DETERMINING THE EFFICACY OF *HELICOVERPA ARMIGERA* NUCLEOPOLYHEDROVIRUS (*Hear*NPV) IN ARKANSAS SOYBEANS

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Abstract

Helicoverpa armigera Nucleopolyhedrovirus (*Hear*NPV) is a viral biocontrol agent utilized in foliar insecticidal applications to control *Helicoverpa zea* populations. The objective of this study was to better understand the efficacy of *Hear*NPV by determining the time to mortality from application, the percent mortality by larval instar, the horizontal transmission potential, and the residual time. Horizontal transmission is the spread of the virus through the current population and subsequent infestations. *Hear*NPV was applied on soybean plants in a greenhouse study and field trials. Time to mortality and percent mortality by instar was determined by monitoring larvae caged on individual soybean plants sprayed with *Hear*NPV within a greenhouse. Viral horizontal transmission and residual time were evaluated by spraying a 50' by 50' area with *Hear*NPV, and then taking samples within zones of distance including 0-25', 25-50', 50-100', and 100-200' from the application. Samples were taken before, 3, 7, 10, 14, and 21 days after application. Polymerase chain reaction (PCR) was conducted to determine the presence of *Hear*NPV within each sample. Data suggests that time to mortality takes between 4.5 and 6 days, and *Hear*NPV is most effective against 1st through 3rd instars. *Hear*NPV can spread as far as 200 feet within 3 days, and lasts at least 13 to 21 days in the canopy.

Introduction

Helicoverpa armigera Nucleopolyhedrovirus (*Hear*NPV) is a viral biopesticide that is specific to Heliothines, including *Helicoverpa zea*, the primary insect pest of soybean (*Glycine max* (L.) Merrill) in Arkansas (Musser et al. 2016). *Hear*NPV is in the viral family Baculoviridae, which contain a protein structure that aids in protection from environmental conditions (Bilimoria 1986; Bilimoria 1991). *Hear*NPV can be applied like other insecticides, and once applied is ingested by the *H. zea* larvae present. The protective structure breaks down upon reaching the midgut, and the viral DNA infects the midgut cells where replication begins, ultimately spreading throughout the host and liquefying it (Hunter-Fujita et al. 1998). Once the host larva liquefies and dies, millions of viral particles are released into the environment where horizontal transmission can occur (Boucias and Pendland 1998).

Horizontal transmission is the movement of the viral particles from the infected larva to a healthy larva. There are several well documented methods for horizontal transmission to occur. A healthy larva can cannibalize an infected larva, or an infected larva can contaminate the food source of a healthy larva through movement or the final liquefaction (Ali et al. 1987a; Ali et al. 1987b; Vasconcelos 1996). A parasitoid that oviposits into or emerges from an infected larva can transmit *Hear*NPV, and a predator that feeds upon an infected larva can defecate frass with high enough viral concentrations to cause infection if ingested by a healthy larva (Young and Yearian 1987; Young and Yearian 1989; Young and Yearian 1990; Lee and Fuxa 2000a; Lee and Fuxa 2000b).

Due to *Hear*NPV killing the host larva after replicating, there has been a less-than desirable time to mortality observed when compared to other insecticides; however, this delay in mortality does not necessarily mean a reduction in efficacy (Luttrell et al. 1982). This study was conducted to determine the efficacy of *Hear*NPV when four factors were considered: time from application to mortality, percent mortality by instar, horizontal transmission potential, and residual time. A greenhouse trial was utilized to determine the time from application to mortality and the percent mortality by instar. Horizontal transmission potential was determined through both field and greenhouse trials, and residual time was determined through field trials.

Materials and Methods

A greenhouse trial was repeated three times during 2017, using untreated Pioneer 47T36 soybean seed. Soybeans were grown in a greenhouse until the initiation of the trial at V3. Thirty randomly selected soybeans were caged with individual sleeve cages and infested with an *H. zea* larva, sixty randomly selected soybeans were temporarily removed from the greenhouse and *Hear*NPV was applied at a concentration of $2.2x10^{11}$ viral particles/oz and a rate of 1.6 oz/acre using a CO_2 backpack sprayer applying at 10 gal/acre. Once the application dried, the plants were moved back into the greenhouse, caged, and infested. A complete randomized block design was utilized with two main factors: larval instar (1^{st}-5^{th}), and *Hear*NPV application (sprayed or unsprayed).

