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Development of microbial control for
lepidopteran pests of the corn ear

by

Randall Lee Pingel

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Entomology
Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

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Development of microbial control for
lepidopteran pests of the corn ear

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The problems associated with chemical pesticide misuse have led to the search for alternative forms of insect pest management such as microbial control that are environmentally and ecologically sound. The efficacy of three *Bacillus thuringiensis* Berliner (Bt) products and *Anagrapha falcifera* multiple nuclear polyhedrosis virus (AfMNPV) to control *Helicoverpa zea* (Boddie) was evaluated in the laboratory and in the field on corn. The virulence of the entomopathogens for *Ostrinia nubilalis* (Hübner) and *Spodoptera frugiperda* (J. E. Smith) was also evaluated in the laboratory.

Three Bt products (HD-1-S-1980, Javelin[®], and XenTari[®]) and AfMNPV were tested in the laboratory. The relative virulence, from estimates of LC₅₀s, of the Bt materials for *H. zea* and *S. frugiperda* was Javelin > XenTari > HD-1-S-1980 and for *O. nubilalis* it was Javelin > XenTari and HD-1-S-1980. AfMNPV was most toxic for *H. zea*, followed by *S. frugiperda*, and then *O. nubilalis*. Mixtures of two entomopathogens (XenTari and AfMNPV) either had no effect on mortality when compared to either pathogen alone or mortality of the virus was reduced with the addition of Bt.

In the field, applications of the three entomopathogens, Javelin, XenTari, and AfMNPV, significantly decreased damage by *H. zea* and increased the percentage of corn ears without damage and of marketable ears; AfMNPV provided as good or better protection than the Bt products. Within this experiment, a starch-sucrose product was also tested for its compatibility with the pathogens. The starch-sucrose product did not affect the efficacy of the pathogens to manage *H. zea*, however additional testing with AfMNPV was recommended.

In addition two sunlight protectants, Congo red and a starch-sucrose product, were mixed singly and in combination with AfMNPV to evaluate their effect on the field persistence of the virus. The treatments were sprayed on corn silks, and the silks were collected immediately after application (Day 0) and 1, 3, 6, and 9 days after application. Bioassay results for viral residuals retrieved from the silks showed no significant differences in persistence between the virus alone and virus+starch-sucrose; the virus alone maintained a high degree of activity ($\approx 80\%$) after 9 days. The addition of Congo red negatively affected results of the residual bioassays.

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CHAPTER 1: INTRODUCTION

Chemical insecticides have been widely used for control of insect pests for many years, but today there is an increasing stance by the general public against the use of pesticides. This position has developed due to concerns of direct contamination, contamination of foods and groundwater, general impact on the environment, and the health and safety of agricultural workers (Parrella et al. 1992). Also, resistance has developed in insect populations to various insecticides, and this resistance has been countered by higher rates and more frequent applications of insecticides (Metcalf 1982). These practices have led to an increase of pest resistance and higher levels of insecticide residuals in foods and the environment. In general, conventional control of insect pests employs broad-spectrum insecticides which is also detrimental to beneficial organisms and leads to outbreaks of secondary pests.

These pervasive and increasing problems associated with chemical pesticide use have led to the search for alternative forms of insect pest management such as biological control that are more environmentally and ecologically sound. Biological control is the regulation by natural enemies (predators, parasites, and pathogens) of another organism's population density at a lower average than would otherwise occur (Debach 1974). Caltagirone & Huffaker (1980) identified two general situations in which natural enemies are used for control of pest populations: introduction and conservation or augmentation.

The purpose of conservation is to increase the efficacy of beneficial organisms through environmental manipulations or by repeated releases of natural enemies (Huffaker et al. 1981). Environmental manipulations include

provision of alternate hosts, attractants, and additional food or other requisites; modification of cropping practices; and even the addition of the pest itself to maintain the natural enemy (Huffaker et al. 1981). Rabb et al. (1976) identified three overlapping categories of release strategies: 1) inoculative releases made with the expectation the pests will be permanently maintained at a lower population densities, 2) inoculative releases made with the expectation the natural enemy will survive and reproduce for a limited number of generations and prevent the pest population from rising above an economic threshold, and 3) periodic inundative releases for immediate control, but no long term regulation. Ignoffo (1985) described the augmentation of pathogens as following the approach of either release of the pathogen to induce an epizootic or the inundative use of the pathogen as a microbial pesticide. Therefore, insect pathogens (entomopathogens)--bacteria, fungi, viruses, etc.--are viewed as possible alternatives to chemical insecticides.

