INVESTIGATING THE POTENTIAL FOR UTILIZING GRAIN SORGHUM AS A NURSERY CROP FOR *HEAR*NPV DISSEMINATION INTO COTTON

W. Calvin
D. L. Kerns
Department of Entomology, Texas A&M University
College Station, TX
J. Greene
Plant and Environmental Sciences Department, Clemson University
Clemson, SC
J. Gore
Mississippi State University Delta Research and Extension Center
Stoneville, MS
L. Perkin
ICCDRU, USDA-ARS
College Station, TX
S. Vyavhare
M. N. Parajulee
Texas A&M AgriLife Research and Extension Center
Lubbock, TX
R. Schnell
Texas A&M AgriLife Extension
College Station, TX

Abstract

Widespread field-evolved resistance of cotton bollworm (*Helicoverpa zea* (Boddie)) to some Bt toxins has resulted in renewed emphasis on integrated pest management approaches to manage the pest. Field experiments were conducted in 2020 and 2021 in College Station, TX; Stoneville, MS; and Blackville, SC to investigate the potential for utilizing grain sorghum as a nursery crop for *Hear*NPV (Heligen®, AgBiTech, Fort Worth, TX) dissemination into near-by cotton to aid in managing *H. zea*. In these experiments, two fields distant of at least 0.25 miles apart were interplanted with grain sorghum and a non-Bt cotton variety. In one field, the blooming grain sorghum was treated with *Hear*NPV at 0.1 l/ha [1.4 fl-oz/ac], targeting 1st and 2nd instar larvae. The untreated field served as a non- Heligen® comparison. The percent fruit large larvae and damaged fruits (square and bolls) were compared between treatments. Bollworm larvae and beneficial arthropod samples were also collected for *Hear*NPV detection using Polymerize Chain Reaction (PCR). The PCR analysis revealed that *Hear*NPV moved from adjacent sorghum strips to the cotton canopy and was detected through the sampling dates and persisted until 21 days after treatment. Additionally, seven beneficial arthropod samples were positive for *Hear*NPV. However, in both years the *Hear*NPV application did not yield a reduction in either percent damaged fruits nor percent fruit larvae. Sorghum may serve as a source for *Hear*NPV dissemination into cotton although the data suggests that the presence of the virus in cotton canopy did not translate in *H. zea* suppression.

Introduction

Helicoverpa zea had largely been relegated as an occasional or minor pest since the introduction and widespread adoption of genetically modified cotton expressing Bt toxins. However, in recent years, due to practical and field-evolved resistance to Cry1 and Cry2 Bt toxins, *H. zea* has re-emerged as a major economic pest of cotton in much of the southern U.S. resulting in renewed emphasis on integrated pest management approaches for *H. zea* management (Tabashnik and Carriere 2015, Dively et al. 2016, Reisig et al. 2018, Reisig et al. 2019, Yang et al. 2019, Kaur et al. 2019). Because of the issues surrounding Bt resistance, *H. zea* reliance on insecticide to manage the pest in Bt cotton has become common and widespread (Kerns et al. 2018, Cook 2018, Reisig et al. 2019). With approximately 100% reliance on diamide insecticides for managing *H. zea* in cotton, along with its long residual and high selection pressure, there is great concern that resistance to diamide insecticides may rapidly develop (Adams 2016). Field-evolved resistance to chlorantraniliprole has already been described for a number of pests, including diamondback moth (*Plutella xylostella* (Linnaeus)), tomato pinworm (*Yuta absoluta* (Meyrick)), and beet armyworm (*Spodoptera exigua* (Hübner)) (Wang et al. 2012, Silva et al. 2018, Yeole et al. 2018). Thus, there is a fundamental need to evaluate insecticide alternative approaches to enhance biological control and alternative biopesticides.

Helicoverpa armigera nucleopolyhedrovirus (*Hear*NPV) is a viral pesticide that is specific to Heliothines, including *H. zea*. In much of the midsouth in recent years, *Hear*NPV is widely adopted for the primary soybean pest, *H. zea* (Musser et al. 2016). In many parts of the world *Hear*NPV is widely utilized for control of *H. armigera* in grain sorghum (Roome 1975, Teakle et al. 1985). Recently, *Hear*NPV has also been marketed for *H. zea* in grain sorghum. However, in cotton, *Hear*NPV persistence has not been sustained. This lack of persistence is thought to be primarily due to the pH of dew on cotton leaves resulting in virus deactivation as the dew dries (Yearian and Young 1974, Young et al. 1977, McLeod et al. 1997). Although initial *Hear*NPV infection of *H. zea* larvae in cotton is possible, 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 source of *Hear*NPV can be initiated for persistent horizontal and/or abiotic transmission into cotton.

