HORIZONTAL TRANSMISSION OF *HELICOVERPA* NUCLEOPOLYHEDROSIS VIRUS (NPV) IN SOYBEAN FIELDS INFESTED WITH CORN EARWORM

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Abstract

Helicoverpa armigera NPV (HearNPV), a viral biocontrol agent, is utilized in foliar insecticidal applications to control corn earworm, *Helicoverpa zea*, populations. The objective of this study was to quantify the horizontal transmission, or spread of the virus through the current population and subsequent infestations, of *Hear*NPV when applied in soybean fields infested with corn earworm. Viral horizontal transmission was evaluated by spraying a 50' by 50' area with *Hear*NPV, and then taking 3 samples within zones of distance including 0-25', 25-50', 50-100', and 100-200' from the application. Samples were taken before, 3, 7, 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 horizontal transmission of *Hear*NPV peaks 7 days after application and dissipates by 21 days.

Introduction

The corn earworm, *Helicoverpa zea* (Boddie), is the most damaging pest of soybeans across the Mid-South (Musser et al 2015a). During the growing season, the corn earworm migrates as far north as Canada using air currents. (Sandstrom et al. 2007). The corn earworm is a pest of several major row crops including cotton, corn, sorghum, and soybeans (Quaintance and Brues 1905). In soybeans, the corn earworm is capable of causing both defoliation and fruit damage, resulting in indirect and direct yield loss (Mueller and Engroff 1980; Adams et al. 2015). There are a few forms of cultural controls growers can implement to avoid corn earworm damage. Planting early can help to miss the later season, larger corn earworm flights (Joshi 1980). Another form of cultural control, fall tillage, can result in local mortality of overwintering pupae (Barber and Dicke 1937; Fife and Graham 1966). Hartstack traps are also used throughout the growing season to monitor the corn earworm population. Once a field is infested with corn earworms, natural enemies can suppress the growing population (Pfannenstiel and Yeargan 2002; Sansone and Smith 2001; Cabanillas and Raulston 1996). However, once threshold is reached an insecticidal application is necessary. Corn earworm has become resistant to several insecticidal classes such as organophosphates and organochlorines (Wolfenbarger et al. 1971; Sparks 1981; Abd-Elghafar et al. 1993; Kanga et al 1996), and is developing resistance to the commonly used pyrethroid class (Musser et al. 2015b). Other avenues of controlling corn earworm have been explored, such as the development of new insecticide classes and research on potential biocontrol agents. One biocontrol agent that has been heavily studied is *Hear*NPV and is used extensively in other countries for control of Heliothines.

*Hear*NPV is a viral biocontrol agent that is used to control corn earworm populations with no known off-target effects. It is in the viral family Baculoviridae which are known for their protective, proteinaceous occlusion bodies (OB) (Bilimoria 1986), which protect the viral DNA from extended exposure to the environment (Bilimoria 1991). Once the OB is ingested by a healthy larva and reaches the midgut, the alkaline conditions begin to break it down releasing virions, which are enveloped nucleocapsids present within the OBs (Bilimoria 1991). The virions infect the midgut epithelial cells, where a simplified version of the virus is produced called budded virus (Bilimoria 1991). The budded virus contains one nucleocapsid enveloped by the nuclear or plasma membrane of the host cell (Bilimoria 1991). The budded virus travels to the fat bodies and throughout the larvae (Hunter-Fujita et al. 1998),

where it will begin to form millions of OBs (Boucias and Pendland 1998). The larvae will then begin to liquefy and release OBs into the environment where it will repeat its life cycle through horizontal transmission (Boucias and Pendland 1998).

Horizontal transmission is capable of occurring through several routes. Abiotic conditions such as rainfall and wind can transport OBs from the soil, which acts as a refuge, to the crop canopy where infection can occur (Fuxa and Richter 2006; Fuxa and Richter 2001; Young 1990). Infected larvae are capable of transmitting *Hear*NPV to healthy larvae by cannibalism of an infected larva by a healthy larva (Vasconcelos 1996). Infected larva can defecate viral DNA in adequate concentrations to initiate infection when consumed (Vasconcelos 1996; Ali et al. 1987b), as well as spread *Hear*NPV through surface contamination through vomiting, liquefaction, or movement (Ali et al. 1987a). Parasitoids, such as *Microplitis croceipes*, transmit *Hear*NPV when they emerge from infected larvae and oviposit into healthy larvae (Young and Yearian 1989), or when they contaminate their ovipositor by laying an egg in an infected larva (Young and Yearian 1990). Predators such as *Nabis roseipennis*, *Podisus maculiventris*, *Sarcophaga bullata*, or *Acheta domesticus*, can feed on an infected larvae and then defecate frass that contains a high enough viral concentration to cause infection when ingested up to 10 days after feeding (Young and Yearian 1987; Lee and Fuxa 2000a; Lee and Fuxa 2000b).

Even though much research has been done, no studies have been conducted to determine the horizontal transmission rate of *Hear*NPV in a field study when all routes are present. The objective of this study was to assess the rate of horizontal transmission of *Hear*NPV in a soybean field infested with *Helicoverpa zea*.