Entomopathogens, in contrast to broad-spectrum chemical insecticides, are relatively host specific. But, host specificity in management of insect pests can be viewed as both advantageous and disadvantageous. The distinct advantage is the safety of their use to beneficial insects. On the negative side, host specificity often limits the market for microbial products and can decrease their usefulness in multiple pest situations (Fuxa 1987).

Growers are accustomed to an immediate response or 'instant death' by insect pests to chemical insecticides (Falcon 1985, Bohmfalk 1986); this does not happen with entomopathogens. Fuxa (1987) identified two categories of insect pathogens based on the speed of their action: those that are slow and those that cause quick damage. Insect viruses are considered examples of

the former category because they disable their hosts after more than 24 hours; the most virulent take from 2-5 days (Payne 1982). In a practical sense, the slow effect on their hosts may limit their use for inundative augmentation. A species of bacterium, *Bacillus thuringiensis* Berliner (Bt) was cited as an example of the latter that causes cessation of feeding within 24 hr, although death may take several days.

Most entomopathogens share a similar constraint--their instability in the environment. The rapid loss of activity is most often attributed to UV radiation (Ignoffo 1985, Starnes et al. 1993), but other factors such as moisture (Ignoffo and Garcia 1992) and leaf surface pH (Andrews and Sikorowski 1973, Young et al. 1977) can enhance inactivation. This instability is complicated by the fact that these pathogens must be ingested, therefore enough material must be placed before a susceptible insect and remain viable long enough for the insect to ingest a lethal dose (Falcon 1985).

The corn earworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie), is one of the most destructive insects of vegetable crops in the United States. The earworm is particularly harmful in fresh market sweet corn, *Zea mays* var. *saccharata* L., due to loss in grade; without control, 100% of the corn ears in a field may be infested. In addition, the European corn borer, *Ostrinia nubilalis* (Hübner), and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), complete the complex of lepidopteran pests attacking the corn ear (Foster 1990). Past research on control of *H. zea* has evaluated the use of the *Heliothis* NPV (Ignoffo et al. 1965, Hamm et al. 1986) and has also compared the efficacy of *Heliothis* NPV and/or Bt to chemical insecticides (Tanada & Reiner 1962, Young & Hamm 1966, Klostermeyer 1968, Oatman et al. 1970, Janes and Greene 1972, Andreadis 1981, Arnold et al. 1986). In

general, the microbial treatments demonstrated an increase in control over untreated corn, but failed to provide the overall control achieved with chemical insecticides.

A recent discovery of a multiple nuclear polyhedrosis virus (MNPV) of the celery looper, *Anagrapha falcifera* (Kirby), has provided encouragement to the possibility of controlling multiple pests because of its broad host range. Thirty-one species of Lepidoptera from 10 families have demonstrated susceptibility to the virus: susceptible insects include *H. zea*, *O. nubilalis*, and *S. frugiperda*. (Hostetter & Puttler 1991). Due to the recent discovery of the virus, little is known about the extent of its virulence toward the various lepidopteran pests of the corn ear or its effectiveness in the field. To put this discovery in perspective, there is only one other known virus with a host range as extensive as *A. falcifera*MNPV.

Another method to attack multiple pests is the combination of two microbials such as Bt and an MNPV. There is the possibility the two pathogens may interact synergistically to increase control compared to either alone (McVay et al. 1977). McEwen and Hervey (1959) first suggested the use of a Bt-NPV combination for control of the cabbage looper, *Trichoplusia ni* (Hübner). Since then, similar combinations have yielded mixed results against various insect pests. Against *H. zea*, the combination proved less effective than the *Heliothis* virus alone (Oatman et al. 1970), and Luttrell et al. (1982) reported no difference between mixtures and the virus alone. However, Bell and Romine (1986) found a synergistic effect at a low concentration of Bt with two concentrations of *Heliothis armigera* NPV for *H.*

zea and *H. virescens*. In conclusion, further information is needed regarding effects of pathogen dosage rates and mixtures to better understand their possible use in insect pest management.

The purpose of this research was to investigate microbial alternatives for control of lepidopteran pests of the sweet corn ear in the laboratory and the field. The objectives of the laboratory research were:

1) to determine LC₅₀s of Bt and AfMNPV against the corn earworm, European corn borer, and the fall armyworm.

