Activity level of Helicoverpa armigera Nucleopolyhedrovirus (HearNPV) and feeding habits of Helicoverpa zea larvae after ingestion of HearNPV in Mississippi soybean

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Activity level of Helicoverpa armigera Nucleopolyhedrovirus (HearNPV) and feeding habits of Helicoverpa zea larvae after ingestion of HearNPV in Mississippi soybean

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A Thesis
Submitted to the Faculty of Mississippi State University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Entomology in the Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology

Mississippi State, Mississippi

May 2023
HearNPV was applied to a soybean field and evaluated for residual efficacy at different time intervals (0, 12, 24, 36, 48, and 72 hours) after application in laboratory bioassays with treated leaves. Larval mortality was rated at five, seven and twelve days after larval infestation. Larval mortality increased as the number of days after infestation increased. To evaluate the feeding habits of Helicoverpa zea at the 2nd and 3rd instar, HearNPV exposed larvae and untreated larvae were weighed at zero (before diet exposure), four, and seven days. At four days after exposure, larval weight gain of specimens exposed to HearNPV was reduced by 80% and 45% for 2nd and 3rd instar larvae, respectively. At seven days after exposure, larval weight gain of 3rd instar specimens exposed to HearNPV was reduced by 60%.
DEDICATION

I would like to dedicate this thesis to my beautiful wife, Lindsay, for her love, support, and patience throughout the entirety of this project. I pursued this degree to ensure a better life for us, as well as for our future family. Your unconditional love and support have been evident throughout the pursuit of this degree. Thank you for standing by me through it all and your willingness to join me in all our crazy adventures.

Also, I would like to dedicate this research and many hours of work that went into the completion of this project to my parents, Rick and Shelby Fortenberry. Words cannot express how grateful I am for the belief you have had in me throughout my life. Your life, faith, and marriage are an encouragement and a beacon of light to more people than you realize. Where I am today is largely accredited to how intentional you were in raising myself, CC, and Maleah. I hope Lindsay and I can model your lives and leadership in all our future endeavors.
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CHAPTER I
INTRODUCTION

Soybean

Soybean History

Soybean, Glycine max (L.) Merrill, is a leguminous crop of significant economic importance throughout the world today. Its domestication by Chinese farmers dates back to 1100 BC. However, it was not until 1765 that soybean seeds were brought from China to be planted for the first time in the United States by a colonist in Georgia (North Carolina Soybean Producers Association 2011). Soybean production in the U.S. quickly spread throughout the Midwest in the 1870s, when farmers realized their benefit as a forage crop for grazing livestock. In 1904, American chemist, George Washington Carver discovered the rotational benefits of soybean cultivation for soil nutrient management and began encouraging cotton farmers across the United States to rotate their cotton crop with soybeans every few years (North Carolina Soybean Producers Association 2011). Throughout the 1940s and 1950s, U.S. farmers began to realize the versatility soybean production offered in terms of its uses for oils, plastics, and livestock consumption (Gibson and Benson 2005).

Soybean Growth and Development

Soybean is a short-day plant sensitive to photoperiod (Jones et al. 2015). Soybean flowering is induced by warmer temperatures and longer night lengths (Berglund et al. 1999). Soybean varieties are separated into thirteen maturity groups, based on the amount of daylight
required to initiate flowering (Pendleton and Hartwig 1973). Soybean cultivars are fundamentally broken into two categories based on their growth characteristics: determinate or indeterminate. Despite their growth habits, all soybeans experience two distinct phases throughout their growth and development, a vegetative (V) phase and a reproductive (R) phase. Indeterminate cultivars continue to grow vegetatively after flowering has begun. Most northern states utilize indeterminate cultivars, maturity groups 00-IV, to account for the shorter growing season experienced in colder climates (Berglund et al. 1999). Determinate cultivars, however, complete all vegetative growth before flowering is initiated. (Berglund et al. 1999). Historically, soybean producers in the southern part of the United States primarily utilized determinate cultivars, maturity groups V-VIII (Purcell and Ashlock 2014). However, due to the severity of drought stress and high temperatures, many southern soybean producers were encouraged to implement Early Soybean Production Systems (ESPS). The adoption of ESPS included utilizing earlier planting dates and earlier maturing varieties (Heatherly and Spurlock 1999). Additionally, indeterminate, early maturing varieties are capable of compensating for significant fruit loss and delayed flowering (Adams et al. 2016, Coelho et al. 2020). Subsequently, indeterminate group IV and V soybeans are now the most commonly planted varieties in many soybean production systems throughout the state of Mississippi.

Throughout its development, soybean progresses through six vegetative (V) stages often followed by eight reproductive (R) stages. The categorization of soybean development into these stages was first described by Walter Fehr and Charles Caviness (1977). While the principal categorization remained the same, Pederson (2004) slightly modified the methodology, by which we still use to describe soybean growth stages today (Fehr and Caviness 1977, Pedersen 2004). Soybean development begins when plant foliage emerges from the soil surface (VE). After
emergence, unifoliate leaves join the cotyledons on the plant stem as they begin to unroll, inducing the second vegetative stage, (VC). The subsequent vegetative phases, V1, V2, V3, Vn, are characterized by the number nodes, possessing fully developed leaves, above the unifoliate nodes. For instance, V3 signifies that the soybean plant has three nodes with fully developed leaves on the main stem above the unifoliate leaves. In accordance with the vegetative phases, soybean progresses through eight reproductive (R) phases beginning with R1 (Fehr and Caviness 1977). Reproductive stage one (R1), beginning bloom, is characterized by the first flowering position on the main stem of the plant, beginning bloom. When multiple flowers can be found in the top two nodes of the plant, the soybean has reached reproductive stage two (R2), full bloom. Reproductive stage three (R3), beginning pod, signifies the development of a pod, 0.5 cm in length, at one of the four uppermost nodes of the plant. Reproductive stage four (R4), full pod, transpires when a full pod, 1.9 cm in length, can be found in the four uppermost nodes. Reproductive stages five (R5), beginning seed, takes place when a seed, 0.3 cm in length, can be found within a pod in the four uppermost nodes. When the seed turns green and fills the pod cavity, Reproductive stage six (R6), full seed, has occurred. As seeds develop, pod maturation begins. Beginning maturity, reproductive stage seven (R7), is characterized by the presence of one pod that has reached pod fill. Full maturity, reproductive stage eights (R8), is the final stage in soybean growth and development. Full maturity has been reached when 95% of the pods have attained their mature color, normally brown or tan, and are ready for harvest (Fehr and Caviness 1977, Pedersen 2004, Plumblee and Harrelson 2022).

National Importance of Soybean

Soybean is the most economically important crop grown for oil and protein production in the United States. Today, soybean producers in the United States are responsible for one third of
the global soybean production (Ritchie and Roser 2021). In 2020, 32% of the total United States crop land was designated to the production of soybeans, second only to field corn, maize (Zea mays L.) (Mourtzinis and Conley 2017). Cumulatively, farmers in the United States planted 33.6 million hectares of soybean, producing a national yield of 112.5 million metric tons. This yield was responsible for over 30 billion USD of revenue, in 2020 (ASA 2021).

**Importance of Soybean in Mississippi**

Soybean is currently the leading row crop commodity produced in the state of Mississippi. Employing over seven thousand Mississippi residents, soybean production is an integral part of the state’s economy. Harvested on almost 850,000 hectares, Mississippi soybean production systems accounted for 1.49 billion USD of revenue, in 2020 (Musser et al. 2021, Robert and Gregory 2021). While Mississippi continues to produce excellent soybean yields from year to year, attainable yields are often inhibited by the intervention of numerous plant and insect pests. Each year, Mississippi soybean producers are required to address infestations of numerous insect pests to prevent economic yield loss. Many yield limiting insect pests such as the soybean looper, *Chrysodeixis includens* (Walker); kudzu bug, *Megacopta cribraria* (F.); stink bugs, *Pentatomid spp.*; green cloverworm, *Hypena scabra* (F.); and corn earworm, *Helicoverpa zea* (Boddie), are known to infest over half of the total soybean hectares planted in Mississippi each year (Musser et al. 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022).
**Helicoverpa zea**

**Taxonomy, Distribution, and Host Species**

*Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), is one of the most prevalent and damaging insect crop pests in North America, causing millions of dollars of economic damage each year (Capinera 2000). *Helicoverpa zea* (corn earworm, bollworm, or tomato fruitworm) is a lepidopteran pest that belongs to the family Noctuidae. The family Noctuidae includes cutworms, noctuid moths, owlet moths as well as many others (Nishida 2002). Its distribution spans from the southern tip of South America, throughout the United States, and into some parts of Canada (Hardwick 1965, McClanahan and Elliott 1976). Although the geographic origin of this insect is unknown, it was first considered an economic pest in North America in 1820 (Quaintance and Brues 1905). This polyphagous insect is known to feed on over one hundred plant species, ranging from a variety of wild hosts to commercially grown row crops (Bragard et al. 2020).

Wild host plant species of *Helicoverpa zea* include palmer pigweed, *Amaranthus palmeri* (S. Wats); wild oats, *Avena sativa* (L. Var Hinoat); and common crown vetch, *Coronilla varia* (L.). However, *Helicoverpa zea* is most well-known for its feeding on commercialized crops such as upland cotton, *Gossypium hirsutum* (L.); soybean, *Glycine max* (L.) Merrill; corn, *Zea mays* (L.); grain sorghum, *Sorghum bicolor* (L.) Moench; peanut, *Arachis hypogaea* (L.); and sugarcane, *Saccharum officinarum* (L.) (Johnson et al. 1975, Sudbrink and Grant 1995).