In Boswana, the application of *Hear*NPV to control *H. armigera* in cotton was found to be more effective and persistent in cotton when applied to sorghum rather directly to cotton (Roome 1975, Roome and Daoust 1975). In India, applications of *Hear*NPV to *H. armigera* trap crops resulted in 14.2-20.2% reduction in *H. armigera* infestation in nearby cotton (Duraimurugan and Repgupathy 2005). Thus, sorghum has the potential to serve as a promoter for *Hear*NVP horizontal and/or abiotic dispersal into nearby cotton.

Methods

Locations, Experimental Design and Treatments

These experiments were conducted at three distinct geographical and environmental locations that are representative of the southern U.S. Cotton Belt. The sites include: College Station, TX; Stoneville, MS; and Blackville, SC.

This experiment was conducted in 2020 and consisted of two treatments at each location. Two fields distant of at least 0.25 miles apart were interplanted with grain sorghum and cotton in replicated strips of 8 rows wide and 60.96 m [200 ft] long. Each field had four replicated strips of cotton and sorghum planted following an alternate pattern. Each location served as a field replicate. A blend of grain sorghum varieties with various maturity rates were utilized. Sorghum were planted 7-10 days after planting cotton to closely correlate expected period of bloom in sorghum with the expected initial weeks of bloom in the cotton. This design was for proof of concept and allowed to maximize the probability of *Hear*NPV dispersal from sorghum into the cotton. Both crops were grown using standard production practices but were not treated with insecticides that may eliminate *H. zea*. In one field, the blooming sorghum were treated with *Hear*NPV (Heligen®, AgBiTech, Fort Worth, TX) at 0.1 l/ha [1.4 fl-oz/ac] targeting 1st and 2nd instar larvae. The treatment was applied by ground using a spray volume of 93.54 l/ha [10 gal/ac]. The untreated field served as a non- Heligen® comparison. Samples were taken before the Heligen® application and at 7, 14, and 21 days post inoculation.

Field Sampling

Helicoverpa zea were sampled from grain sorghum using the beat-bucket method (Merchant and Teetes 1992). Four locations within each replicate were sampled. At each location 25 heads were sampled (100 heads total per replicate) by bending the sorghum into a 2.5-gallon bucket and vigorously shaking it against the bucket walls to dislodge *H. zea* larvae and natural enemies. Samples were collected into 1-gallon Zip-Loc bags and returned to the laboratory for counting. The number of *H. zea* larvae were recorded and sized as small (1st and 2nd instar) or large (3rd, 4th, and 5th instar). *Helicoverpa zea* were pooled and stored at -80 ºC and the collected samples were tested for *Hear*NPV infection utilizing polymerize chain reaction (PCR).

Cotton within the cotton-sorghum interplanting were sampled using three methods: visual sampling, beat-bucket sampling and drop cloth sampling. The visual sampling method is primarily aimed at detecting incidences of fruit injury and eggs and the drop cloth method is intended as a mean to capture *H. zea* larvae that were used to determine infected *H. zea*. Using the visual sampling method, each replicated strip was sampled by sampling 25 individual plants. For each plant, the terminal was inspected for evidence of *H. zea* feeding and the presence of *H. zea* larvae. Four squares were sampled from each plant, 2 small upper canopy (first 5 nodes), and 2 lower canopy squares for evidence of injury and the presence of *H. zea* larvae. Four bolls were sampled on each plant, 2 small bolls (approximately 1-cm in diameter) with bloom tags (dried-attached blossoms), and 2 larger bolls (approximately 2-2.5 cm in diameter) with no bloom tag. Injury to squares and bolls was only recorded as positive 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

were recorded as small (1st and 2nd instar) or large (3rd, 4th and 5th instar). Additionally, when inspecting the various plant structures, the presence or absence and number of Heliothine eggs were recorded for each plant. Additionally, four drop cloth samples were collected per replicated strip. Five feet of cotton were vigorously shaken causing *H. zea* to dislodged and drop on the drop cloth. Dislodged fruits and leaves were examined for presence of *H. zea* larvae. The larvae samples collected from each replicated strip were pooled and stored at -80 ºC and were later analyzed to estimate *Hear*NPV infection using PCR. Throughout the sampling period, precautions were taken to minimize anthropogenic dispersal of *Hear*NPV.

Cotton-Sorghum-Heligen Validation

At each location in 2021, two approximately 5 acres non-Bt cotton field distant of at least 0.25 miles apart were utilized. The two fields were bordered on the predominantly upwind side with 8-12 rows of grain sorghum. A blend of sorghum varieties with various maturity rates were utilized. Sorghum were planted 7-10 days after planting cotton to closely correlate expected period of bloom in sorghum with the expected initial weeks of bloom in the cotton. Planting the sorghum upwind from the cotton allowed to minimize the potential for herbicide drift from cotton into the sorghum and maximize 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 may eliminate *H. zea*. Heligen® application and data collection were performed as described in 2020 experiment.