2) to examine the effects of a Bt and AfMNPV mixture on the mortality of corn ear pests.

Field research objectives were:

1) to evaluate the relative effectiveness of two subspecies of Bt and AfMNPV to control *H. zea* on the corn ear

2) to determine the compatibility of a starch formulation with the efficacy of the entomopathogens.

3) to measure the residual activity of AfMNPV on corn silks

4) to determine the effect of two UV protectorants on viral persistence on sweet corn silks.

Dissertation Organization

This dissertation contains three manuscripts written according to the following journal styles and specifications:

Chapter 2 - Journal of Invertebrate Pathology

Chapter 3 - Journal of Economic Entomology

Chapter 4 - Environmental Entomology

The papers are preceded by a literature review and followed by a general conclusion chapter, a review of literature (for the introduction, literature review, and conclusion chapter), acknowledgements, and appendices.

Literature Review

Bacillus thuringiensis Berliner

Bacillus thuringiensis (Bt) is the most commonly applied bacterium for microbial control, and the current worldwide market for Bt products is estimated to be \$20-25 million U. S. (Beegle and Yamamoto 1992). This species of gram positive, spore-forming bacterium is a complex of bacterial subspecies which is further separated by isolates. The subspecies and isolates (within subspecies) can differ in respect to their pathogenicity (ability to infect host) and their virulence (extent of disease-causing ability measured by LC₅₀ or LD₅₀), and thus differ in their host range. The bacterium has a vegetative stage in which it multiplies when nutrients are abundant and a reproductive stage in which a sporangium is formed. The sporangium contains a spore and one or more crystalline proteins which are termed protoxins (Tanada and Kaya 1993). When protoxins are ingested by a host insect, they are reduced in size and activated into toxic polypeptides by the combination of the high alkalinity of the midgut and the enzymes present in the midgut. The activated toxins (endotoxins) interact with the epithelial cells lining the midgut causing paralysis and eventually death of the host.

The protoxins are not the only toxins produced by Bt: three other types of toxins are produced, one of which is not toxic to insects. Of these, a group of related heat stable toxins that are called β -exotoxins (thuringiensin) is of particular interest for insect pest control (Mohd-Salleh et al. 1980). These

exotoxins are typically produced during the vegetative phase of growth and are secreted into the culture medium. Not all subspecies produce thuringiensin, and neither do all isolates within a subspecies. Thuringiensin interferes with RNA transcription and has a broad host range: susceptible insects are found in seven orders of insects and its effect is not limited to insect herbivores (Tanada and Kaya 1993). Unfortunately, thuringiensin is also toxic to vertebrates, and it is currently not legal for Bt products to contain measurable levels of β -exotoxins in Western Europe and the U. S. (Beegle and Yamamoto 1992).

The Bt spore also can contribute to its toxicity; Bt-susceptible insects vary in their response to spores. Some insects only respond to the protein crystal, while mortality in others is greatly increased with certain ratios of spores to crystals; increased susceptibility is isolate dependent (Mohd-Salleh and Lewis 1982). It is not known whether increased mortality is due to additional crystal protein in the spore coat or to another factor, but the effect of the addition of the spore is probably due to a biological rather than a toxin factor (Beegle and Yamamoto 1992).

The toxic activity of the Bt crystals is limited to the immature stage of mainly three orders of insects: Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), and Coleoptera (beetles). Subspecies that are active against lepidopterans usually form protein crystals which are bipyramidal in shape. The proteins forming the crystal have molecular weights of approximately 130 kDa and there may be several closely related proteins in a crystal; if a second protoxin is present, it is cuboidal in shape and contains smaller proteins of about 70 kDa (Hofte and Whiteley 1989). The formation of the proteins is controlled by genes located on plasmids (extrachromosomal

DNAs), and many subspecies have multiple protoxin genes. Because of their location, the genes are easily transmissible between bacterial cells and can also be lost. Eleven genes of two types that encode for lepidopteran protoxins have been cloned: Type I and Type II genes encode the 130 and 70 kDa protoxins, respectively (Yamamoto and Powell 1993). The naming of the genes correspond to the proteins that they encode, for example, the CryIA(a) protein is encoded by cryIA(a). As groups, cryI genes produce proteins toxic to lepidopterans while cryII genes produce proteins toxic to both lepidopterans and dipterans. The insect specificity of Bt isolates results in part from the combined action of their gene products, and there are differences in levels of expression of the genes among isolates (Yamamoto and Powell 1993).

