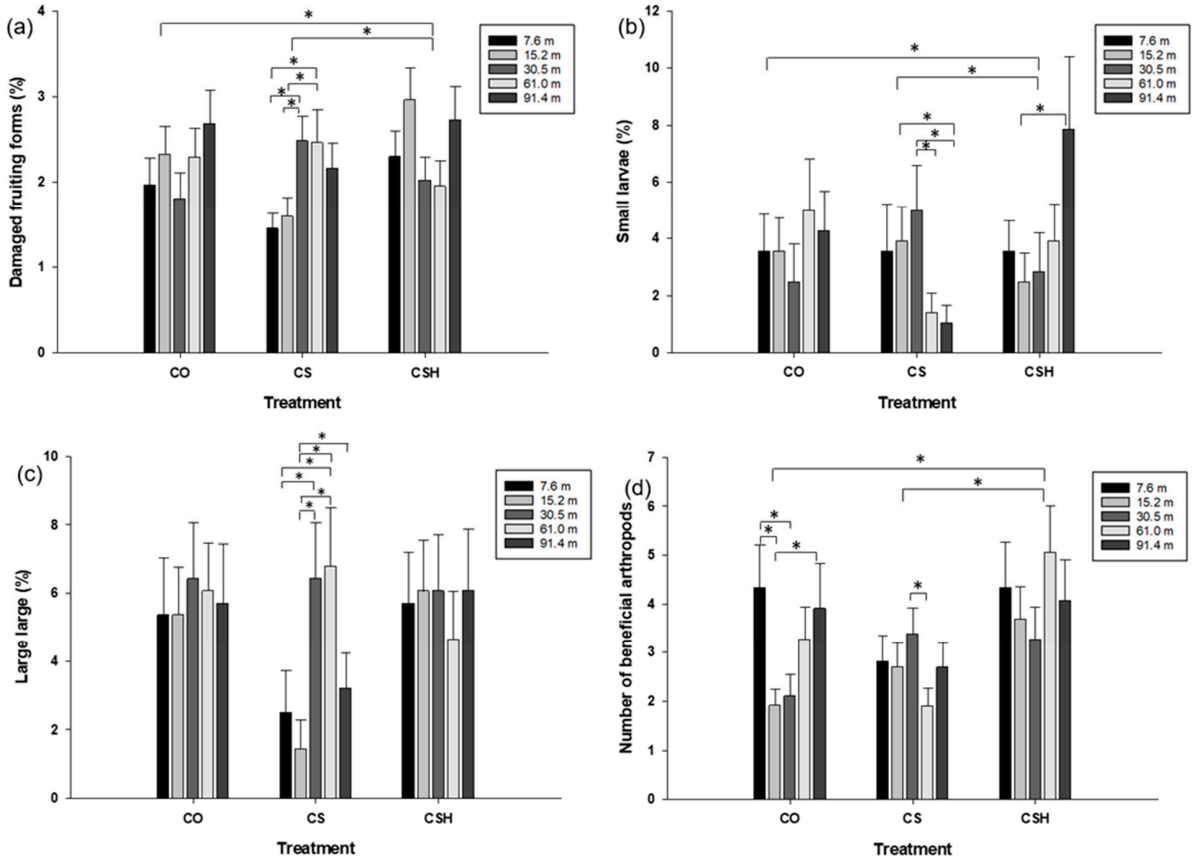

Figure 3. Means (±SE) for percentage of damaged fruiting forms (**a**), percentage of small larvae (**b**), percentage of large larvae (**c**), and number of beneficial arthropods (**d**) between paired treatments and between paired distance within treatment affected by grain sorghum and *Hear*NPV in 2021. CO = cotton only, CS = cotton intercropped with grain sorghum, and CSH = cotton intercropped with grain sorghum treated with *Hear*NPV. The asterisks indicate the comparisons were significantly different ($p \leq 0.05$).

*Hear*NPV-treated cotton–sorghum resulted in a significantly greater number of injured fruiting forms (Figure 3a; $t = -2.39$, df = 398, $p = 0.0174$) and small larvae (Figure 3b; $t = -2.23$, df = 365.84, $p = 0.0265$), as well as a greater number of beneficial arthropods (Figure 3d; $t = -3.27$, df = 357.57, $p = 0.0012$) than the non-treated cotton–sorghum, but the two treatments did not differ in large larvae incidence (Figure 3c; $t = -1.78$, df = 398, $p = 0.0766$).