Materials and Methods

Soybean fields that had corn earworm infestation levels of at least 2 larvae per 10 sweeps were utilized for this study. Once a field was found with a proper infestation level, an application area of 50’ by 50’ was flagged off on the edge of the field, and sample distances of 0-25’, 25-50’, 50-100’, and 100-200’ from the plot were flagged off as well. Before the *Hear*NPV application, 3 field samples and 3 soil samples were taken randomly from the trial area and analyzed to determine if the field contained any naturally occurring *Hear*NPV. Soil samples were analyzed using the extraction methods described in Evans et al. (1980). *Hear*NPV was applied at a rate of 1.6 fl oz/ acre at a concentration of 2.22×10^{11} OBs/oz using a backpack sprayer applied at a rate of 10 gal/acre. The applicator did not travel outside of the application area and exited directly out of the field to minimize the potential for anthropogenic transmission.

Three samples were taken for each distance 3, 7, 14, and 21 days after the application of *Hear*NPV. Each sample consisted of 10 sweeps, using a new sweep net for each distance, and samples were taken from the farthest distance towards the application area. Once the samples were taken, the sampler left the field in a manner that reduced the potential for anthropogenic transmission. Samples were frozen for at least 48 hours. Each sample was analyzed by counting and identifying all the arthropods present and then placing them in a 15 mL test tube. The OBs 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 OBs 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. Data was then mapped using ArcGIS 10 (Esri, Redlands, CA).

Results and Discussion

Three days after application of *Hear*NPV the virus was found in the application area but was not found in any of the other sample areas indicating that the virus had not spread (Figure 1). Seven days after application, *Hear*NPV was found in the application area, at 0-25’, and at 50-100’ (Figure 2). The corn earworm population in this soybean field crashed, going from 1-2 larvae per sweep to 0-2 larvae per 10 sweeps. Fourteen days after application the virus was only present in the application area and in the 50’-100’ area. (Figure 3). At 21 days after the application, there was no virus present in the crop canopy (Figure 4).

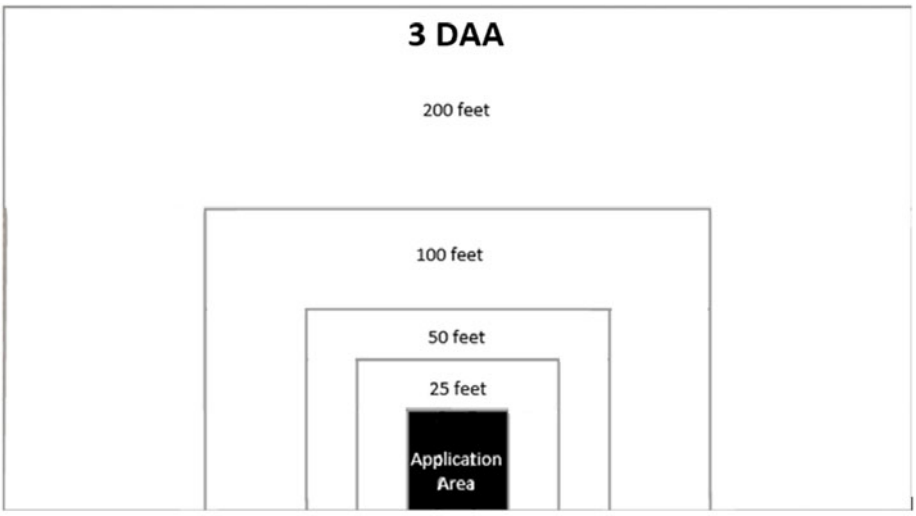

Figure 1: The horizontal transmission of *Hear*NPV three days after the application. Positive samples are depicted in black, negative in white.

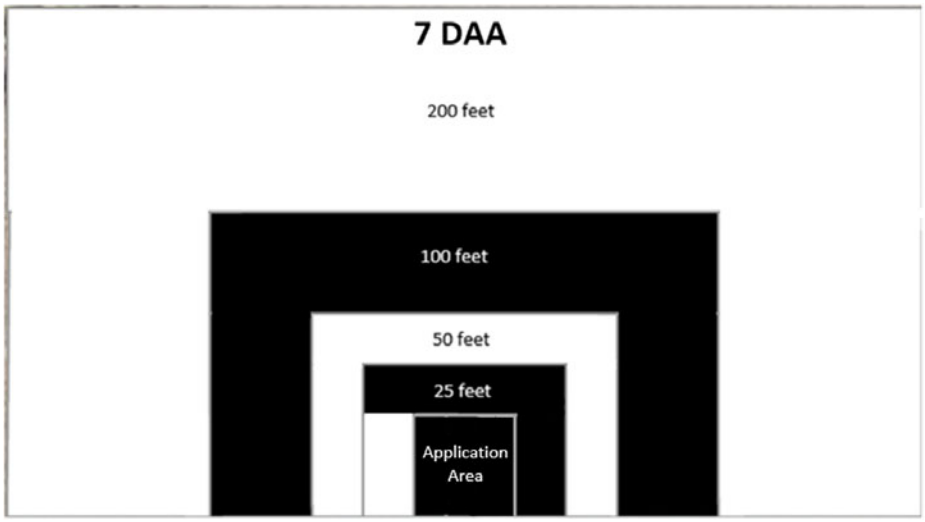

Figure 2: The horizontal transmission of *Hear*NPV seven days after the application. Positive samples are depicted in black, negative in white.