Most protein crystals are insoluble except at high pH (Knowles 1994), and pH values in excess of 12 have been found in the lepidopteran midgut (Dow 1986). The dissolution of the crystal into endotoxins is accompanied by activation of the toxins by the midgut proteases, and the same protoxin may produce endotoxins with different host specificities depending on the kinds of proteolytic enzymes present (Tanada and Kaya 1993). Once activated, the endotoxins pass through the peritrophic membrane and attach to specific sites or receptors on the cells lining the midgut; CryI toxins bind specifically to receptors on the brush border of the columnar cells (Knowles 1994). A certain toxin may have more than one binding site and several toxins may compete for the same site (Gill et al. 1992). The attachment of the toxin causes the formation of a pore in the plasma membrane which allows for movement of ions and molecules disrupting the osmotic balance of the cell. The influx of ions with water causes cell swelling and lysis. The process,

from ingestion of Bt toxins to the destruction of the midgut epithelial cells, occurs within 30-60 minutes; eventual death of the insect occurs within 1-3 days (Knowles 1994).

The isolate HD-1 of the Bt subspecies *kurstaki* is the active ingredient of the first commercial product in the United States. To control for day-to-day variations in insect susceptibility and compute potencies of new and different isolates, a preparation of HD-1 was adopted as the U. S. reference standard and named HD-1-S-1971 (Beegle et al. 1986). The fermentations of this standard were not similar to those produced by industry so it was replaced by HD-1-S-1980. This fermentation remains as the standard, and it has been shown to be homologous with other HD-1 fermentations (Beegle et al. 1986).

The Baculoviridae

The virus family Baculoviridae causes disease in more than 500 insect species (Martignoni and Iwai 1981). This most widely studied and utilized viral family contains three subgroups: the nuclear polyhedrosis viruses (NPV), granulosis viruses (GV) and non-occluded viruses. All baculoviruses have virus particles or virions of the same basic structure: a center core containing circular double-stranded DNA and protein which is called the nucleocapsid; the rod-shaped nucleocapsid is contained in an envelope. Two types of NPVs have been recognized: 1) those with a single nucleocapsid (SNPV) per envelope and 2) those with multiple nucleocapsids (MNPV), as many as 39, within a common envelope (Heimpel and Adams 1966). The enveloped nucleocapsids are packaged in occlusion bodies referred to as polyhedra from their component protein polyhedrin. The GVs normally possess one enveloped nucleocapsid in a ovoid capsule containing

granulin. As their name suggests, the non-occluded viruses lack an occlusion body surrounding a single enveloped nucleocapsid. The baculoviruses are regarded as the insect virus group with the most potential as pest control agents because of lack of taxonomic similarities to vertebrate viruses (Evans 1986).

The first virus detected in insects was an NPV; the SNPVs have been found in 7 orders of insects, but MNPVs have only been isolated from lepidopterans (Tanada and Kaya 1993). Both types of NPVs are distinguished by the polyhedral inclusion body (PIB) in which the many enveloped, single or multiple nucleocapsids are embedded. The PIB provides protection for the virus particles in the environment (Payne 1982).

Infection in a susceptible insect usually begins with the ingestion of the PIB. The dissolution of the PIB is dependent on the high alkalinity, pH 9.5 to 11.5, of the lepidopteran larval midgut and possibly the gut enzymes (Granados and Williams 1986). This process releases the infectious virus particles which then pass through the peritrophic membrane. Derkson and Granados (1988) identified a 'viral enhancing factor' associated with polyhedron that caused a structural change in the peritrophic membrane allowing for the passage of the virus particles. The virus particle enters into susceptible midgut cells by fusion of the viral envelope with the microvilli of columnar epithelial cells (Harrap and Robertson 1968). These early events in the infection process take from 1/4 to 4 hours after ingestion of the PIB (Granados and Williams 1986). The nucleocapsid then moves through the cell cytoplasm, enters the cell nucleus, and begins the viral replication process; thus producing other infective units that invade the cells of other tissues. In lepidopterans, most NPV infections are systemic, and secondary

infections develop particularly in the fat body, hypodermis, trachea, and blood cells (Tanada and Kaya 1993). Larval death from the most virulent of NPVs occurs within 2-5 days.