Field Sampling

Sorghum were sampled as described in 2020 experiment. Four locations with 25 sorghum heads per location, were sampled within the block of sorghum. As previously described, *H. zea* larvae and beneficial arthropod density were determined for each sample date. In both sorghum-cotton interplanting fields, cotton was sampled based on replicated transects originating from the sorghum edge. Each field were divided into equally spaced grids and the transect were divided into 4 equally spaced transect along those grids (Fig. 1). Data were collected along each transect at 25 ft, 50 ft, 100 ft, 200 ft, and 300 ft. At each transect location, 10 plants were visually sampled, 5 beat-bucket and 2 drop cloth samples were taken as previously described. *Hear*NPV infection were determined for each sample transect distance by replicate by sample date. Precautions were taken to minimize anthropogenic dispersal of *Hear*NPV. Samples were taken in the untreated field first then in the Heligen® treated field starting from the furthest to the closest transect to the sorghum block.

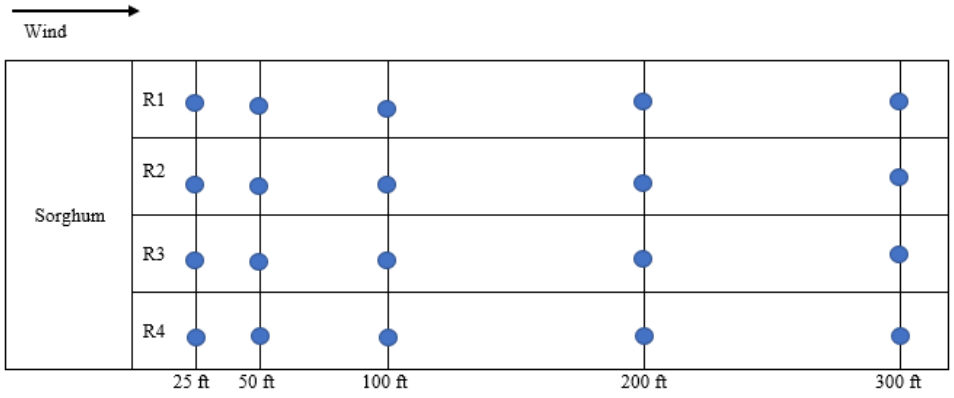

Figure 1. Distribution of transect locations within the cotton fields.

***Hear*NPV Infection Analysis**

*Hear*NPV infection of *H. zea* larvae were determined using methods described by Black et al. (2019). *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). Extracted DNA were amplified with *Hear*NPV polyhedrin-specific primers HzSpolh-2F (5′-CCCTACTTTGGGCAAAACC-3′) and HzSpolh-2R (5′-TCGGTTTGGTTGGTCGCATA-3′) (IDT, Coralville, IA) utilizing a Veriti™ 96-Well Thermal Cycler (Applied Biosystem, Foster City, CA). A volume of 50 ul of PCR mixture were 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). In order to confirm effective amplification of the target gene, a positive control

and a negative control consisting of Heligen® 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 bp.

Statistical Analysis

For year 2020 experiment, *H. zea* larvae counts on a per strip basis were compared using an Analysis of Variance (ANOVA) with ± = 0.05 (PROC GLIMMIX. Version 9.4, SAS Institute Inc., Cary, NC). Presence or absence of *Hear*NPV was a fixed effect and location was a random effect. For year 2021 experiment, *H. zea* larvae counts were compared using a two-way Analysis of Variance (ANOVA) with ± = 0.05 (PROC GLIMMIX. Version 9.4, SAS Institute Inc., Cary, NC). Factor A consisted of the treatments (presence or absence of *Hear*NPV) and factor B consisted of the distances (25 ft, 50 ft, 100 ft, 200 ft, 300 ft). Both factors were fixed effects and location was a random effect. To alleviate differences in the response of the variables among locations (blocks), the data were normalized by treatment by setting the maximum value to that of the highest replicate to 100%. The Kenward-Roger method (Kenward and Roger 1997) were used to compute denominator degrees of freedom for the test of fixed effects for all variables. Fisher's exact test (GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, CA) was used to compare the virus detection frequency between treatments.

Results

Cotton Sampling

In 2020, significant differences were not detected in neither percent damaged fruits (*F=2.71; DF=1, 68; P=0.1043*) nor percent fruit large larvae (*F=0.58; DF=1, 68; P=0.4507*). However, cotton-sorghum treated with Heligen® favored 18% lower fruit large larvae relatively to the untreated (Fig. 2b). In 2021, significant differences were not detected in percent damaged fruits between treatments (*F=0.12; DF=1, 268; P=0.733*) and among distances (*F=1.08; DF=4, 268; P=0.3651*). Although the distances were not statistically different, distances closer to sorghum had lower incidence of damaged fruit (Fig. 3b). There was a significant treatment by distance effect (*F=4.04; DF=4, 268; P=0.0034*), however, the Tukey post-hoc test indicated that all interactions were comparable (Fig. 3c). The treatments did differ in percentage large larvae (*F=10.24; DF=1, 268.3; P=0.0015*). Surprisingly, the *Hear*NPV treated field exhibited the highest incidence of large larvae. Although the two treatments were statically different, in practicality they were comparable considering the small differences in numbers and they were both above the recommended action threshold based on percent fruit large larvae (Fig. 4a). Neither distances (*F=0.69; DF=4, 268.3; P=0.5987*) nor treatment by distance interactions (*F=1.41; DF=4, 268.3; P=0.2307*) differed in percentage large larvae. However, occurrence of large larvae was lower in distances closer to sorghum (Fig. 4b).