3.2.2. Comparing Damaged Fruit Incidence, *H. zea* Larvae, and Beneficial Arthropod Density between Transect Locations within Cotton-Only, Non-Treated Cotton–Sorghum, and *Hear*NPV-Treated Cotton–Sorghum

Within the cotton-only field, there was no difference between any of the distances for injured fruiting forms, small larvae, or large larvae ($p > 0.05$; Figure 3a–c). However, beneficial arthropod incidence was statistically greater at 7.6 m from the grain sorghum than at 15.2 and 30.5 m and significantly greater at 91.4 m than at 15.2 m ($p < 0.05$; Figure 3d).

Within the non-treated cotton–sorghum, a lower incidence of injured fruiting forms was observed in cotton at 7.6 and 15.2 m than at 30.5 m; fewer injured fruiting forms were found at 7.6 and 15.2 m than at 61.0 m ($p < 0.05$; Figure 3a). Fewer small *H. zea* larvae were detected at 61.0 m than at 15.2 or 30.5 m, and the 30.5 m distance exhibited a greater incidence of small larvae ($p < 0.05$; Figure 3b). Fewer large larvae were observed at 7.6 m than at 30.5 and 61.0 m, and significantly fewer large larvae were found at 15.2 m than at 30.5, 61.0, and 91.4 m from the grain sorghum ($p < 0.05$; Figure 3c). Significantly more beneficial arthropods were detected at 30.5 m than at 61.0 m ($p < 0.05$; Figure 3d).

Within the *Hear*NPV-treated cotton–sorghum, none of the distances differed in the number of injured fruiting forms, large larvae, or beneficial arthropods ($p > 0.05$; Figure 3a–c). However, significantly fewer small larvae were observed at 15.2 m than at 91 m from the grain sorghum ($p < 0.05$; Figure 3b).

3.3. Beneficial Arthropods Observed

A variety of predators and parasitoids of *H. zea* were observed in the cotton during both years of the study (Table 2). Minute pirate bugs (Hemiptera: Anthocoridae), fire ants (Hymenoptera: Formicidae), lady beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae, Hemerobiidae), cotton fleahoppers (Hemiptera: Miridae), big-eyed bugs (Hemiptera: Geocoridae), and spiders (Araneae: Thomisidae, Salticidae, Araneidae, and Oxyopidae) were the most common predators. Tachinid flies (Diptera: Tachinidae) and braconid wasps (Hymenoptera: Braconidae) were the most abundant parasitoids.

Table 2. Beneficial arthropods that occurred in cotton and grain sorghum in 2020 and 2021.

Order	Family	Common Name	Benefit
Araneae	Thomisidae	Crab spider	Predator
	Salticidae	Jumping spider	Predator
	Araneidae	Orb-weaver spider	Predator
	Oxyopidae	Lynx spider	Predator
Coleoptera	Coccinellidae	Lady beetle	Predator
Diptera	Syrphidae	Hoverfly	Predator
	Tachinidae	Tachinid fly	Parasitoid
Hemiptera	Pentatomidae	Spined soldier bug	Predator
	Reduviidae	Assassin bug	Predator
	Geocoridae	Big-eyed bug	Predator
	Anthocoridae	Minute pirate bug	Predator
	Miridae	Cotton fleahopper	Predator
	Nabidae	Damsel bug species	Predator
Hymenoptera	Formicidae	Fire ant	Predator
	Braconidae	Braconid wasp	Parasitoid
Neuroptera	Chrysopidae	Green lacewing	Predator
	Hemerobiidae	Brown lacewing	Predator

3.4. HearNPV Infection Analysis

3.4.1. *Helicoverpa zea* Samples

In 2020, *Hear*NPV was not detected in the *H. zea* samples collected from pre-treated cotton of any treatment. However, the virus was detected in *H. zea* samples collected throughout the subsequent sampling dates for all treatments (Figure 4a). Based on the Kruskal–Wallis test results, there was a difference in the *Hear*NPV prevalence between the cotton-only and non-treated cotton–sorghum (χ^2 = 3.8571, df = 1, p = 0.0495), with cotton-only having a greater prevalence of *Hear*NPV (Figure 4b). Additionally, there was a significant difference between the non-treated cotton–sorghum and *Hear*NPV-treated cotton–sorghum (χ^2 = 3.8571, df = 1, p = 0.0495), with the *Hear*NPV-treated cotton–sorghum exhibiting greater incidence of *Hear*NPV (Figure 4b). There was no statistical difference in virus detection in *H. zea* between the cotton-only and *Hear*NPV-treated cotton–sorghum (χ^2 = 0, df = 1, p = 1).