In the first run, *Hear*NPV spread to several unsprayed larvae; therefore, wooden barriers 3' x 5' were used to separate the unsprayed from the sprayed plants for the two remaining runs. Larvae were monitored twice a day for mortality and percent defoliation. Fourteen days after the application, all cages were re-infested with a second instar larva to determine horizontal transmission potential across generations.

Data concerning the mortality of each larva was subject to an ANOVA (α= 0.05), and Fisher's Exact Test, as the response variable was considered categorical. Main effects consisted of larval instar, treatment, and interaction effects between larval instar and treatment. Block number and run number were analyzed as random effects. To determine differences in the time to mortality an ANOVA (α= 0.05) which utilized a Tukey's post hoc analysis was used. Mortality of the second generation was subject to an ANOVA (α= 0.05) that utilized Tukey's post hoc analysis. All data was analyzed using SAS 9.4 (PROC GLIMMIX. Version 9.4, SAS Institute Inc., Cary, NC).

A field trial was conducted over two years on four grower fields during 2016 and 2017. Fields that had a *H. zea* larval population of at least 5 larvae per 25 sweeps were utilized: one field in 2016 and three fields in 2017. Three samples consisting of 10 sweeps each were taken before initiating the trial to verify no natural *Hear*NPV populations. Then, a 50 x 50' area was designated for *Hear*NPV application at 1.6 oz/acre. Prior to the application, sampling zones of 0-25, 25-50, 50-100, and 100-200 feet from the application area were set up. In 2016, samples were taken 3, 7, 14, and 21 days after application, and each sample date consisted of 3 samples of 10 sweeps for each sample zone. In 2017, samples were taken 3, 7, 10, 14, and 21 days after application, and each sample date consisted of 6 samples of 58 sweeps for the 100-200' zone, 5 samples of 21 sweeps for the 50-100' zone, 3 samples of 12 sweeps for the 25-50' zone, and 3 samples of 10 sweeps for the 0-25' zone and application area. All samples were frozen for a minimum of 48 hours prior to sample analysis.

Each sample was analyzed by counting and identifying all the arthropods present and then placing them in a 15 mL test tube. The viral particles were then extracted using a modified extraction technique (O'Reilly et al. 1992), and stored in a 4°C freezer. The viral DNA was then extracted from the particles by a DNA extraction kit, DNeasy Blood and Tissue Kit (Quiagen, Hilden, Germany). Following viral DNA extraction, polymerase chain reaction (PCR) was used to replicate any viral DNA present using *Hear*NPV specific primers (IDT, Coralville, IA), and a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA). A known positive was also added to the thermocycler before PCR to confirm the amplification process was successful. After the amplification of the DNA by PCR the samples were processed using gel electrophoresis, loading 20µL of each sample PCR product into individual wells, including the positive control. The gel was run for 1 hour at 90 volts using Sybr Safe DNA gel stain (Life Technologies Corporation, Carlsbad, CA), and was then visualized under a UV baselight (UPV LLC., Upland, CA). If a band was present at 450 base pairs *Hear*NPV was positive for that corresponding sample.

The mean number of *H. zea* larvae was analyzed using an ANOVA (α=0.05) and Tukey's post hoc. SAS 9.4 was used for all data analysis (PROC GLIMMIX. Version 9.4, SAS Institute Inc., Cary, NC).

Results and Discussion

In the greenhouse trials, regardless of larval instar, all larvae placed on a sprayed plant that died from *Hear*NPV died between 4.5 and 6 days after the application, and averaged 5 days (Figure 1). However, percent mortality varied by instar (Table 1). Only one 1st instar larva out of 36 placed on sprayed plants survived to pupation, and all the 2nd and 3rd instars died from *Hear*NPV. There was a 35% mortality rate in the 4th instars, and only one 5th instar died from *Hear*NPV. This reveals that the target population when using *Hear*NPV as a control tactic should be 1st through 3rd instar larvae.