**Life Cycle and Identification**

While *Helicoverpa zea* is broadly distributed across North America, its distribution and survival are largely dependent on its life cycle. *Helicoverpa zea* develops through complete metamorphosis in four distinct life stages: egg, larva, pupa, and adult or moth (Quaintance and Brues 1905). Eggs are laid singly on host plants and begin as pearly, milky white spheres.
However, as the egg matures it develops into a yellowish gray color before hatching (Quaintance and Brues 1905). Complete egg development varies greatly based on environmental conditions, however eclosion typically occurs within two to eight days after oviposition (Quaintance and Brues 1905). After eclosion, larvae progress through six instars over a period of 14-25 days (CABI 2016). Larval color is variable and inconsistent. *Helicoverpa zea* larva may appear light brown, dark brown, creamy white, green, or orange. While larval color may not always be an effective identification characteristic, larva have an “inverted Y” on the crown of the head capsule that can be used to distinguish them from other species (Porter and Ludwick 2020). Also, larva often have a dark stripe above the spiracles that runs lengthwise of the body (Capinera 2000). Each larval instar is characterized by head capsule width and larval size. Estimated head capsule widths for each instar are 0.29, 0.47, 0.77, 1.30, 2.12, and 3.10 mm, respectively (Capinera 2000). Under normal climate conditions, approximately 32° C, this species will progress through instars one to six over a period of nine to twenty days, respectively (Quaintance and Brues 1905). Once the larvae are full grown, about 32 mm long, they climb down from their feeding sites and burrow into the ground to pupate (Eaton 2016). Bollworm pupae are reddish-brown in color and measure 25mm in length (Mayer 2003). Pupa can be found five to thirteen centimeters underground, often below crop cover (Drost 2021). Pupal development is highly dependent upon temperature. During the summer, pupation lasts ten to twenty-five days, however, as temperatures decrease and winter approaches, *Helicoverpa zea* can diapause in its pupal form and can remain under ground until spring (Mayer 2003). While *Helicoverpa zea* is capable of overwintering as pupa, it is not thought to overwinter at latitudes greater that 40 degrees, or about Kansas, Ohio, Virginia, and southern New Jersey (Capinera 2008). Adults then emerge as moths to mate and produce offspring. The forewings of *Helicoverpa zea* moths are
often a yellowish brown (female) to greenish tan (male) color, while the hindwings are milky white basally and darken distally (Capinera 2000, Reisig 2022). *Helicoverpa zea* moths possess a small dark spot near the middle of their forewings that can be used for species identification. The head, thorax, and abdomen typically measure fourteen to eighteen mm in length, cumulatively, with a wingspan of thirty to forty-seven mm in width (Edde 2022). Female moths are capable of ovipositing up to 3000 eggs (Capinera 2000). Fecundity has also been shown to be dependent on factors such as crop irrigation and fertilization (Braswell et al. 2019). The average life span of an adult moth is five to fifteen days, however under optimal conditions, moths have been reported to live up to 30 days after eclosion (Capinera 2000).

**Helicoverpa zea in Soybean**

*Helicoverpa zea* is one of the most economically important pests of soybean in the U.S. (Schug et al. 2022). Infestations of *Helicoverpa zea*, in soybeans, typically occur when field corn begins to dry down, rendering it unattractive for bollworm suitability. In soybeans, adult moths prefer to oviposit in the upper portion of the plant, primarily in the terminal and on the underside of leaves (Luttrell and Jackson 2012). After emergence from the chorion, first and second instar larva tend to feed in the top of the canopy on young foliage and terminals (Swenson et al. 2013). As larvae continue to grow and develop, they begin feeding lower into the canopy, primarily targeting reproductive structures, such as the pods and flowers of the soybean plant (Luttrell and Jackson 2012, Olmstead et al. 2016). Consumption by larvae increase as the larvae progress through instars and consume more during the last instar than during all previous instars combined (McWilliams 1983).
*Helicoverpa zea* in Mississippi Soybean

*Helicoverpa zea* infestations in Mississippi soybean vary considerably from one year to the next. However, *Helicoverpa zea* continue to be among the top yield limiting insect pests of soybean in Mississippi each year. In some areas, economic infestations occur every year. In some cases, over 70% percent of the total soybean hectares planted, within a given year, encountered infestations of bollworms (Musser et al. 2012). In 2020, populations of *Helicoverpa zea* cost Mississippi producers over 22 million dollars in yield loss as well as the cost required to control these populations (Musser et al. 2021). The Mississippi State University Extension Service offers economic thresholds that describe when control measures are necessary to prevent economic yield loss from specified insect pests.

**Economic Threshold**

Currently, there are economic thresholds for *Helicoverpa zea* in Mississippi soybean for when infestations occur before bloom and during bloom. Before bloom, treatment is recommended when soybeans have sustained 35% defoliation. After bloom, the threshold is three to five *Helicoverpa zea* larva per row meter when using a drop cloth, and nine larva per 25 sweeps when using a sweep net (Crow et al. 2023). The Mississippi State University Extension Service also offers dynamic thresholds for corn earworm infestations in soybean that account for fluctuations in crop protection costs, as well as commodity prices, based on economic injury levels reported in Adams et al. 2016 (Adams et al. 2016, Crow et al. 2023).

**Control Tactics**

When populations of insect pests, such as *Helicoverpa zea*, reach or surpass the economic threshold, treatment of some sort is required to prevent economic yield loss. A well-designed
integrated pest management strategy allows producers to combat the effects of harmful pests in their environment through the use of several different control methods. While integrated pest management strategies are multifaceted, pest control methods generally fall under three categories: cultural, chemical, and biological control (Olmstead et al. 2016).

Cultural Control

Yield limiting populations of *Helicoverpa zea* can be regulated using a variety of diverse cultural control methods. Fall tillage, for instance, is a form of cultural control that has been shown to produce results up to 100% mortality of overwintering *Helicoverpa zea* (Fife and Graham 1966). Variety selection is also known to play a key role in pest presence. Selecting varieties that quickly develop closed canopies can likely reduce the probability of reaching the economic threshold for corn earworms (Alston et al. 1991). In addition, practices such as intercropping can provide a habitat more suitable for natural enemies, conceivably reducing populations of *Helicoverpa zea* (Alston et al. 1991).

Chemical Control

Once populations of *Helicoverpa zea* reach the economic threshold, action must be taken to prevent economic yield loss. Currently, the primary control method of *Helicoverpa zea* is the use of synthetic insecticides. Foliar applications of select chemistries are an integral part of soybean production in many southern soybean production systems (Adams et al. 2016). Synthetic insecticides have been the leading control measure for *Helicoverpa zea* since 1943, when G.V. Johnson began testing DDT for control of the corn earworm (Johnson 1944). Several insecticide classes are currently registered for the control of *Helicoverpa zea*, however repetitive, widespread foliar applications of many of these classes have rendered them ineffective (Musser
Insecticides such as organophosphates, carbamates, pyrethroids, chlorinated hydrocarbons, and benzoylphenylureas have all encountered resistance or inefficiency in recent years (Musser and Shelton 2003, Walsh et al. 2022). In 2008, the Environmental Protection Agency approved a new class of insecticides, known as diamides, which possessed a new mode of action classified as ryanodine receptor modulators (Adams et al. 2016). Due to the history of insecticidal resistance, it has become necessary to develop new modes of action that have low possibilities of resistance while maintaining efficacious on populations of *Helicoverpa zea* (Ginting et al. 2019).

**Biological Control**

Biological control methods are often separated into three categories: predation, parasitism, and viral infection. Several species such as spotted lady beetles, *Coleomegilla maculate* (De Geer); big-eyed bugs, *Geocoris punctipes* (Say); insidious flower bugs, *Orius insidiosus* (Say); and tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), have been documented as predators of *Helicoverpa zea* (Pfannenstiel and Yeargan 2002). Similarly, other species are known to regulate populations of *Helicoverpa zea* through parasitism. These species include *Archytas marmoratus* (Townsend), *Carcelia illota* (Curran), as well as *Microplitis*, *Telenomus*, and *Trichogramma* spp. (Reisig 2022). While many species are capable of predation and parasitism of *Helicoverpa zea*, perhaps the leading form of biological control for *Helicoverpa zea* is the implementation of insect viruses. There are more than 1,100 species of viruses, most of which can infect insects (Fuxa 1991). Most insect viruses belong to the family Baculoviridae, characterized by the infection of an insect after viral occlusion bodies have been ingested (Cory and Myers 2003, Abbas 2020). Baculoviruses are divided into two groups, nucleopolyhedroviruses (NPVs) and granuloviruses (GVs), based on the quantity of virions and...
the size of occlusion bodies (Cory and Myers 2003). Baculoviruses are further divided into four genera, *Alphabaculovirus* (lepidopteran-specific NPV), *Betabaculovirus* (lepidoptera-specific GV), *Gammabaculovirus* (hymenopteran-specific NPV), and *Deltabaculovirus* (dipteran-specific NPV) (Eroglu et al. 2020). Nucleopolyhedroviruses, in particular, have been found to infect seven insect orders, as well as Crustacea (Federici 1997). While baculoviruses infect numerous insect orders, the most commonly studied baculoviruses are lepidopteran-specific NPV’s (Davis 2019). Viruses in all genera are highly host specific and are therefore considered harmless to non-target species (Landwehr 2021). Many baculoviruses have been commercialized for sale as group 31 insecticides (biological insecticides). *Helicoverpa armigera* Nucleopolyhedrovirus (*Hear* NPV) is an *Alphabaculovirus* commercially available for the control of *Helicoverpa* spp. *Hear* NPV has been commercially available since the release of Elcar®, manufactured by Sandoz Incorporated in the mid-1970s (Teakle et al. 1986). The use of commercialized insect viruses should be at least be considered when developing an integrated pest management strategy because of their potential ability to reduce the rate at which species develop resistance to synthetic insecticide classes (Landwehr 2021).