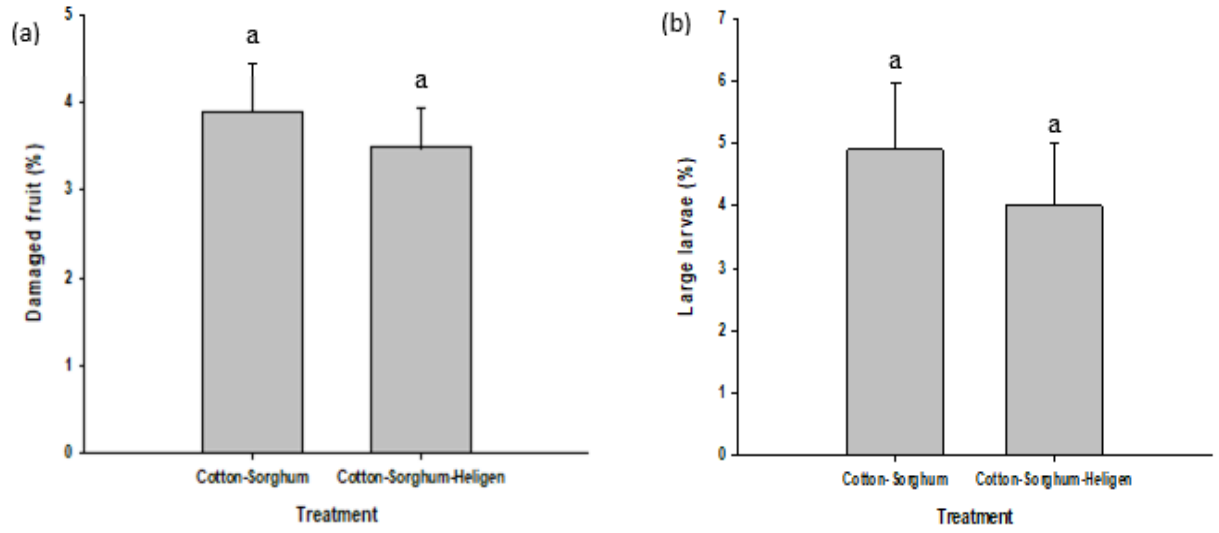

Figure 2. Percentage damaged fruit (a) per 100 fruits and percentage large larvae (b) per 100 plants as affected by *Hear*NPV.