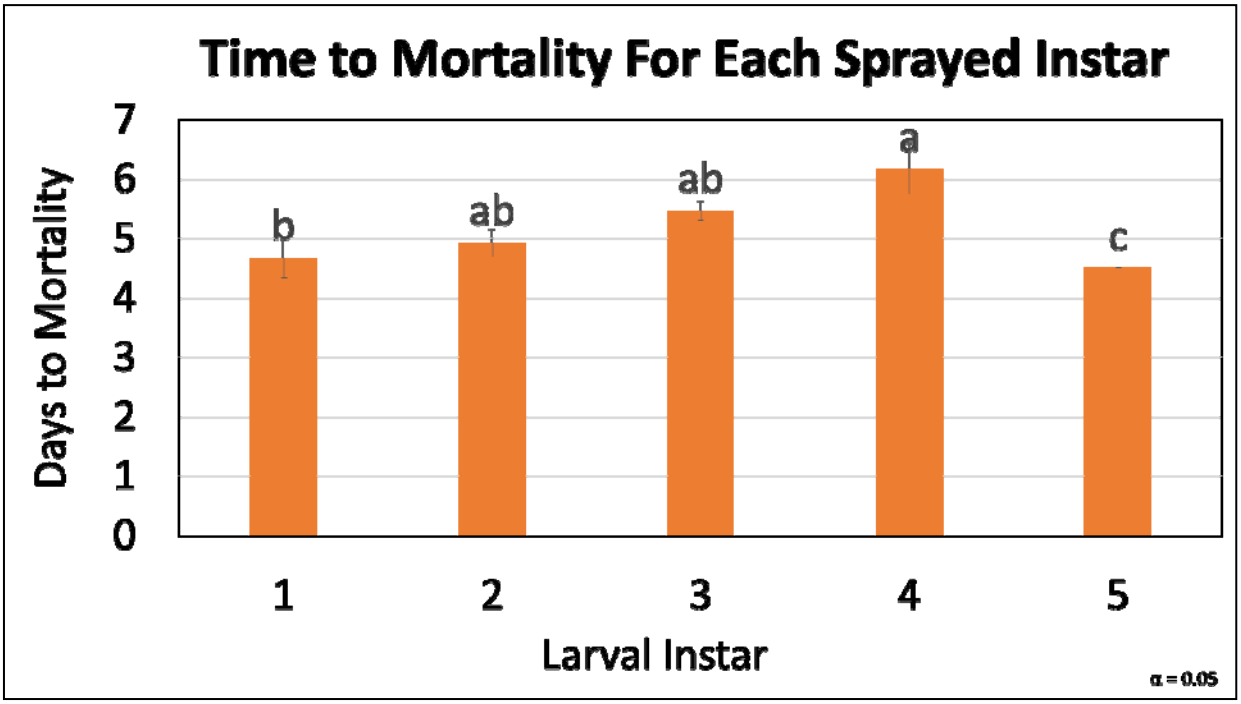

Figure 1: Time to mortality for each *Helicoverpa zea* larval instar when placed on a soybean plant sprayed with 1.6 oz/acre of *Hear*NPV.
*Lowercased letters denote a significantly different value within a given field using an ANOVA (α=0.05), and a Tukey's post hoc analysis (p<0.05).

Table 1: The percent mortality observed for each instar placed on a soybean plant sprayed with 1.6 oz/acre of *Hear*NPV.

Instar	% Mortality*
1	97 a
2	100 a
3	100 a
4	35 b
5	3 c

*Lowercased letters denote a significantly different value within a given field using an ANOVA (α=0.05), and a Tukey's post hoc analysis (p<0.05).

When a second infestation was added to the sprayed plants 14 days after the application, 100% mortality was observed, with no significant differences between time to mortality (Figure 2). All larvae died on average within 3.5 days, significantly faster than the first generation, regardless of the larval instar of the previous generation, indicating that *Hear*NPV is capable of transmitting across generations, and causing faster mortality.