*Helicoverpa armigera* Nucleopolyhedrovirus

Nuclear polyhedrosis viruses consist of rod-shaped nucleocapsids with double-stranded DNA (Bilimoria 1986). They form large occlusion bodies, which range from 1-15 mm These occlusion bodies contain many virions and serve as a protection mechanism from environmental factors (Fuxa 2004). Once occlusion bodies of *Hear* NPV particles have been ingested, they dissolve in the midgut epithelium, releasing the occlusion-derived virus (Bilimoria 1991). Infection then spreads to various tissues, primarily fat body, epithelial cells, hemocytes, hypodermis, and the tracheal matrix. Within these tissues, enormous quantities of virions are
produced and occluded that the host-cell nuclei become completely packed with occlusion bodies (Fuxa 2004). After infection, larvae may appear swollen, glossy, or lethargic (Kumar et al. 2011). During the later phase of viral infection, NPV-infected larvae tend to climb to the highest point in the crop canopy where liquification of the integument occurs, releasing mass quantities of viral occlusion bodies into the environment (Fuxa 2004). The first three larval instars of *Helicoverpa zea* are highly susceptible to the virus, and often experience 100% mortality at low LT50 values. The average viral incubation period for third instar larva is five to seven days after ingestion of *Hear*NPV (Bandi et al. 2021). However, after larvae reach the fourth instar, they become much less susceptible to viral infection. This indicates that NPV efficacy is directly correlated to the age of larva at the time of infection, limiting its usefulness as a pest management tool (Kumar et al. 2011).

Several studies have been conducted to evaluate the impact of environmental and agricultural factors such as irrigation timing, dew pH, leaf pH, temperature, distribution on the plant, and ultraviolet radiation on residual activity of *Hear*NPV (McLeod et al. 1976, Young et al. 1977, Young 1990, Young and Yearian 1992). *Hear*NPV is not easily deactivated by water, but is capable of being dispersed throughout the crop canopy by light rains and overhead irrigation (Young et al. 1977, Young 1990). While occlusion bodies protect the virus in transit, they do not provide protection against UV degradation. This is a substantial disadvantage of *Hear*NPV compared to chemical insecticides (Benz 1987). The probability of rapid degradation of *Hear*NPV is one reason the use of biological insecticides has not been widely implemented in agro-ecosystems (Ignoffo et al. 1972). Most viral degradation occurs during daylight hours from UV exposure and temperature. This indicates that sunlight is the most limiting factor with regard to *Hear*NPV persistence in the environment (McLeod et al. 1976, Young and Yearian 1992).
HearNPV persists in the environment through replication within hosts and infection of new larvae (Kumar et al. 2011). Horizontal transmission is believed to be the primary form of viral persistence and dissemination. This occurs when infected larvae interact with susceptible larvae in a manner capable of transferring occlusion bodies, resulting in exposure and potential infection (Cory and Myers 2003). Horizontal transmission of HearNPV has several well documented transmission paths including abiotic factors, larva-to-larva, predator-to-larva, and parasitoid-to-larva (Black 2017).

Abiotic factors potentially responsible for the dissemination of HearNPV include wind, rain, and soil profile (Fuxa 2004, Fuxa and Richter 2007). While HearNPV does not cause mortality in predators of the Helicoverpa zea, predacious species such as big-eyed bugs as well as many spiders (Arachnid spp.) are known to be carriers of the virus, and are capable of dispersing infectious occlusion bodies through their frass (Black 2017). In addition, larval parasitoids, namely Trichogramma spp. and Microplitis croceripes (Cresson), have been documented as a potential mode of horizontal transmission via ovipositor contamination (Black 2017). However, parasitoids are not considered to be a primary source of horizontal transmission, of HearNPV to Helicoverpa zea larvae (Young and Yearian 1989).

The most prevalent source of virus sustainability within the environment is the transmission of HearNPV from one larva to the next. Infected larvae are capable of dispersing occlusion bodies of HearNPV in concentrations high enough to infect susceptible larvae through fecal contamination, cannibalism, and postmortem liquification (Ali et al. 1987, Chapman et al. 1999, Vasconcelos 1996). However, low frequencies of larval infection from fecal contamination and cannibalism suggest these mechanisms are unlikely to be responsible for significant epizootic events (Ali et al. 1987, Dhandapani et al. 1993, Chapman et al. 1999, Cory and Myers
Postmortem liquification is widely accepted as the predominant mode of horizontal transmission from one larva to the next (Ali et al. 1987, Dhandapani et al. 1993, Chapman et al. 1999, Cory and Myers 2003). Lysis, also referred to as liquification, is the disintegration of larval integument during the final stages of NPV replication (Ali et al. 1987). Liquification of the virus-killed larvae will spread infectious occlusion bodies over a vast area, via wind and rain. This increases the likelihood that susceptible hosts will encounter and ingest them, providing *HearNPV* an additional host allowing the virus to persist and replicate within the environment (Cory and Myers 2003).

**Objectives**

Currently, there are little data on the length of time *HearNPV* will remain active within a soybean canopy after initial application occurs. Also, it has been proposed that additives, such as surfactants, are capable of increasing the viral persistence of *HearNPV* in the environment. It is well-documented that mortality may not occur for several days after ingestion of *HearNPV*. However, the feeding habits of infected *Helicoverpa zea* larvae has not been fully investigated. In response to this, studies were conducted to determine how long *HearNPV* would remain active in a soybean canopy, and if a surfactant, currently recommended for co-application with *HearNPV* would aid in viral persistence. Studies were also conducted to determine the feeding habits of *Helicoverpa zea* larvae after ingestion of *HearNPV*.  

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**Literature Cited**


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CHAPTER II

ACTIVITY LEVEL OF *HELICOVERPA ARMIGERA* NUCLEOPOLYHEDROVIRUS

WITHIN A SOYBEAN FIELD AT DISTINCT TIME INTERVALS FOLLOWING

APPLICATION

Abstract

*Helicoverpa armigera* Nucleopolyhedrovirus (*HearNPV*) is an insect virus in the family *baculoviridae* and is commercially available as a biological pesticide for the control of *Helicoverpa zea* (Boddie). *HearNPV* offers potential benefits such as host specificity, economic affordability, and the ability to create epizootic events leading to the viral infection of multiple generations. However, a notable disadvantage of *HearNPV* as a biopesticide is its susceptibility to degradation by ultraviolet radiation. Ultraviolet light degradation is a well-documented phenomenon in the world of biopesticides, however few studies have been conducted to evaluate the length of time *HearNPV* will remain active in a soybean field under natural conditions. This study was conducted to determine the activity level of *HearNPV* at different time intervals over a period of three days, and to determine if the addition of a molasses-based surfactant would increase residual mortality of *H. zea* larvae. This was accomplished by obtaining leaf samples from a soybean field treated with *HearNPV* at different time intervals following application. Susceptible larvae were allowed to feed on contaminated leaf tissue, as well as artificial diet overlayed with a rinsate acquired from leaf tissue collections. Susceptible larvae were rated for mortality at five, seven, and twelve days after exposure. The molasses-based surfactant was co-
applied with *HearNPV* as a separate treatment. Results were compared at each time interval across all treatments. When applied at the recommended rate, *HearNPV* resulted in greater than 50% mortality, out to 36 hours after application when mortality was rated at twelve days after exposure. However, the molasses-based surfactant did not increase persistence or mortality of *HearNPV*.