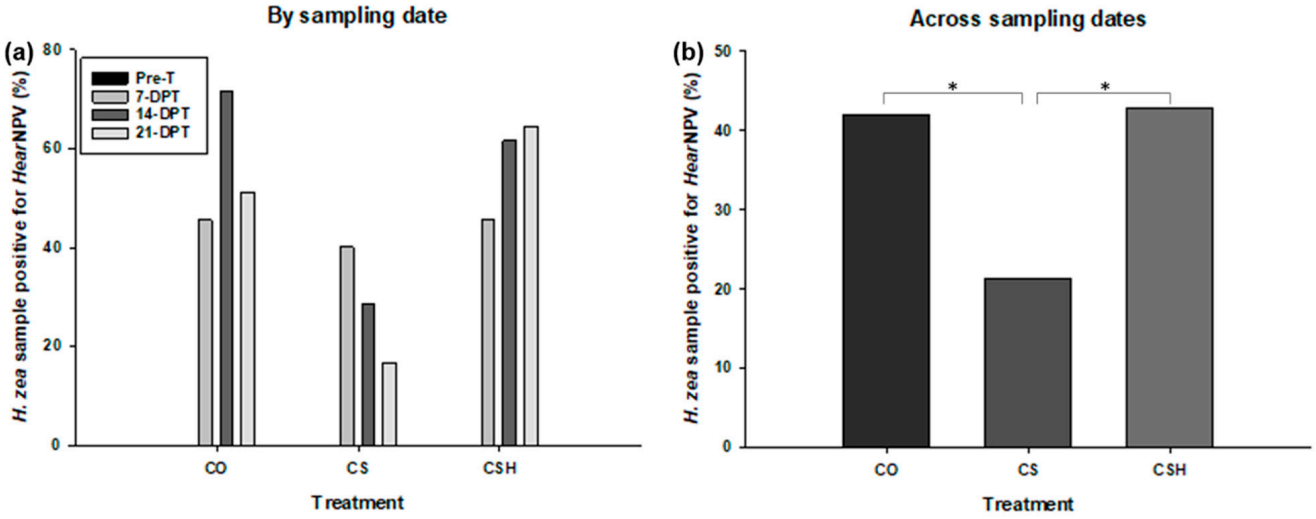

Figure 4. Percentage of *H. zea* samples that tested positive for *Hear*NPV in 2020. CO = cotton only, CS = cotton intercropped with grain sorghum, and CSH = cotton intercropped with grain sorghum treated with *Hear*NPV. Only data across sampling dates were considered for statistical analysis. The asterisks indicate the comparisons were significantly different ($p \leq 0.05$).

In 2021, *Hear*NPV was detected in *H. zea* samples collected from cotton at all sampling dates for both treated and non-treated cotton–sorghum. Additionally, throughout the subsequent sampling dates, the virus was detected in *H. zea* samples collected from both fields and across most distance locations, except at 91.4 m from the *Hear*NPV-treated grain sorghum. However, *Hear*NPV was not detected in any *H. zea* samples collected from the cotton-only field (Figure 5a). We observed a statistical difference in *Hear*NPV frequency between the cotton-only and non-treated cotton–sorghum (χ^2 = 7.8125, df = 1, p = 0.0052), with non-treated cotton–sorghum exhibiting greater *Hear*NPV incidence (Figure 5b). Additionally, there was a significant difference between the non-treated cotton–sorghum and *Hear*NPV-treated cotton–sorghum (χ^2 = 6.9018, df = 1, p = 0.0086), with the non-treated cotton–sorghum exhibiting greater *Hear*NPV incidence (Figure 5b). No statistical difference between the cotton-only and *Hear*NPV-treated cotton–sorghum was observed (χ^2 = 3.7156, df = 1, p = 0.0539).