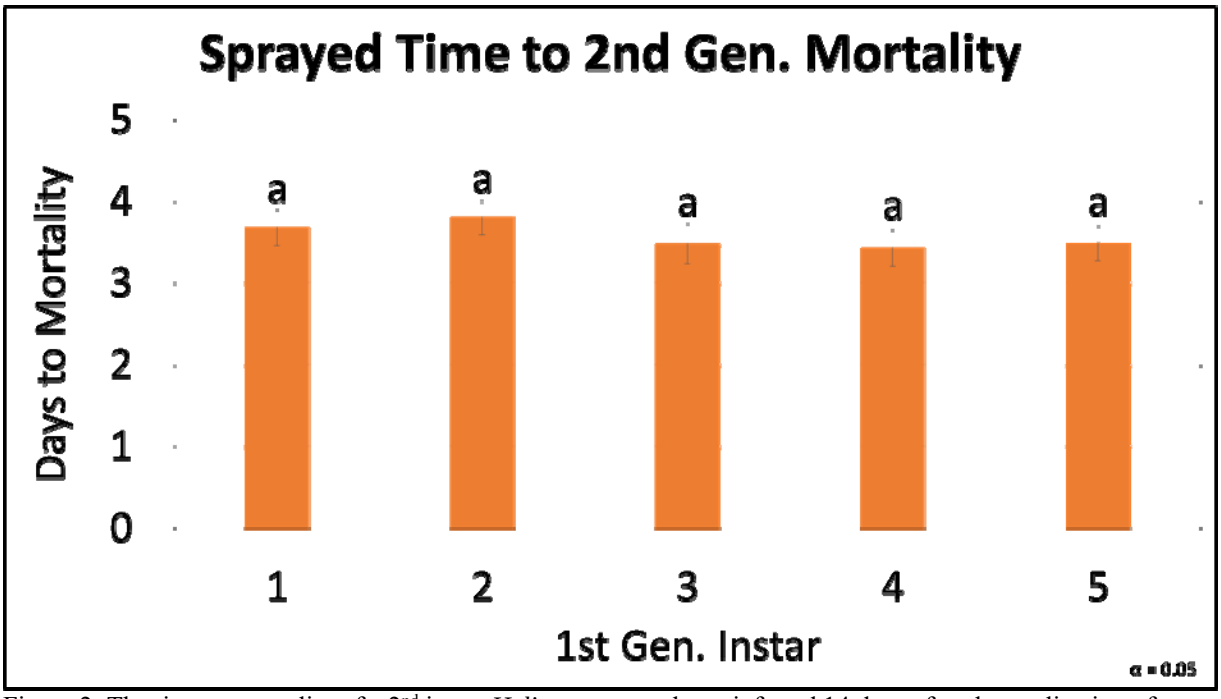

Figure 2: The time to mortality of a 2nd instar *Helicoverpa zea* larva infested 14 days after the application of *Hear*NPV and initial infestation of a 1st – 5th instar larva.
*Lowercased letters denote a significantly different value within a given field using an ANOVA (α=0.05), and a Tukey's post hoc analysis ($p<0.05$).

In the field trials in 2016, *Hear*NPV was observed 100 feet from the application area 7 days after the application, and remained in the crop canopy 14 days (Table 2). In 2017, *Hear*NPV was observed 200 feet from the application area 3 days after the application, and remained in the crop canopy 13-21 days, depending on the field. This movement could allow for growers to implement *Hear*NPV in a grid or strip application, rather than applying *Hear*NPV to the entire field. *Helicoverpa zea* populations differed across fields and sample dates, with 3 fields having larval populations drop to almost zero by the 14 day sample date, but one field had sustained populations for the duration of the study. In the fields where *H. zea* populations dropped, *Hear*NPV presence declined at the same time. In the field where a sustained population of *H. zea* was observed, a sustained presence of *Hear*NPV was also observed. This could be revealing a possible dependency of *Hear*NPV presence on *H. zea* population levels; however, this could not be proven statistically due to a low sample size.

Table 2: *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) larval populations evaluated at 25 sweeps, and maximum observed distance *Helicoverpa armigera* Nucleopolyhedrovirus spread for all fields and sample dates where *Hear*NPV was applied.

Days After App.	Mean # *H. zea*/25 sweeps	Max Distance (ft)
	2016 Field 1	
3	36.7 a, A	0
7	24.3 b, A	100
14	2.3 c, B	100
21	2.3 c, B	-
	2017 Field 1	
2	5.6 a, B	200
6	2 b, C	0
9	1.7 b, B	100
13	1.1 b, B	100
20	0.4 b, C	-
	2017 Field 2	
2	6.9 a, B	200
6	1.7 b, C	-
9	0.5 b, B	-
13	0.3 b, B	-
20	0.6 b, C	200
	2017 Field 3	
3	9.8 a, B	200
7	13.9 a, B	200
10	12.8 a, A	200
14	9 a, A	200
21	9.3 a, A	200

*Lowercased letters denote a significantly different value within a given field using an ANOVA (α=0.05), and a Tukey's post hoc analysis ($p<0.05$).
**Capital letters denote a significantly different value across fields for a given sample date using an ANOVA (α=0.05), and a Tukey's post hoc analysis ($p<0.05$).

Summary

*Hear*NPV should be applied when *H. zea* populations are mainly 1st through 3rd instars to maximize efficacy (Mortality within 4.5-6 days after the application). *Hear*NPV has been observed moving up to 200 feet and lasted in the crop canopy between 13 and 21 days. In some cases, as seen in the greenhouse, *Hear*NPV can transmit across generations, and even kill a second generation (3.5 days) significantly faster than the first generation (4.5–6 days). Future studies should focus on the importance of *H. zea* larval population levels on *Hear*NPV presence, and focus on the efficacy of applying *Hear*NPV in a grid- or strip-application to capitalize on the movement recorded in this study.

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