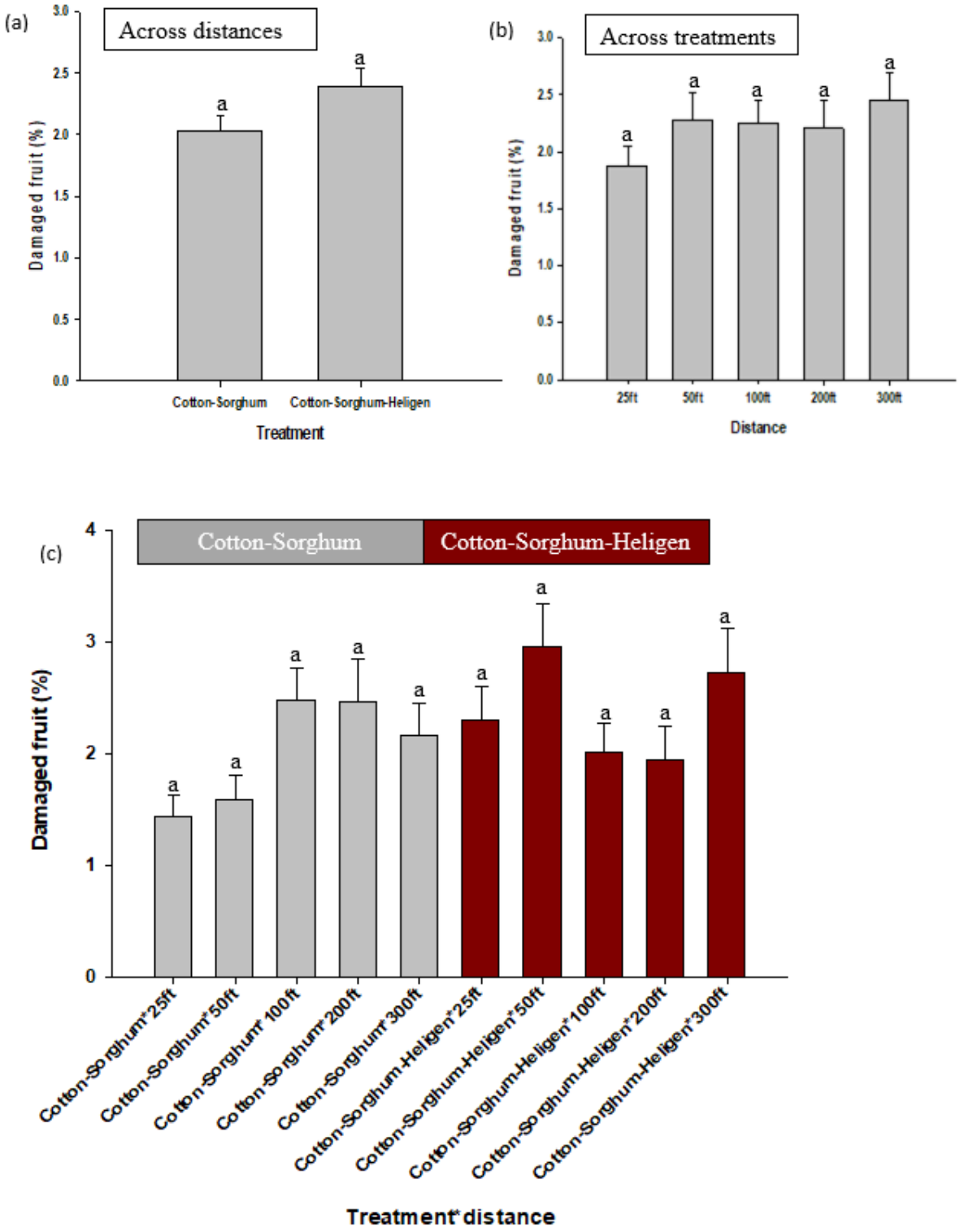

Figure 3. Percentage damaged fruit per 100 fruits among treatment, distance, and treatment by distance interactions as affected by *Hear*NPV.