**Introduction**

*Helicoverpa zea* (Boddie), (Lepidoptera: Noctuidae), is one of the most prevalent and damaging insect crop pests in North America, causing millions of dollars of economic damage each year (Capinera 2000). In 2021, *H. zea* was responsible for over $33 million of soybean damage in the state of Mississippi (Musser et al. 2022). Infestations of *H. zea* are commonly managed by applications of foliar insecticides (Viteri and Linares-Ramírez 2022). Over time, *H. zea* has developed resistance to insecticides in the organophosphate, carbamate, pyrethroid, chlorinated hydrocarbon, and benzoylphenylurea classes (Musser and Shelton 2003). During 2008, insecticides representing the diamide class, a new class with a novel mode of action, were registered. Diamide insecticides act on the ryanodine receptor in insect muscle cells (Cordova et al. 2006, Teixeira and Andaloro 2013). Due to the history of insecticidal resistance, effective insecticide resistance management (IRM) is critical to maintain the efficacy of current and future insecticides (Sparks et al. 2020). In pursuit of effective IRM, it has become necessary to utilize new modes of action with low probabilities of resistance while maintaining efficient control of *Helicoverpa zea* (Ginting et al. 2019). Recently, interest in biological control agents for the control of *Helicoverpa zea* has increased (Black et al. 2022). One biological control agent that has received extensive attention in recent years is a host specific *Alphabaculovirus, Helicoverpa armigera* Nucleopolyhedrovirus (*HearNPV*).
HearNPV is a commercially available insect virus (group 31 insecticide) that is exclusively active against *Chloridea virescens* and *Heliothis spp.* (Sparks et al. 2020, Landwehr 2021). The double-stranded DNA of *HearNPV* form occlusion bodies which provide some protection during host-to-host transfer (Fuxa 2004). However, these occlusion bodies do not provide adequate protection from sunlight ultraviolet radiation (UV) (McLeod et al. 1976). Ultraviolet light is the primary cause of *HearNPV* inactivation (Bullock 1967, McLeod et al. 1976, Young and Yearian 1992). Inactivation of *HearNPV* is directly related to the period of UV radiation exposure (McLeod et al. 1976). Residual viral activity in many commercially grown crops has been extensively studied with *HearNPV*. However, the results of these studies are highly variable. *HearNPV* activity can range from twelve hours up to three days when targeting *Helicoverpa spp.* (Bullock 1967, McLeod et al. 1976, Young and McNew 1994). The impact of surfactants, including cotton seed kernel extract, carbon, and aluminum oxide, on UV degradation of *HearNPV* has been evaluated (Ignoffo and Batzer 1971, Ignoffo et al. 1976, Muthuswami et al. 1994). Some surfactants have been shown to provide protection to *HearNPV* from UV degradation (Ignoffo et al. 1976, Muthuswami et al. 1994). These surfactants provide more protection from UV degradation when co-applied with wettable powder formulations of *HearNPV*, rather than water-based formulations (Ignoffo et al. 1976). Additionally, when co-applied with *HearNPV*, some surfactants, such as corn extract, cotton seed extract, and crude sugar, can increase larval feeding and virus consumption (Montoya et al. 1966, Muthuswami et al. 1994). Increased viral consumption can result in higher mortality rates and reduced viral incubation periods (Allen and Pate 1965, Bell and Romine 1980, Muthuswami et al. 1994).

The objective of this study was to determine the persistence/residual activity of a commercial formulation of *HearNPV* (Heligen®, AgBiTech Corporation, Queensland,
Australia) in Mississippi soybean production systems, and to investigate the effects of a feeding stimulant/surfactant (Optimol®, AgBiTech Corporation, Queensland, Australia) on persistence of *HearNPV*.

**Materials and Methods**

Trials were conducted at the R. R. Foil Plant Science Research Center in Starkville, MS to accomplish these objectives. Trials were arranged as a randomized complete block design with three replications. Treatments included an untreated check, *HearNPV* alone, and *HearNPV* + feeding stimulant/surfactant. Plots were four rows by 12.19 m, with 0.96 m row spacing. The indeterminate maturity group V soybean variety (Asgrow 55XFO®, Bayer CropScience, St. Louis, MO) was planted at 272,000 seeds per hectare, into raised conventional tilled beds on 3 June 2021, and 31 May 2022. The middle two rows of each plot were treated and sampled, leaving the exterior rows untouched to act as a border between plots. The trial was repeated four times using one half of the labeled rate of *HearNPV* (Heligen®, 58 mL/ha) and four times using the labeled rate of *HearNPV* (Heligen®, 116 mL/ha). The labeled rate of the feeding stimulant/surfactant (Optimol®, 232 mL/ha) was used in all trials. Trials were repeated over a period of two years, 2021 and 2022. Two half rate trials and two full rate trials were conducted each year. Each year, trials were initiated when soybeans reached the R3 stage. All corn earworm larvae were obtained from the Mississippi State Insect Rearing Center in Starkville, MS. The Mississippi State corn earworm colony originated from a collection in non-Bt corn in 2008. The colony has been infused with wild individuals collected from non-Bt corn on a biannual basis.

Plots were sprayed using a CO₂ backpack sprayer equipped with TX6 hollow cone nozzles (TeeJet®, Glendale Heights, IL) calibrated to deliver 93.46 L/ha at 275.79 kPa. The untreated control plots were sprayed first with uncontaminated water, *HearNPV* treatment plots
were sprayed second, and the *HearNPV* + feeding stimulant/surfactant plots were sprayed last to ensure no cross-contamination would occur between plots. In addition, Tyvek suits and surgical gloves were worn and discarded after spraying each treatment to ensure no cross-contamination would occur during application. When the solution had dried on the leaf surface, approximately 20 minutes after application, a 0-hour interval collection was conducted by collecting forty leaves from the top of the canopy throughout each plot that were then sealed in a 3.79-liter plastic bag (Ziploc®, S.C. Johnson and Johnson, Inc. Racine, WI), labeled and separated by plot number and treatment.

**Leaf Rinsate Bioassay**

Twenty leaves from each plot were collected and transported to the laboratory and placed in 0.95-liter glass jars. The untreated checks were handled first, then *HearNPV*, and lastly *HearNPV* + feeding stimulant/surfactant, while changing and discarding surgical gloves between treatments. 100 mL of distilled water were added to each mason jar, the jar was shaken vigorously for thirty seconds to dislodge any residual virus that may have been present on the leaves. 200µL of the solution acquired from each jar was dispensed into 36.97 mL cups (Dart Container, Mason, MI) containing artificial diet (F9781B, Frontier Scientific Services, Newark, DE). Ten diet cups were used for each plot resulting in 30 samples per treatment per time interval. The solution was allowed to absorb into the diet for three hours before cups were infested with one neonate. The larvae were sealed in the cups and rated for mortality at five, seven, and twelve days after infestation. In order to test the persistence/residual of infectious levels of *HearNPV*, these procedures were repeated at 12, 24, 36, 48, and 72 hours after application.
**Leaf Feeding Bioassay**

Twenty leaves from each plot were collected and placed in an aluminum foil pan (25 cm x 36 cm) labeled by plot number and treatment. Twenty-five 2nd instar larvae were placed directly on the leaves and allowed to feed for 48 hours. Each container was covered with a clear lid to prevent larval escape. Six holes, 6mm in diameter, were drilled into the sides of each lid to allow airflow and to minimize leaf tissue degradation. The holes were covered with 100-micron mesh screens to prevent larval escape. After 48 hours, ten larvae from each plot/container were removed and placed individually in separate diet cups containing artificial diet. Larvae were rated for mortality at five, seven, and twelve days after infestation. These procedures were repeated at 3, 6, 12, and 24 hours after application.

**Data Analyses**

Data from the leaf rinsate and leaf feeding studies were subjected to quadratic regression analyses using the Proc GLIMMIX procedure in SAS 9.4 (SAS Institute, 2016). Larval mortality resulting from *HearNPV* and *HearNPV* + feeding stimulant/surfactant treatments were corrected for control mortality using Abbott's formula (Abbott 1925). Trial and replication nested within trial were considered random effects. Orthogonal contrasts were used to compare the slopes and intercepts of the regression equations for *HearNPV* and *HearNPV* + feeding stimulant/surfactant. *HearNPV* and *HearNPV* + feeding stimulant/surfactant data were combined and means were separated using the Proc MEANS procedure in SAS 9.4 (SAS Institute, 2016). Quadratic and linear term slopes of the regression equations were compared by rating intervals using the contrast function. Additionally, the contrast function was used to compare the intercepts of the regression equations by rating intervals.
Results

Leaf Rinsate Bioassay

When the lower rate of HearNPV was used, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed for both HearNPV and HearNPV + feeding stimulant/surfactant, when mortality was rated at five days after infestation (DAI) (Table 2.1). The slopes of the regression equations for larval mortality at five DAI with HearNPV and HearNPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.2). When mortality was rated at seven DAI, a significant quadratic relationship was observed between *H. zea* mortality and hours after application before larval exposure for both HearNPV and HearNPV + feeding stimulant/surfactant (Table 2.1). The slopes of the regression equations for larval mortality at seven DAI with HearNPV and HearNPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.2). Additionally, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed for both HearNPV and HearNPV + feeding stimulant/surfactant, when mortality was rated at twelve DAI (Table 2.1). The slopes of the regression equations for larval mortality at twelve DAI with HearNPV and HearNPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.2). No differences were detected between the slopes or intercepts of regression equations for larval mortality and hours after application for HearNPV and HearNPV + feeding stimulant/surfactant, when mortality was rated at five, seven, or twelve DAI. Therefore, mortality data for HearNPV and HearNPV + feeding stimulant/surfactant were combined within a rating date (five, seven, or twelve DAI). These combined data were subjected to regression analyses. A significant quadratic relationship
between *H. zea* mortality and hours after application before larval exposure was observed when larval mortality was rated at five DAI ($x^2: F=33.16, df=1,130, P<0.01$; $x: F=70.40, df=1,130, P<0.01$), seven DAI ($x^2: F=30.68, df=1,130, P<0.01$; $x: F=80.19, df=1,130, P<0.01$), and twelve DAI ($x^2: F=12.03, df=1,130, P<0.01$; $x: F=44.52, df=1,130, P<0.01$) (Figure 2.1). When comparing different rating intervals, no differences were detected in the slopes of the regression equations for five, seven, and twelve DAI (Table 2.3). However, as shown in Table 2.3, differences were observed between the intercepts of the five DAI and seven DAI regression equations, the five DAI and twelve DAI regression equations, and the seven DAI and twelve DAI regression equations (Table 2.3).