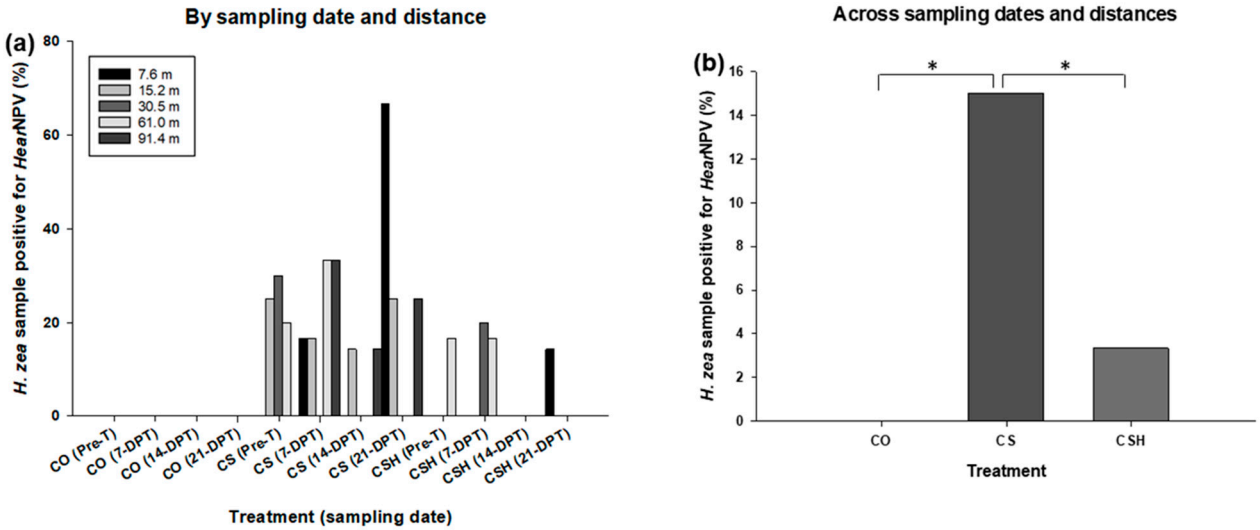

Figure 5. Percentage of *H. zea* samples that tested positive for *Hear*NPV in 2021. CO = cotton only, CS = cotton intercropped with grain sorghum, CSH = cotton intercropped with grain sorghum treated with *Hear*NPV, Pre-T = pre-treatment, and DPT = days post-treatment. Only data across sampling dates and distances were considered for statistical analysis. The asterisks indicate the comparisons were significantly different ($p \leq 0.05$).

3.4.2. Beneficial Arthropod Samples

In 2020, none of the beneficial arthropod samples collected from the cotton-only and non-treated cotton–sorghum fields were positive for *Hear*NPV, while the virus was detected in seven samples collected from the treated cotton–sorghum treatment. Arthropods in the families Chrysopidae, Coccinellidae, Pentatomidae, and Reduviidae were the only arthropod groups that appeared to be carriers for *Hear*NPV (Table 3). In 2021, the virus was detected in beneficial arthropod samples collected from both the treated and non-treated cotton–sorghum fields. The arthropod groups that carried the virus included spiders (Thomisidae, Salticidae, Araneidae, and Oxyopidae), Formicidae, Anthocoridae, Reduviidae, Coccinellidae, and Pentatomidae. Coccinellids, pentatomids, and reduviids were the only arthropod groups in which the virus was detected consistently in both years of the study (Table 3).

Table 3. Beneficial arthropods that tested positive for *Hear*NPV in 2020 and 2021.