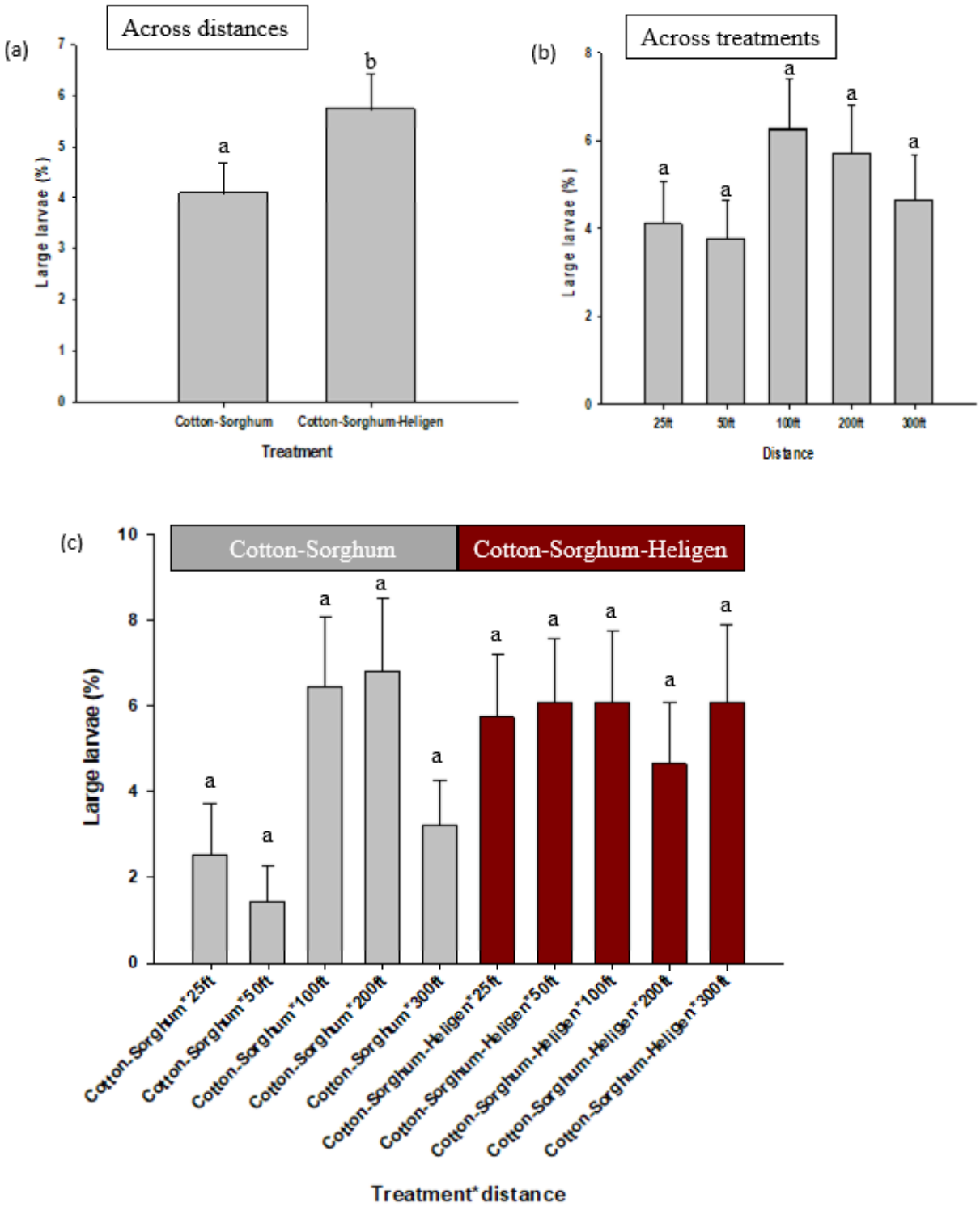

Figure 4. Percentage large larvae per 100 plants among treatment, distance, and treatment by distance interactions as affected by *Hear*NPV.

PCR Analysis

In 2020, *Hear*NPV was not detected in *H. zea* samples collected from pre-treated cotton. However, *Hear*NPV was detected in 28.6% and 50% of *H. zea* samples collected from pre-treated sorghum of untreated and treated field respectively. Throughout the subsequent sampling dates, the virus was detected in *H. zea* samples collected from both crops within both treatments. Additionally, the virus was detected in samples collected 21 days post *Hear*NPV application (Table 1). Based on the frequency analysis result, *Hear*NPV positive samples were more frequent in treated cotton relative to the untreated (P=0.0044). None of the beneficial arthropod samples collected from the untreated field were positive for *Hear*NPV while the virus was detected in 7 samples collected from the treated field. Chrysopidae, Coccinellidae, Pentatomidae and Reduviidae were the only arthropod families that appeared to be carriers for *Hear*NPV (Table 2).

Table 1. Number of *H. zea* larvae samples and percentage of *Hear*NPV infected samples in cotton and sorghum by sampling dates within treatment.

Treatment	Crop	Sampling date	No. sample	No. positive for HearNPV	% positive for HearNPV
Untreated	Cotton	Pre-inoculation	3	0	0
	Cotton	7-DPI	5	2	40
	Cotton	14-DPI	7	2	28.6
	Cotton	21-DPI	6	1	16.7
	Sorghum	Pre-inoculation	7	2	28.6
	Sorghum	7-DPI	8	5	62.5
	Sorghum	14-DPI	8	5	62.5
	Sorghum	21-DPI	4	4	100
*Hear*NPV	Cotton	Pre-inoculation	1	0	0
	Cotton	7-DPI	11	5	45.5
	Cotton	14-DPI	13	8	61.5
	Cotton	21-DPI	14	9	64.3
	Sorghum	Pre-inoculation	8	4	50
	Sorghum	7-DPI	16	14	87.5
	Sorghum	14-DPI	14	10	71.4
	Sorghum	21-DPI	9	6	66.7

Table 2. Number of beneficial arthropod samples and percentage of *Hear*NPV positive samples across dates within treatment.

Treatment	Arthropod groups	Sample size	No. positive for *Hear*NPV	% positive for *Hear*NPV
Untreated	Chrysopidae	15	0	0.0
	Coccinellidae	28	0	0.0
	Pentatomidae	1	0	0.0
	Reduviidae	1	0	0.0
	Formicidae	14	0	0.0
	Geocoridae	18	0	0.0
	Anthocoridae	37	0	0.0
	Syrphidae	4	0	0.0
	Miridae	5	0	0.0
	Nabidae	1	0	0.0
	Spiders*	40	0	0.0
	Total	164	0	0.0
HearNPV	Chrysopidae	31	4	12.9
	Coccinellidae	52	1	1.9
	Pentatomidae	4	1	25.0
	Reduviidae	2	1	50.0
	Formicidae	10	0	0.0
	Geocoridae	19	0	0.0
	Anthocoridae	30	0	0.0
	Syrphidae	…	…	…
	Miridae	11	0	0.0
	Nabidae	1	0	0.0
	Spiders*	42	0	0.0
	Total	202	7	3.5

*Spiders include Thomisidae, Salticidae, Araneidae, and Oxyopidae

Summary

This study is the first to investigate the potential of utilizing grain sorghum as a source for *Hear*NPV dissemination into cotton canopy to manage *H. zea* infestation in cotton in the U.S. The virus has demonstrated high efficacy against the pest in another crop in the U.S (Black et al. 2022). In both years of this study, the *Hear*NPV application did not result in significant decrease in fruit large larvae nor in damaged fruit. The only measurable outcome was an 18% reduction in *H. zea* population in the *Hear*NPV treated field in 2020 (Fig. 2b) which is in accordance with a previous study conducted in India where they found applications of *Hear*NPV to *H. armigera* trap crops occasioned up to 20.2% reduction in *H. armigera* infestation in nearby cotton (Duraimurugan and Repgupathy 2005).