In trials when the labeled rate of *Hear*NPV was used, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed for both *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant, when mortality was rated at five DAI (Table 2.4). The slopes of the regression equations for larval mortality at five DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.5). When mortality was rated at seven DAI, a significant quadratic relationship was observed between *H. zea* mortality and hours after application before larval exposure for both *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant (Table 2.4). The slopes of the regression equations for larval mortality at seven DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.5). Additionally, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed for both *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant, when mortality was rated at twelve DAI (Table 2.4). The slopes of the regression equations for larval mortality at
twelve DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.5). No differences were detected between the slopes or intercepts of regression equations for larval mortality and hours after application for *Hear*NPV and *Hear*NPV+ feeding stimulant/surfactant, when mortality was rated at five, seven, or twelve DAI. Therefore, mortality data for *Hear*NPV and *Hear*NPV+ feeding stimulant/surfactant were combined within a rating date (five, seven, or twelve DAI). These combined data were subjected to regression analyses. A significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed when larval mortality was rated at five DAI (x^2: F=18.72, df=1,130, P=<0.01; x: F=60.13, df=1,130, P=<0.01), seven DAI (x^2: F=20.03, df=1,130, P=<0.01; x: F=77.07, df=1,130, P=<0.01), and twelve DAI (x^2: F=4.85, df=1,130, P=0.03; x: F=35.12, df=1,130, P=<0.01) (Figure 2.2). When rating intervals were compared, no differences were detected in the slopes of the regression equations for five DAI and seven DAI, five DAI and twelve DAI, or seven DAI and twelve DAI (Table 2.6). As shown in Table 2.6, statistical differences were observed between the intercepts of the five DAI and seven DAI regression equations, and the five DAI and twelve DAI regression equations. The intercepts of the seven DAI and twelve DAI regression equations did not statistically differ (Table 2.6).

**Leaf Feeding Bioassay**

When the lower rate of *Hear*NPV was used in the leaf feeding bioassay, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed for both *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant, when larval mortality was rated at five DAI (Table 2.7). The slopes of the regression equations for larval mortality at five DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were
not different, and there were no differences between the intercepts (Table 2.8). When mortality was rated at seven DAI, a significant quadratic relationship was observed between *H. zea* mortality and hours after application before larval exposure for both *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant (Table 2.7). The slopes of the regression equations for larval mortality at seven DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.8). When mortality was rated at twelve DAI, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed for both *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant (Table 2.7). The slopes of the regression equations for larval mortality at twelve DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.8). No differences were detected between the slopes or intercepts of regression equations for larval mortality and hours after application for *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant, when mortality was rated at five, seven, or twelve DAI. Therefore, mortality data for *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were combined within a rating date (five, seven, or twelve DAI). These combined data were subjected to regression analyses. A significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed when larval mortality was rated at five DAI (*x^2*: $F=37.57$, $df=1,106$, $P=<0.01$; $x$: $F=67.43$, $df=1,106$, $P=<0.01$), seven DAI (*x^2*: $F=32.90$, $df=1,106$, $P=<0.01$; $x$: $F=64.29$, $df=1,106$, $P=<0.01$), and twelve DAI (*x^2*: $F=37.22$, $df=1,106$, $P=<0.01$; $x$: $F=74.29$, $df=1,106$, $P=<0.01$) (Figure 2.3). When rating intervals were compared, no differences were detected in the slopes of the regression equations between five DAI and seven DAI, five DAI and twelve DAI, or seven DAI and twelve DAI (Table 2.9). No differences were detected between the intercepts of the five DAI
and seven DAI regression equations, or the regression equations of seven DAI and twelve DAI (Table 2.9). However, as shown in Table 2.9, differences were observed between the intercepts of the five DAI and twelve DAI regression equations.

In the leaf feeding bioassay, when the labeled rate of *Hear*NPV was used, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed for both *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant, when mortality was rated at five DAI (Table 2.10). The slopes of the regression equations for larval mortality at five DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.11). When mortality was rated at seven DAI, a significant quadratic relationship was observed between *H. zea* mortality and hours after application before larval exposure for *Hear*NPV but not for *Hear*NPV + feeding stimulant/surfactant (Table 2.10). The slopes of the regression equations for larval mortality at seven DAI with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.11). Similarly, a significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed at twelve DAI for *Hear*NPV, but not for *Hear*NPV + feeding stimulant/surfactant (Table 2.10). However, at twelve DAI, the slopes of the regression equations for larval mortality with *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were not different, and there were no differences between the intercepts (Table 2.11). No differences were detected between the slopes or intercepts of regression equations for larval mortality and hours after application for *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant, when mortality was rated at five, seven, or twelve DAI. Therefore, mortality data for *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant were combined within a rating date (five, seven, or twelve DAI). These
combined data were subjected to regression analyses. A significant quadratic relationship between *H. zea* mortality and hours after application before larval exposure was observed when larval mortality was rated at five DAI ($x^2$: $F=21.47$, $df=1,118$, $P=<0.01$; $x$: $F=52.62$, $df=1,118$, $P=<0.01$), seven DAI ($x^2$: $F=10.72$, $df=1,118$, $P=<0.01$; $x$: $F=43.05$, $df=1,118$, $P=<0.01$), and twelve DAI ($x^2$: $F=11.43$, $df=1,118$, $P=<0.01$; $x$: $F=39.51$, $df=1,118$, $P=<0.01$) (Figure 2.4). When rating intervals were compared, no differences were detected in the slopes of the regression equations for five DAI and seven DAI, five DAI and twelve DAI, or seven DAI and twelve DAI (Table 2.12). Differences were detected between the intercepts of five DAI and seven DAI and five DAI and twelve DAI, but not between the intercepts of seven DAI and twelve DAI (Table 2.12).

**Discussion**

Degradation by ultraviolet light is a major limitation on the activity of *HearNPV* in the field (McLeod et al. 1976, Young and Yearian 1992). The longevity or residual activity of *HearNPV* against *H. zea* and *C. virescens* under field conditions in cotton, grain sorghum, and soybean ranges from 12 to 72 hours (Bullock 1967, McLeod et al. 1976, Young and McNew 1994). In the current studies, *HearNPV* resulted in greater than or equal to fifty percent mortality of *H. zea* out to ca. twenty-nine hours and thirty-eight hours in the leaf rinsate and leaf feeding bioassays, respectively. Several adjuvants have been shown to increase mortality of *C. virescens* and *H. zea* when applied with *HearNPV* (Allen and Pate 1965, Montoya et al. 1966, Muthuswami et al. 1994). In the current studies, molasses-based feeding stimulant/surfactant did not increase larval mortality in any instance. Also, in the current studies, higher larval mortality was observed
at ≥ seven days after exposure compared to < seven days. This was similar to that observed in previous studies (Muthuswami et al. 1994).

The *HearNPV* formulation used in the current studies performed similar to other formulations evaluated by McLeod et al. (1976) and Young and McNew (1994) with regard to speed of kill and ultraviolet light stability. These factors have been an impediment in the use of *HearNPV* across many different crops. However, in some crops, such as soybean, *HearNPV* has a potential fit in management programs for *H. zea*, because thresholds are higher than in some other crops. Also, *HearNPV* is unlikely to flare other insect pests and can be economically attractive compared to other management tools. However, the short residual of *HearNPV* could be problematic in situations where oviposition occurs over an extended period of time (greater than three to four days). Also, the utility of *HearNPV* can be diminished if multiple pest species are present in the field at the same time.
Table 2.1  Regression equations and statistics for the relationship between *H. zea* mortality and time between application and larval infestation for *HearNPV* applied at 58 mL/ha and *HearNPV* at 58 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf rinsate bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
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<th>5 DAI</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
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<td>Treatment</td>
<td>Regression Equation</td>
<td>F</td>
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<td><em>HearNPV</em></td>
<td>y = 0.0132x^2 (0.0031) - 1.3914x (0.2322) + 37.9754</td>
<td>18.48</td>
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<tr>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>y = 0.0134x^2 (0.0035) - 1.5328x (0.2616) + 45.0839</td>
<td>14.88</td>
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<table>
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<th>7 DAI</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Regression Equation</td>
<td>F</td>
</tr>
<tr>
<td><em>HearNPV</em></td>
<td>y = 0.0154x^2 (0.0036) - 1.7720x (0.2755) + 57.0252</td>
<td>17.73</td>
</tr>
<tr>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>y = 0.0155x^2 (0.0042) - 1.9928x (0.3141) + 69.8365</td>
<td>13.84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12 DAI</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Regression Equation</td>
<td>F</td>
</tr>
<tr>
<td><em>HearNPV</em></td>
<td>y = 0.0125x^2 (0.0048) - 1.7210x (0.3591) + 71.4177</td>
<td>6.85</td>
</tr>
<tr>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>y = 0.0117x^2 (0.0051) - 1.7845x (0.3869) + 80.7920</td>
<td>5.20</td>
</tr>
</tbody>
</table>
Table 2.2  Test for equal slopes and intercepts of the regression equations for *Hear*NPV applied at 58 mL/ha and *Hear*NPV at 58 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf rinsate bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
<thead>
<tr>
<th></th>
<th>Slope-Quadratic Term</th>
<th>Slope-Linear Term</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5 DAI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for Equality</td>
<td>0.01</td>
<td>1,138</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>7 DAI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for Equality</td>
<td>0.01</td>
<td>1,138</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>12 DAI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for Equality</td>
<td>0.01</td>
<td>1,138</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Table 2.3  Relationship between larval mortality rated at five, seven, and twelve DAI with *Hear*NPV applied at 58 mL/ha and *Hear*NPV at 58mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf rinsate bioassay.