Year	Arthropod Groups	n [a]	No. Positive Sample	% Positive Sample	n [a]	No. Positive Sample	% Positive Sample	n [a]	No. Positive Sample	% Positive Sample
		Cotton-Only			Non-Treated Cotton–Sorghum			Treated Cotton–Sorghum		
2020	Chrysopidae	5	0	0	15	0	0	31	4	12.9
	Coccinellidae	10	0	0	28	0	0	52	1	1.9
	Pentatomidae	0	0	0	1	0	0	4	1	25
	Reduviidae	0	0	0	1	0	0	2	1	50
	Combined	15	0	0	45	0	0	89	7	7.9
2021	Coccinellidae	24	0	0	50	3	6	43	2	4.7
	Pentatomidae	0	0	0	3	2	66.7	2	0	0
	Reduviidae	1	0	0	4	1	25	1	1	100
	Formicidae	19	0	0	57	2	3.5	72	3	4.2
	Anthocoridae	4	0	0	31	0	0	40	1	2.5
	Spiders *	11	0	0	58	6	10.3	60	1	1.7
	Combined	59	0	0	203	14	6.9	218	8	3.7

[a] Denotes sample size. * Spiders include Thomisidae, Salticidae, Araneidae, and Oxyopidae.

4. Discussion

This study is the first to investigate the potential of utilizing grain sorghum as a source for *Hear*NPV dissemination into the cotton canopy to manage *H. zea* infestation in cotton in the U.S. Several studies have reported the utility of intercropping for insect pest management. Growing crops in an intercropping setting may favor pest diversion and increase natural enemy populations [21,24,30–32]. Based on the results of our study, growing cotton in an intercropping system with grain sorghum did not result in a consistent increase in *H. zea* control and beneficial arthropods relative to the cotton-only treatment. Surprisingly, the cotton–sorghum treatment exhibited a significantly lower percentage of parasitized larvae relative to the cotton-only. Hence, the results of this study did not show evidence that sorghum could serve as a *H. zea* trap crop and a source of *H. zea* natural enemies. However, a previous study found sorghum to be a desirable diversionary *H. zea* trap crop and favored measurable *H. zea* control, but, similarly to our study, sorghum did not serve as a source for *H. zea* natural enemies [24].

Additionally, the results of our current study did not provide sufficient evidence to support our hypothesis that grain sorghum interplanted with cotton will serve as a source of *Hear*NPV that would favor persistent dissemination of the virus into the cotton canopy. In the first year of the study, *Hear*NPV was more prevalent in the treated cotton–sorghum field compared with the non-treated cotton–sorghum field, but the virus became more prevalent in the non-treated cotton–sorghum fields in the second year of the study. However, we observed an interesting pattern. When *Hear*NPV was more prevalent in the treated field, reduced incidence of injured fruiting forms and fewer larvae were detected, and when *Hear*NPV was more prevalent in the non-treated field, there was also a reduction in injury to fruiting forms and lower larval counts. This indicated that the presence of *Hear*NPV that originated from either natural sources or nearby *Hear*NPV-treated grain sorghum favored some level of *H. zea* suppression in cotton. Previous studies have demonstrated that *Hear*NPV applied to nearby grain sorghum favored a greater level of *H. armigera* control in cotton compared with direct applications to cotton and facilitated the persistence of the virus in the cotton canopy [30–32].

Several factors could have impacted the results of this study. For instance, to maintain isolation, the fields (treatments) were planted distantly from each other. Thus, the field for each individual treatment could have been exposed to significantly different levels of *H. zea* infestation and had considerably varied densities of beneficial arthropods. The natural occurrence of the virus could have also been inherently varied among the field locations. In College Station, we observed higher *H. zea* pressure in the *Hear*NPV-treated cotton–sorghum field than the non-treated cotton–sorghum and cotton-only fields in 2021. This condition might have caused the data to be biased. Additionally, populations of *H. zea* in these locations could have had varied levels of susceptibility to *Hear*NPV [42]. Resistance to Cry Bt proteins in *H. zea* is widespread [3,4], and laboratory bioassays show that *H. zea* strains resistant to Cry *Bt* proteins are significantly less susceptible to *Hear*NPV relative to a *Bt*-susceptible strain [42]. This situation has caused this study to be extremely challenging.

Our data suggest that the effectiveness of using grain sorghum as a trap crop and a nursery for natural enemies and *Hear*NPV will not consistently result in beneficial outcomes. However, we suggest that further investigation on virus efficacy against *H. zea* in cotton using the sorghum–cotton system, as well as the ability of grain sorghum to serve as a trap crop and source of natural enemies for *H. zea,* be considered in future studies, which may allow a better understanding of these systems.

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acquisition, D.L.K., J.G. (Jefferey Gore) and J.G. (Jeremy Greene). All authors have read and agreed to the published version of the manuscript.

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