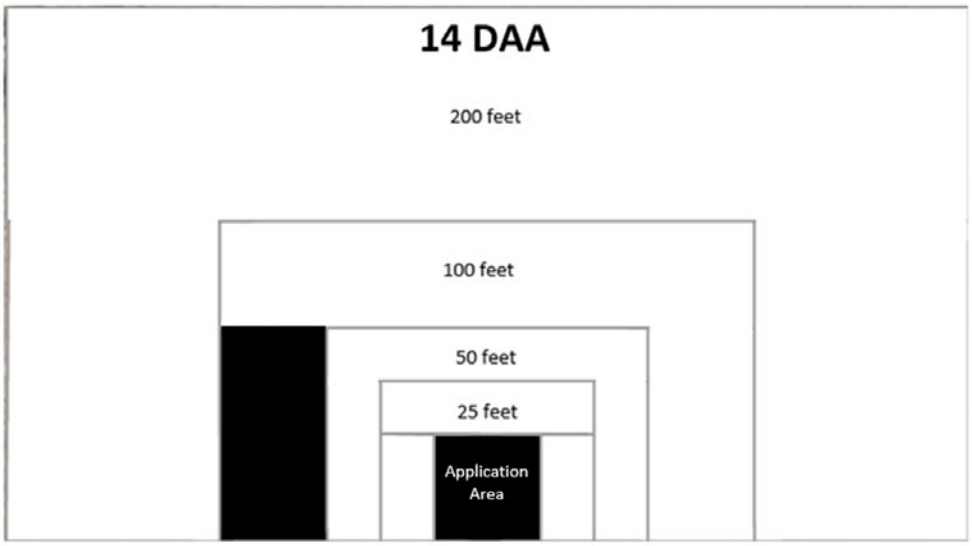

Figure 3: The horizontal transmission of *Hear*NPV fourteen days after the application. Positive samples are depicted in black, negative in white.

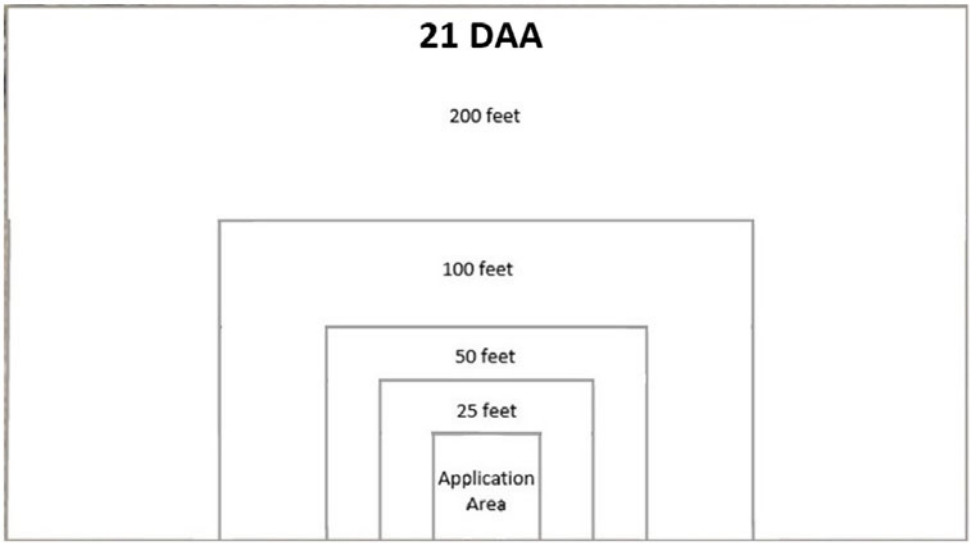

Figure 4: The horizontal transmission of *Hear*NPV twenty-one days after the application. Positive samples are depicted in black, negative in white.

Summary

This data indicated that horizontal transmission of *Hear*NPV peaks around 7 days after application, and is absent from the crop canopy by 21 days after application. However, it is possible that this peak is due to the decline in corn earworms present in the field after that sample date. This study helps to better understand the distance *Hear*NPV can spread which reveals potential alternative application methods such as grid and strip application of this virus. Also, understanding how long *Hear*NPV is active within the crop canopy reveals the potential for cross-generational transmission. Although this data shows us important information about the use of this virus, it is important to note that this is from one field in one growing season. This study will be replicated in the summer of 2017. In addition to the previously stated sample dates, samples will be taken 10 and 17 days after the application, to get a better understanding of how quickly the virus spreads and dissipates from seven days after application. Also, samples will be divided and analyzed by species rather than grouped together. This will allow for an understanding of the impact of each species in the horizontal transmission of *Hear*NPV.

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