<table>
<thead>
<tr>
<th>DAI</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F</em></td>
<td>df</td>
<td><em>P</em></td>
</tr>
<tr>
<td>5 vs 7</td>
<td>0.20</td>
<td>1,423</td>
<td>0.65</td>
</tr>
<tr>
<td>5 vs 12</td>
<td>0.07</td>
<td>1,423</td>
<td>0.80</td>
</tr>
<tr>
<td>7 vs 12</td>
<td>0.50</td>
<td>1,423</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 2.4  Regression equations and statistics for the relationship between *H. zea* mortality and time between application and larval infestation for *HearNPV* applied at 116 mL/ha and *HearNPV* at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf rinsate bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
<thead>
<tr>
<th>5 DAI</th>
<th>Treatment</th>
<th>Quadratic Term</th>
<th></th>
<th>Linear Term</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Regression Equation</td>
<td>F</td>
<td>df</td>
<td>P</td>
</tr>
<tr>
<td><strong>HearNPV</strong></td>
<td>y=0.0097x² (0.0039) -1.4037x (0.2976) +60.8227</td>
<td>6.05</td>
<td>1,58</td>
<td>0.02</td>
<td>22.25</td>
</tr>
<tr>
<td><strong>HearNPV + Feeding Stimulant/Surfactant</strong></td>
<td>y=0.0146x² (0.004) -1.8876x (0.3058) +67.5477</td>
<td>13.05</td>
<td>1,58</td>
<td>&lt;0.01</td>
<td>38.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7 DAI</th>
<th>Treatment</th>
<th>Quadratic Term</th>
<th></th>
<th>Linear Term</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HearNPV</strong></td>
<td>y= 0.0108x² (0.004) -1.7201x (0.3018) +85.7055</td>
<td>7.29</td>
<td>1,58</td>
<td>0.01</td>
<td>32.49</td>
</tr>
<tr>
<td><strong>HearNPV + Feeding Stimulant/Surfactant</strong></td>
<td>y=0.0148x² (0.0041) -2.0740x (0.3116) +90.2907</td>
<td>12.91</td>
<td>1,58</td>
<td>&lt;0.01</td>
<td>44.29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12 DAI</th>
<th>Treatment</th>
<th>Quadratic Term</th>
<th></th>
<th>Linear Term</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HearNPV</strong></td>
<td>y=0.0066x² (0.0046) -1.3291x (0.3489) +88.9786</td>
<td>2.06</td>
<td>1,58</td>
<td>0.16</td>
<td>14.51</td>
</tr>
<tr>
<td><strong>HearNPV + Feeding Stimulant/Surfactant</strong></td>
<td>y=0.0075x² (0.0045) -1.537x (0.3384) +95.1482</td>
<td>2.78</td>
<td>1,58</td>
<td>0.10</td>
<td>20.63</td>
</tr>
</tbody>
</table>
Table 2.5  Test for equal slopes and intercepts of the regression equations for *HearNPV* applied at 116 mL/ha and *HearNPV* at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf rinsate bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
<thead>
<tr>
<th></th>
<th>Slope-Quadratic Term</th>
<th></th>
<th>Slope-Linear Term</th>
<th></th>
<th>Intercept</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>df</td>
<td>P</td>
<td>F</td>
<td>df</td>
</tr>
<tr>
<td>5 DAI</td>
<td>Test for Equality</td>
<td>0.49</td>
<td>1,138</td>
<td>0.49</td>
<td>0.82</td>
<td>1,138</td>
</tr>
<tr>
<td>7 DAI</td>
<td>Test for Equality</td>
<td>0.27</td>
<td>1,138</td>
<td>0.60</td>
<td>0.37</td>
<td>1,138</td>
</tr>
<tr>
<td>12 DAI</td>
<td>Test for Equality</td>
<td>0.01</td>
<td>1,138</td>
<td>0.92</td>
<td>0.11</td>
<td>1,138</td>
</tr>
</tbody>
</table>
Table 2.6  Relationship between larval mortality rated at five, seven, and twelve DAI with *Hear*NPV applied at 116 mL/ha and *Hear*NPV at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf rinsate bioassay.

<table>
<thead>
<tr>
<th>DAI</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F</em> df <em>P</em></td>
<td><em>F</em> df <em>P</em></td>
<td><em>F</em> df <em>P</em></td>
</tr>
<tr>
<td>5 vs 7</td>
<td>0.01 1,423 0.90</td>
<td>0.38 1,423 0.54</td>
<td>14.33 1,423 &lt;0.01</td>
</tr>
<tr>
<td>5 vs 12</td>
<td>0.88 1,423 0.35</td>
<td>0.27 1,423 0.61</td>
<td>19.65 1,423 &lt;0.01</td>
</tr>
<tr>
<td>7 vs 12</td>
<td>1.12 1,423 0.29</td>
<td>1.28 1,423 0.26</td>
<td>0.42 1,423 0.52</td>
</tr>
</tbody>
</table>
Table 2.7  Regression equations and statistics for the relationship between *H. zea* mortality and time between application and larval infestation for *HearNPV* applied at 58 mL/ha and *HearNPV* at 58 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf feeding bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
<thead>
<tr>
<th>5 DAI</th>
<th>Treatment</th>
<th>Regression Equation</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F</em></td>
<td>df</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em></td>
<td>( y = 0.0608x^2 ) (0.0111) -3.8474x (0.5677) + 52.2665 )</td>
<td>29.68</td>
<td>1,46</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>( y = 0.0429x^2 ) (0.01237) -3.2152x (0.6287) + 60.0730 )</td>
<td>12.06</td>
<td>1,46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7 DAI</th>
<th>Treatment</th>
<th>Regression Equation</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F</em></td>
<td>df</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em></td>
<td>( y = 0.0544x^2 ) (0.0116) -3.5586x (0.5902) + 58.4174 )</td>
<td>21.94</td>
<td>1,46</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>( y = 0.0437x^2 ) (0.0116) -3.4067x (0.5919) + 70.8919 )</td>
<td>14.05</td>
<td>1,46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12 DAI</th>
<th>Treatment</th>
<th>Regression Equation</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F</em></td>
<td>df</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em></td>
<td>( y = 0.0613x^2 ) (0.0115) -4.1540x (0.5825) + 70.4137 )</td>
<td>28.58</td>
<td>1,46</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>( y = 0.0458x^2 ) (0.0119) -3.5332x (0.605) + 78.4533 )</td>
<td>14.78</td>
<td>1,46</td>
</tr>
</tbody>
</table>
Table 2.8  Test for equal slopes and intercepts of the regression equations for *Hear*NPV applied at 58 mL/ha and *Hear*NPV at 58 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf feeding bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
<thead>
<tr>
<th></th>
<th>Slope-Quadratic Term</th>
<th>Slope-Linear Term</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F</em></td>
<td>df</td>
<td><em>P</em></td>
</tr>
<tr>
<td><strong>5 DAI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for Equality</td>
<td>0.85</td>
<td>1,114</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>7 DAI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for Equality</td>
<td>0.27</td>
<td>1,114</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>12 DAI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for Equality</td>
<td>0.63</td>
<td>1,114</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Table 2.9  Relationship between larval mortality rated at five, seven, and twelve DAI with *Hear*NPV applied at 58 mL/ha and *Hear*NPV at 58mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf feeding bioassay.

<table>
<thead>
<tr>
<th>DAI</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F</em></td>
<td>df</td>
<td><em>P</em></td>
</tr>
<tr>
<td>5 vs 7</td>
<td>0.09</td>
<td>1,351</td>
<td>0.77</td>
</tr>
<tr>
<td>5 vs 12</td>
<td>0.01</td>
<td>1,351</td>
<td>0.99</td>
</tr>
<tr>
<td>7 vs 12</td>
<td>0.08</td>
<td>1,351</td>
<td>0.77</td>
</tr>
</tbody>
</table>

43
Table 2.10  Regression equations and statistics for the relationship between *H. zea* mortality and time between application and larval infestation for *HearNPV* applied at 116 mL/ha and *HearNPV* at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf feeding bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
<thead>
<tr>
<th>5 DAI</th>
<th>Treatment</th>
<th>Regression Equation</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F</em></td>
<td>df</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em></td>
<td>( y = 0.0487x^2 ) (0.0112) -3.606x (0.5756) +70.7883</td>
<td>19.04</td>
<td>1,46</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>( y = 0.0284x^2 ) (0.0101) -2.4864x (0.5069) +66.8658</td>
<td>7.89</td>
<td>1,46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>7 DAI</th>
<th>Treatment</th>
<th>Regression Equation</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F</em></td>
<td>df</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em></td>
<td>( y = 0.0299x^2 ) (0.0094) -2.8042x (0.4841) +82.8949</td>
<td>10.17</td>
<td>1,46</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>( y = 0.0187x^2 ) (0.0106) -2.1047x (0.5301) +84.3059</td>
<td>3.12</td>
<td>1,58</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12 DAI</th>
<th>Treatment</th>
<th>Regression Equation</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>F</em></td>
<td>df</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em></td>
<td>( y = 0.0337x^2 ) (0.0098) -2.8792x (0.512) +87.0028</td>
<td>11.52</td>
<td>1,46</td>
</tr>
<tr>
<td></td>
<td><em>HearNPV</em> + Feeding Stimulant/Surfactant</td>
<td>( y = 0.0192x^2 ) (0.0113) -2.0688x (0.5667) +87.0986</td>
<td>2.88</td>
<td>1,58</td>
</tr>
</tbody>
</table>
Table 2.11  Test for equal slopes and intercepts of the regression equations for *Heliothis NPV* applied at 116 mL/ha and *Heliothis NPV* at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf rinsate bioassay when mortality was rated at five, seven, and twelve days after infestation.