Although the application of *Hear*NPV did not result in suitable control of *H. zea* in cotton, our results show evidence that spraying nearby grain sorghum with *Hear*NPV facilitates the dissemination and the persistence of the virus into the cotton canopy. Moreover, the virus persisted until 21 days after being applied which is consistent with Black et al. (2019) earlier research on soybean. The PCR analysis also detected the *Hear*NPV in samples collected from the control field indicating that the virus is naturally occurred in the location where the tests were conducted. Nevertheless, *Hear*NPV was more prevalent in the treated field. Additionally, only the treated field had some beneficial arthropod

samples that carried the virus. These data suggest that spraying *Hear*NPV in sorghum adjacent and up-wind of adjacent cotton will increase the likelihood of larvae within the cotton becoming infected and thus allowing the replication of the virus which is essential for an epizootic event.

Several factors could have impacted the results of this study. First of all, because the virus is naturally occurred in the locations of the test, natural suppression is likely to be provided by the *Hear*NPV already present in the environment and prevented us from having a true untreated control. Additionally, *H. zea* in cotton in these locations could have been less susceptible. Resistance to Cry Bt proteins in *H. zea* is widespread (Tabashnik and Carriere 2015, Dively et al. 2016) and laboratory bioassays showed that Cry resistant *H. zea* strains are significantly less susceptible to the *Hear*NPV relatively to the Bt susceptible strain (Unpublished data). Our experimental design also might have affected the results of this experiment in that each field (each treatment) were planted at a significantly large distance apart to ensure isolation. Given that, the field for each individual treatment could have been exposed to significantly different level of *H. zea* pressure. For instance, in College Station, we observed a higher *H. zea* pressure in the treated field in 2021 which might have caused the data to be biased. Another factor could have been the composition of cotton phytochemicals. It is reported that variation in plant host phytochemicals such as phenolics and terpenoids may induce variability in susceptibility of the pest to the virus (Channakeshava and Sannaveerappanavar 2018).

We can conclude that sorghum can serve as a source for *Hear*NPV dissemination into cotton although the data suggests that the presence of the virus in cotton canopy did not result in detectable *H. zea* suppression. Knowing that the virus is effective against bollworm in soybean (Black et al. 2022) and if the persistence of the virus in the cotton canopy is maintainable, it is hopeful that *Hear*NPV can potentially provide some level of control against *H. zea* in cotton. Therefore, future studies need to further investigate the virus efficacy against *H. zea* in cotton.

Acknowledgements

This work is supported by the Crop Protection and Pest Management Competitive Grants Program [grant no. 2019-70006-30449/project accession no. 1021163] from the USDA National Institute of Food and Agriculture. We thank all the graduate students, student workers and Lab staff in Dr. David Kerns Lab, Dr. Jeff Gore Lab, and Dr. Jeremy Greene Lab for their participation in the success of this project.

References

Adams, A., J. Gore, A. Catchot, F. Musser, D. Cook, N. Krishnan, and T. Irby. 2016. Susceptibility of *Helicoverpa zea* (Lepidoptera: Noctuidae) neonates to diamide insecticides in the Midsouthern and Southeastern United States. J. Econ. Entomol. 109: 2205-2209.

Black, J.L., G.M. Lorenz, A.J. Cato, T.R. Faske, H.J.R. Popham, K.J. Paddock, N.R. Bateman and N.J. Seiter. 2019. Field Studies on the horizontal transmission potential by voluntary and involuntary carriers of *Helicoverpa armigera* nucleopolyhedrovirus (Baculoviridae). J. Econ. Entomol. https://doi-org.srv-proxy2.library.tamu.edu/10.1093/jee/toz012.

Black, J.L., G.M. Lorenz, A.J. Cato, N.R. Bateman, and N.J. Seiter. 2022. Efficacy of *Helicoverpa armigera* Nucleopolyhedrovirus on Soybean for Control of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in Arkansas Agriculture. *Insects.* 13: 91. https://doi.org/10.3390/ insects13010091

Channakeshava, R., and V.T. Sannaveerappanavar. 2018. Effect of host plants on the susceptibility of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae to *Hear*NPV. J. Entomol. Zool. Stud. 6: 475-479.

Cook, D.R. 2018. 2017. Cotton insect loss estimates. In Proceedings, 2018 Beltwide Cotton Conferences, 3-5 January 2018, San Antonio, TX, National Cotton Council of America, Memphis, TN, pp. 721-780.

Dively G.P., P.D. Venugopal, C. Finkenbinder. 2016. Field-evolved resistance in corn earworm to Cry proteins expressed by transgenic sweet corn. PLoS ONE 11: e0169115. doi:10.1371/journal.pone.0169115.

Duraumurugan, P. and A. Regupathy. 2005. Push-pull strategy with trap crops, neem and nuclear polyhedrosis virus for insecticide resistance management in *Helicoverpa armigera* (Hubner) in cotton. Amer. J. of Applied Sci. 2: 1042-1048.