Field Persistence of Baculoviruses

The use of baculoviruses as microbial insecticides has been limited due to the lack of field persistence. Viruses inactivate rapidly on a variety of crops, and therefore frequent applications would have to be made. On cabbage leaves, David et al. (1968) found total inactivation of the *Pieris* granulosis virus took between 12 and 19 hrs. The half-life of the nuclear polyhedrosis virus (NPV) of *Heliothis* on soybean foliage was $>2<3$ days (Ignoffo et al. 1974); Ignoffo et al. (1973) also found less than 10% of the original activity of the virus on corn silks after six days. In all of these tests, purified virus was used. From their earlier work, David et al. (1968) suggested the activity of crude preparations of viruses was better preserved due to protection of the virus by dried up insect fluids and tissue fragments. However, a partially purified viral preparation of the *Heliothis* NPV also was rapidly inactivated on the upper leaf surfaces of cotton, soybean, and tomato (Young and Yearian 1974). Smirnoff (1972) also found pronounced inactivation of a partially purified viral preparation of the NPV of *Neodiprion swainei* Middleton when exposed to solar radiation.

Sunlight is probably the major factor contributing to the rapid inactivation of viruses in the field (David et al. 1968, Jaques 1972, Smirnoff 1972, Young and Yearian 1974). Ultraviolet radiation (UV), ranging from 250 to 400 nm although only wavelengths >290 nm actually strike the earth (David et al.

1968, Jaques 1971), has been determined as the principle component of sunlight causing inactivation (David 1969, Bullock et al. 1970, Witt and Stairs 1975).

The amount of UV radiation reaching a virus on plant surfaces, or the opposite of this, the amount of shading that prevents sunlight penetration, affects viral persistence. Viral applications on plants maintained in the dark were inactivated more slowly than others maintained under four other light conditions (Jaques 1972); Smirnoff (1972) also found the activity of dried viral suspensions to remain constant when kept in the dark. These examples were certainly the most extreme form of shading, but physical shielding of dried partially purified and purified viral suspensions from simulated UV radiation in the laboratory also maintained viral activity for the duration of exposure (Smirnoff 1972, Ignoffo and Garcia 1992). Young and Yearian (1974) found viral persistence to be greater on tomato than cotton or soybean leaves in field trials; they suggested the physical structure of the plant (whether the leaves are hirsute, curved, pilose, etc.) to which virus was applied could provide mechanical shading and extend persistence. Shading can also occur in the field from adjacent plants and within the plant from leaves and stems higher in the crop canopy. Measurements of sunlight penetrating the corn canopy showed that only 20% reached a depth of 60 cm (Dunkle and Shasha 1989).

Normally encountered field temperatures and relative humidities do not affect viral persistence (Morris 1971, Ignoffo and Garcia 1992). However, other factors have been reported as influencing the breakdown of viral polyhedra in the environment. The addition of water in combination with UV light increased inactivation of virus by three times compared with dry

exposed virus (Ignoffo and Garcia 1992). The increased persistence of dry versus wet films was also previously reported by David (1969). The chemical nature of leaf surfaces may also affect viral persistence. The pH of dew from cotton leaves was reported to range from 8.8 to 9.8 and evaporation of dew continued to increase the pH; however, it was suggested that the interaction of changing pH along with other factors could be responsible for loss of viral activity (Andrews and Sikorowski 1973, Young et al. 1977).

Various materials have been added to protect viruses (from UV radiation as well as other factors) and thus increase persistence after application. Young and Yearian (1974) found some protection of partially purified NPV with the addition of Shade® and lignin sulfate. Activated carbon extended the half-life of a commercial preparation of *Heliothis* NPV from <1 day to ≈3 days (Ignoffo et al. 1973). Charcoal mixed with skim milk powder, egg albumen, or Brewer's yeast extended the activity of an NPV of *Trichoplusia ni* and *Pieris* granulosis virus, but the protection of the mixtures was attributed for the most part to the charcoal; thus not only the carbon particles, but their dark color were viewed as advantageous (Jaques 1972). Jaques (1971) also found that India ink as well as several stains and colors possessed protective activity. The UV absorbing qualities of various dyes have also been tested in a laboratory experiment: five were found very effective and Congo red provided complete protection (100% of the original activity remained) after 14 days of exposure (Shapiro 1989, Shapiro and Robertson 1990).