<table>
<thead>
<tr>
<th></th>
<th>5 DAI</th>
<th>7 DAI</th>
<th>12 DAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope-Quadratic Term</td>
<td>Slope-Linear Term</td>
<td>Intercept</td>
</tr>
<tr>
<td></td>
<td>$F$  df  $P$</td>
<td>$F$  df  $P$</td>
<td>$F$  df  $P$</td>
</tr>
<tr>
<td>Test for</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equality</td>
<td>1.43  1,126  0.23</td>
<td>1.78  1,126  0.19</td>
<td>0.79  1,126  0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.76  1,126  0.38</td>
<td>1.24  1,1262  0.27</td>
<td>0.30  1,126  0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.04  1,126  0.31</td>
<td>1.44  1,126  0.23</td>
<td>0.49  1,126  0.49</td>
</tr>
</tbody>
</table>
Table 2.12  Relationship between larval mortality rated at five, seven, and twelve DAI with *HearNPV* applied at 116 mL/ha and *HearNPV* at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha in the leaf feeding bioassay.

<table>
<thead>
<tr>
<th>DAI</th>
<th>Quadratic Term</th>
<th>Linear Term</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F</em></td>
<td>df</td>
<td><em>P</em></td>
</tr>
<tr>
<td>5 vs 7</td>
<td>0.99</td>
<td>1,387</td>
<td>0.32</td>
</tr>
<tr>
<td>5 vs 12</td>
<td>0.73</td>
<td>1,387</td>
<td>0.39</td>
</tr>
<tr>
<td>7 vs 12</td>
<td>0.02</td>
<td>1,387</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Figures

Figure 2.1  Relationship between larval mortality and number of hours after application of *HearNPV* and *HearNPV* + feeding stimulant/surfactant at five, seven, and twelve DAI in the leaf rinsate bioassay.

Neonate larvae were exposed to artificial diet treated with the rinsate of soybean leaves from plots treated with *HearNPV* at 58 mL/ha, *HearNPV* at 58 mL/ha + feeding stimulant/surfactant at 232 mL/ha, and an untreated control. Percent mortality of *H. zea* resulting from exposure to *HearNPV* and *HearNPV* + feeding stimulant/surfactant was corrected for control mortality.
Figure 2.2  Relationship between larval mortality and number of hours after application of *HearNPV* and *HearNPV* + feeding stimulant/surfactant at five, seven, and twelve DAI in the leaf rinsate bioassay.

Neonate larvae were exposed to artificial diet treated with the rinsate of soybean leaves from plots treated with *HearNPV* at 116 mL/ha, *HearNPV* at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha, and an untreated control. Percent mortality of *H. zea* resulting from exposure to *HearNPV* and *HearNPV* + feeding stimulant/surfactant was corrected for control mortality.
Figure 2.3  Relationship between larval mortality and number of hours after application of *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant at five, seven, and twelve DAI in the leaf feeding study.

For 48 hours, second instar larvae were allowed to feed directly on soybean leaves from plots treated with *Hear*NPV at 58 mL/ha, *Hear*NPV at 58 mL/ha + feeding stimulant/surfactant at 232 mL/ha, and an untreated control. After 48 hours, larvae were transferred onto artificial diet for mortality ratings. Percent mortality of *H. zea* resulting from exposure to *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant was corrected for control mortality.
Figure 2.4  Relationship between larval mortality and number of hours after application of *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant at five, seven, and twelve DAI in the leaf feeding study.

For 48 hours, second instar larvae were allowed to feed directly on soybean leaves from plots treated with *Hear*NPV at 116 mL/ha, *Hear*NPV at 116 mL/ha + feeding stimulant/surfactant at 232 mL/ha, and an untreated control. After 48 hours, larvae were transferred onto artificial diet for mortality ratings. Percent mortality of *H. zea* resulting from exposure to *Hear*NPV and *Hear*NPV + feeding stimulant/surfactant was corrected for control mortality.
Literature Cited


CHAPTER III
IMPACT OF HEARNPV AND MOLASSES-BASED FEEDING STIMULANT/SURFACTANT ON GROWTH OF HELICOVERPA ZEA LARVAE

Abstract

Studies were conducted to investigate the effects of HearNPV, with and without a molasses-based feeding stimulant/surfactant, on the feeding habits of 2nd and 3rd instar Helicoverpa zea larvae. This study was conducted under the premise that larval weight gain is directly related to the quantity of diet consumed. The formulation of HearNPV, Heligen® (AgBiTech Corporation, Queensland, Australia), as well as, the feeding stimulant/surfactant, Optimol® (AgBiTech Corporation, Queensland, Australia), was applied directly to, and absorbed by, artificial corn earworm diet. Weight gain of larvae that fed on HearNPV and the feeding stimulant/surfactant treated diet was compared to that of larvae that fed on untreated diet at four and seven days after infestation. Exposure to HearNPV reduced weight gain of 2nd and 3rd instar larvae at four days after infestation and 3rd instar larvae at seven days after infestation compared to larvae that fed on untreated diet. Also, larvae that fed on diet treated with the feeding stimulant/surfactant gained less weight than larvae that fed on untreated diet at four and seven days after infestation.
Introduction

*Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), commonly referred to as the corn earworm, bollworm, and tomato fruitworm, is a polyphagous insect regarded as one of the most economically important pests in many commercially grown crops throughout the United States (Schug et al. 2022). Producers of soybean, *Glycine max* (L.) Merrill, often encounter yield limiting populations of *H. zea* as field corn, *Zea mays* (L.), begins to dry down in the later part of the growing season. Soybean yield loss, induced by *H. zea*, is predominantly caused by a decrease in the number of harvestable pods per individual plant (Eckel et al. 1992). However, *H. zea* larvae also cause damage in soybean by feeding on foliage and developing flowers (Eckel et al. 1992). Infestations of *H. zea* in soybean are most commonly controlled with applications of synthetic insecticides (Adams et al. 2016). Over time, *H. zea* has developed resistance to organophosphates, carbamates, pyrethroids, chlorinated hydrocarbons, and benzoylphenylureas (Musser and Shelton 2003, Walsh et al. 2022). In search for alternative control methods, extensive research has been conducted to determine the potential benefits that commercial formulations of *Helicoverpa armigera* Nucleopolyhedrovirus (*HearNPV*) may have to offer. *HearNPV* is a naturally occurring baculovirus that infects *Helicoverpa spp.* exclusively (Bulach et al. 1999). There have been several commercial *HearNPV* products, with the first one registered in 1976 (Ignoffo 1973, Pande et al. 2001).

*HearNPV* has several limitations compared to more traditional insecticides. These include its susceptibility to ultraviolet (UV) light, sporadic efficacy, and slow speed of kill. Substantial work has been conducted to address these issues. Some commercial adjuvants are capable of providing marginal protection against UV degradation of *HearNPV* (Ignoffo et al. 1976, Valizadeh et al. 2020, Wilson et al. 2020). Additionally, more recent formulations of
HearNPV have used more virulent NPV isolates, that provide greater mortality and are more UV stable than the wild-type virus (Arrizubieta et al. 2013). HearNPV must be ingested before infection can occur, and the potential benefit of some feeding stimulant additives have been evaluated (Farrar and Ridgway 1994). Feeding stimulants, such as fresh corn flour and cottonseed flour, applied in conjunction with HearNPVs, have been shown to increase larval mortality through increased consumption of plant tissue and virus particles (Farrar and Ridgway 2000, Ignoffo et al. 1976, Luna-Santillana et al. 2011). Increased efficacy has been observed when HearNPV was applied with crop oil concentrates, such as Coax® (Traders Oil Mill Co., Fort Worth, TX), fresh corn, and cottonseed flour additives (Bell and Romine 1980, Montoya et al. 1966).

The remaining disadvantage of HearNPV is its extended incubation period, which results in the slower speed of kill compared to traditional insecticides (Arrizubieta et al. 2013, Ignoffo 1973). After ingestion of HearNPV, larvae become lethargic and may require up to nine days to die, depending on larval size at the time of ingestion (Bandi et al. 2021, Ignoffo 1973). The extended incubation period is particularly important in crops such as cotton, Gossypium hirsutum (L.), and tomato, Lycopersicon esculentum (Miller), that have a low tolerance for bollworm feeding. The slow speed of kill is likely one of the primary reasons HearNPV has not been considered a preferred method of bollworm management across the U.S. (Beas-Catena et al. 2014, Fuxa 1995). Many growers rationally assume that corn earworm larvae are capable of continuing to cause economical damage during the incubation period, after ingestion of HearNPV. Currently, there does not seem to be sufficient research available to refute this belief. Therefore, the first objective of this study was to determine the effect HearNPV infection has on larval feeding habits of H. zea, using bioassay procedures. A commercial HearNPV product
(Heligen®, AgBiTech Corporation, Fort Worth, TX) with a viral occlusion body concentration of $7.51 \times 10^9$ /mL was utilized in these studies. Also, a molasses-based surfactant (Optimol®, AgBiTech Corporation, Fort Worth, TX), marketed as a larval feeding stimulant for co-application with biological insecticides was evaluated.