Kaur, G., J. Guoa, S. Brown, G.P. Head, P.A. Priced, S. Paula-Moraese, X. Nif, M. Dimasea, and F. Huanga. 2019. Field-evolved resistance of *Helicoverpa zea* (Boddie) to transgenic maize expressing pyramided Cry1A.105/Cry2Ab2 proteins in northeast Louisiana, the United States. J. Invertebr. Pathol. 163: 11–20.

Kenward, M.G., and J.H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics. 53: 983-997.

Kerns, D.L., F. Yang, G.M. Lorenz, J. Gore, A.L. Catchot, S.D. Stewart, S.A. Brown, D.R. Cook and N. Seiter. 2018. Value of Bt technology for bollworm management. In Proceedings, 2018 Beltwide Cotton Conferences, 3-5 January 2018, San Antonio, TX, National Cotton Council of America, Memphis, TN, pp. 805-809.

McLeod, P.J., W.C. Yearian, and S.Y. Young III. 1977. Inactivation of *Baculovirus heliothis* by ultraviolet irradiation, dew, and temperature. J. Invertebr. Pathol. 30: 237-241.

Merchant, M.E., and G.L. Teetes. 1992. Evalutaion of selected sampling methods for panicle infesting insect pests of sorghum. J. Econ. Entomol. 85: 2418-2424.

Musser, F.R., A.L. Catchot, Jr., J.A. Davis, D.A. Herbert, Jr., G.M. Lorenz, T. Reed, D.D. Reisig, and S.D. Stewart. 2016. 2015 Soybean insect losses in the southern US. Midsouth Entomol. 9: 5-17.

Reisig, D.D., A.S. Huseth, J.S. Bachelor, M. Aghaee, L. Braswell, H.J. Burrack, K. Flanders, J.K. Greene, D.A. Herbert, A. Jacobson, S.V. Palua-Moraes, P. Roberts, and S.V. Taylor. 2018. Long-term empirical and observational evidence of practical *Helicoverpa zea* resistance to cotton with pyramided Bt toxins. J. Econ. Entomol. 111: 1824-1833.

Reisig, D., D. Kerns, J. Gore, and F. Musser. 2019. Managing pyrethroid- and Bt-resistant bollworm in southern U.S. cotton. Crops & Soils. doi:10.2134/cs2019.52.0108.

Roome, R.E. 1975. Field trials with a nuclear polyhedrosis virus and Bacillus thuringiensis against larvae of *Heliothis armigera* (Hb.) (Lepidoptera, Noctuidae) on sorghum and cotton in Botswana. Bull. Entomol. Res. 65: 507-514.

Roome, R.E. and R.A. Daoust. 1975. Survival of the nuclear polyhedrosis virus of *Heliothis armigera* on crops and in soil in Botswana. J. Invert. Path., 27: 7-12.

SAS Institute. 2011. User's manual. Version 9.3. SAS Institute, Cary, NC.

Silva, J.E., L.M. da S. Ribeiro, N. Vinasco, R.N.C. Guedes and H.A.A. Siqueira. 2018. Field-evolved resistance to chlorantraniliprole in the tomato pinworm *Tuta absoluta*: inheritance, cross-resistance profile, and metabolism. J. Pest Sci. https://doi.org/10.1007/s10340-0181064-z.

Stewart, S.D. 2007. Bt cotton. University of Tennessee Extension publication W129, Knoxville, TN.

Tabashnik, B.E., and Y. Carrière. 2010. Field-evolved resistance to Bt cotton: *Helicoverpa zea* in the US and pink bollworm in India. Southwest. Entomol. 35: 417–424.

Teakle, R.E., J.M. Jensen, and J.C. Mulder. 1985. Susceptibility of *Heliothis armigera* (Lepidoptera: Noctuidae) on sorghum to nuclear polyhedrosis virus. J. Econ. Entomol. 78: 1373-1378.

Wang, X., S.K. Khakame, C. Ye, Y. Yang and Y. Wu. 2012. Characterisation of field-evolved resistance to chlorantraniliprole in the diamondback moth, *Plutella xylostella*, from China. Pest Manag. Sci. 69: 661-665.

Yang, F., J.C.S. Gonzales, J. Williams, D.C. Cook, R.T. Gilreath and D.L. Kerns. 2019. Occurrence and ear damage of *Helicoverpa zea* on transgenic *Bacillus thuringiensis* maize in the field in Texas, U.S. and its susceptibility to Vip3A protein. Toxins. 11, 102; doi:10.3390/toxins11020102.

Yearian, W.C., and S.Y. Young. 1974. Persistence of *Heliothis* nuclear polyhedrosis virus on cotton plant parts. Environ. Entomol. 3: 1035-1036.

Yeole, R.S., P.N. Sangale, and S.B. Damal. 2018. Chlorantraniliprole resistance in diamondback moth on different locations of Maharashtra. J. Pharm. Phytochem. 7: 523-525.

Young, S.Y., W.C. Yearian, and K.S. Kim. 1977. Effect of dew from cotton and soybean foliage on activity of *Heliothis* nuclear polyhedrosis virus. J. Invert. Path. 29: 105-111.