**Materials and Methods**

Biological assays were conducted to determine the influence of *Hear*NPV (Heligen®) and a molasses-based surfactant (Optimol®) on feeding habits of *H. zea* larvae. Two trials were conducted to accomplish this objective, one trial utilized 2\(^{nd}\) instar larvae, and another trial utilized 3\(^{rd}\) instar larvae. Each trial was replicated four times. Two replications of the 2\(^{nd}\) instar larvae trial and one replication of the 3\(^{rd}\) instar larvae trial were conducted in 2021. During 2022, two replications of the trial that used 2\(^{nd}\) instar larvae were conducted, while three replications of the trial that used 3\(^{rd}\) instar larvae were conducted. The feeding stimulant/surfactant (Optimol®) was included as a treatment in addition to the untreated control and *Hear*NPV, during 2022. A solution containing *Hear*NPV equivalent to a foliar spray solution of 116.82 mL of *Hear*NPV (Heligen®) per hectare at an application volume of 93.46 L/ha was made by adding 1.25 mL of formulated *Hear*NPV to 1 L of water. A solution of the feeding stimulant/surfactant was made using similar procedures, 2.48 mL of the feeding stimulant/surfactant (Optimol®) was added to 1 L of water to yield the same concentration of 232.1 mL of formulated feeding stimulant/surfactant per hectare at an application volume of 93.46 L/ha. Approximately 18 mL of commercial *heliothine* meridic diet (F9781B, Frontier Scientific Services, Newark, DE) was placed into 36.97 mL plastic cups (Dart Container, Mason, MI). Each replicate consisted of 30 cups. 200 µL of the appropriate solution was pipetted onto the surface of the diet in each cup. The diet was allowed to dry for three hours before larvae were infested.
Larvae used in these trials were obtained from the Mississippi State University corn earworm colony and provided by the Mississippi State Insect Rearing Center (Mississippi State, MS). This colony began in 2008 from a collection of a natural population of corn earworms inhabiting non-Bt corn in Starkville, MS. Subsequent populations were obtained from non-Bt corn and incorporated into the colony on a biannual basis. For each replication for both trials, 30 larvae were used per treatment. For the 2\textsuperscript{nd} instar larvae trial, initial larval weights ranged from 0.0012 g to 0.01 g (mean 0.0043 g). For the 3\textsuperscript{rd} instar larvae trial, initial larval weights ranged from 0.0101 g to 0.055 g (mean 0.0349 g). All larvae were weighed on an Ohaus® Precision Balance Scale (PR523N, Ohaus®, Parsippany, NJ) utilizing grams (g) as the unit of measurement. When larvae were infested, the diet cups were labeled so that weight gain of individual larvae could be determined. At four days after infestation, larval weight was determined. Larvae in the 2\textsuperscript{nd} instar trial were removed from their diet cups and weighed four days after infestation. The 2\textsuperscript{nd} instar larvae trial was terminated after four days due to high larval mortality (>50\%) in the HearNPV treatment. Weights of the larvae in the 3\textsuperscript{rd} instar larvae trial were determined at four and seven days after infestation. At four and seven days, only live larvae were weighed. Larval initial weights and weights at four and seven days after infestations were used to calculate weight gain.

**Data Analyses**

Data were analyzed using the generalized linear mixed model analysis of variance in SAS 9.4 (SAS Institute 2009) using replication as the random variable and individual insect as a subsample. Degrees of freedom were estimated using the Kenward-Roger method. Means and standard errors were calculated using LSMEANS and subjected to a Tukey (HSD) test. Statistical significance was identified at an alpha level of 0.05.
Results

2\textsuperscript{nd} Instar Larvae

At four days after infestation, \textit{HearNPV} and the feeding stimulant/surfactant resulted in less weight gain than that in the untreated control ($F=21.39; df=2,272; P<0.0001$) (Figure 3.1). Larvae feeding on untreated diet gained 82\% and 54\% more weight in four days than larvae feeding on \textit{HearNPV} and feeding stimulant/surfactant treated diet, respectively. This trial was terminated at four days after infestation due to 100\% mortality observed for larvae exposed to the \textit{HearNPV} treated diet.

3\textsuperscript{rd} Instar Larvae

At four days after infestation, larvae exposed to \textit{HearNPV}, and the feeding stimulant/surfactant gained less weight than those in the untreated control ($F=27.17; df=2,315; P<0.0001$) (Figure 3.2). Larvae feeding on \textit{HearNPV} and feeding stimulant/surfactant treated diet gained 45\% and 40\% less weight at four days of infestation than larvae exposed to untreated diet.

At seven days after infestation, \textit{HearNPV} resulted in significantly less larval weight gain than the untreated control and the feeding stimulant/surfactant treatment ($F=34.65; df=2, 269; P<0.0001$) (Figure 3.3). \textit{HearNPV} reduced weight gain by 60\% and 41\% when compared to the untreated control and feeding stimulant/surfactant treatment. Additionally, larvae exposed to the feeding stimulant/surfactant treated diet gained 32\% less weight than those exposed to the untreated diet.
Discussion

Studies show that feeding stimulants can significantly increase the consumption of diet and viral particles by corn earworm larvae when applied with *HearNPV* (Bell and Romine 1980, Montoya et al. 1966, Kumar et al. 2008). When *HearNPV* is combined with a suitable feeding stimulant, such as fresh corn or cottonseed oil, larvae are more quickly attracted to the solution and ingest higher quantities of viral particles, resulting in higher mortality rates as well as a shorter incubation period (Montoya et al. 1966, Bell and Romine 1980, Muthuswami et al. 1994). However, in the current study, the molasses-based feeding stimulant/surfactant resulted in lower weights of *H. zea* larvae compared to those fed untreated diet. This indicates that the molasses-based feeding stimulant/surfactant deterred larval feeding. In these studies, a combination of *HearNPV* and the molasses-based surfactant was not evaluated, but a combination of the two was evaluated in chapter two.

Little information is available on feeding behavior of *H. zea* after *HearNPV* ingestion. However, it is well-documented that larvae become lethargic after infection occurs (Ahmad et al. 2022, Andreadis 1987, Bandi et al. 2021). In the current study, weight gain, which was used as a proxy for feeding, was reduced by 80% and 45% for 2nd and 3rd instar larvae at four days after infestation, and 60% for 3rd instar larvae at seven days after infestation. Soybean can tolerate some damage without impacting yield (Adams et al. 2015). Additionally, larvae feed on foliage and reproductive structures (Eckel et al. 1992, Resig et al. 2020). With larvae not feeding exclusively on reproductive structures and the reduced feeding of infected larvae, economic damage to soybean should not result from feeding by infected larvae.

In the current study a molasses-based surfactant (Optimol®), marketed as a feeding stimulant for co-application with biological insecticides, did not increase larval feeding.
Although this study is limited in scope, it further indicates that the molasses-based surfactant provides little benefit to *H. zea* management in soybean with *HearNPV.*
Figure 3.1  Weight gain of 2\textsuperscript{nd} instar larvae four days after diet infestation.

Relationship between larvae exposed to untreated diet and diet treated with *HearNPV* at 1.25 mL/gal and Feeding Stimulant/Surfactant at 232.1 mL/gal. Bars with a different letters signify a significant difference in weight gain between treatments ($P \geq 0.05$, Tukey HSD).
Figure 3.1  Weight gain of 3rd instar larvae four days after diet infestation.

Relationship between larvae exposed to untreated diet and diet treated with *Hear*NPV at 1.25 mL/gal and Feeding Stimulant/Surfactant at 232.1 mL/gal. Bars with a different letters signify a significant difference in weight gain between treatments ($P \geq 0.05$, Tukey HSD).
Figure 3.2  Weight gain of 3rd instar larvae seven days after diet infestation.

Relationship between larvae exposed to untreated diet and diet treated with *HearNPV* at 1.25 mL/gal and Feeding Stimulant/Surfactant at 232.1 mL/gal. Bars with a different letters signify a significant difference in weight gain between treatments (*P*≥0.05, Tukey HSD).
Literature Cited


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CHAPTER IV
SUMMARY

*Helicoverpa zea* (Boddie) is a yield limiting insect pests in Mississippi soybean. *Helicoverpa armigera* Nucleopolyhedrovirus (*HearNPV*) is a form of biological control that is efficacious against *H. zea* populations. The primary disadvantage of *HearNPV* is its susceptibility to ultraviolet light degradation resulting a relatively short residual after application. Larval mortality was significantly impacted by the number of hours after application in both the leaf rinsate and leaf feeding studies. *HearNPV* resulted in greater than or equal to fifty percent mortality of *H. zea* out to ca. twenty-nine hours and thirty-eight hours in the leaf rinsate and leaf feeding bioassays, respectively. The addition of a molasses-based surfactant co-applied with *HearNPV* did not increase the persistence or efficacy of *HearNPV*.

Larvae exposed to *HearNPV* gained significantly less weight than larvae exposed to untreated diet. Weight gain, which was used as a proxy for feeding, was reduced by 80% and 45% for 2\(^{nd}\) and 3\(^{rd}\) instar larvae at four days after infestation, and 60% for 3\(^{rd}\) instar larvae at seven days after infestation. This implies that after ingestion of *HearNPV*, larval feeding is substantially reduced. However, the feeding stimulant/surfactant resulted in reduced weight gain compared to the untreated diet.

The implications of these results suggest that *HearNPV* is a capable control method for the suppression of *Helicoverpa zea* populations in soybean when applied at the full labeled rate. However, *HearNPV* should not be expected to infect satisfactory quantities of *H. zea* after 18 to
41 hours following application. This can limit the utility of *Hear*NPV when *H. zea* oviposition continues for periods greater than this. These data suggest that the addition of a feeding stimulant/surfactant co-applied with *Hear*NPV should not be expected to increase residual activity or efficacy of *Hear*NPV. These studies demonstrate that feeding by infected larvae is substantially reduced and should not be considered as a yield limiting threat. Therefore, *Hear*NPV infected larvae, but still alive and often present in the top of a soybean canopy, do not require additional control measures.