Other entomopathogens are inactivated by sunlight, i.e. *Bacillus thuringiensis* (Bt). Rapid inactivation of bacterial spores has been demonstrated in the laboratory and the field (Pinnock et al. 1971, Griego and

Spence 1978, Beegle et al. 1981). Encapsulation of Bt within a starch matrix, a technology developed for chemical insecticides but modified for use of entomopathogens, was tested and found successful in the laboratory in maintaining activity for the European corn borer, *Ostrinia nubilalis* (Dunkle and Shasha 1988). One advantage to this type of formulation is that UV protectants can be easily added. McGuire et al. (1994) first compared persistence of a granular encapsulated Bt with and without two other UV additives, Congo red and Coax[®], to a commercial formulation of Bt in the field: in two of three years of the study, the commercial product's residual activity was significantly lower than the other treatments, and the reduction was particularly pronounced in a year with heavy rainfall. A starch encapsulation procedure used by Dunkle and Shasha (1988) was also tested in the laboratory for extending residual activity of the *Heliothis* NPV. Four other UV protectorants were added to the starch; results indicated that Congo red provided the best protection (Ignoffo et al. 1991). A new process for a sprayable starch encapsulation has been developed: it incorporates a commercially available starch product with sucrose and has never been evaluated with baculoviruses or with any pathogens on corn (M. R. McGuire, USDA/ARS, Plant Polymer Research, National Center for Agricultural Research, Peoria, IL, personal communication)

**CHAPTER 2: EFFECT OF *BACILLUS THURINGIENSIS*,
ANAGRAPHA FALCIFERA MULTIPLE NUCLEAR
POLYHEDROSIS VIRUS, AND THEIR MIXTURE ON THREE
LEPIDOPTERAN CORN PESTS**

A Paper to be Submitted to Journal of Invertebrate Pathology

Randall L. Pingel and Leslie C. Lewis

The first step to develop microbials as alternatives to chemical controls is to test susceptibility of insects and determine estimates of virulence of the microbials in the laboratory. To this end, *Anagrapha falcifera* multiple nuclear polyhedrosis virus (AfMNPV) and three *Bacillus thuringiensis* (Bt) products, HD-1-S-1980, XenTari[®], and Javelin[®], were bioassayed against neonate *Ostrinia nubilalis*, *Helicoverpa zea*, and *Spodoptera frugiperda* using a droplet feeding method. In addition, the effect of XenTari and AfMNPV mixtures on the mortality of three corn ear pests was examined. The relative virulence, from estimates of LC₅₀s, of the Bt materials for *H. zea* and *S. frugiperda* was Javelin > XenTari > HD-1-S-1980 and for *O. nubilalis* it was Javelin > XenTari and HD-1-S-1980. AfMNPV was most toxic to *H. zea*, followed by *S. frugiperda*, and then *O. nubilalis*. Mixtures of the two entomopathogens either had no effect on mortality when compared to either pathogen alone or mortality of the virus was reduced with the addition of Bt.

Key Words: *Helicoverpa zea*; *Ostrinia nubilalis*; *Spodoptera frugiperda*; *Bacillus thuringiensis*; *Anagrapha falcifera* MNPV; multiple nuclear polyhedrosis virus; bioassay; modified droplet method.

Introduction

The corn earworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie), is one of the most destructive insects in vegetable crops in the United States; it is particularly harmful in sweet corn, *Zea mays* var. *saccharata* L. (Tanada and Reiner, 1962). The European corn borer, *Ostrinia nubilalis* (Hübner), and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), complete the complex of lepidopteran pests attacking the corn ear (Foster, 1990).

The discovery of a multiple nuclear polyhedrosis virus (MNPV) of the celery looper, *Anagrapha falcifera* (Kirby), has provided encouragement to the management of multiple pests because of its relatively broad host range. Thirty-one species of Lepidoptera from 10 families are susceptible to the virus; susceptible insects include *H. zea*, *O. nubilalis*, and *S. frugiperda*. (Hostetter & Puttler, 1991). Due to the recent discovery of the virus, little is known about the extent of its virulence to many of the pests.

A combination of two microbials, such as *Bacillus thuringiensis* Berliner (Bt) and a nuclear polyhedrosis virus (NPV), has been suggested as a means to increase the host spectrum of insect pathogens and thus manage multiple pests. There is also the possibility the two pathogens may interact synergistically to increase virulence compared to either alone (McVay et al., 1977). Hall and Dunn (1958) first suggested the use of a Bt and NPV combination for control of the cabbage looper, *Trichoplusia ni* (Hübner); similar combinations have yielded mixed results against various insect pests in the laboratory and the field. Against *H. zea*, the combination proved less effective than the *Heliothis* virus alone (Oatman et al., 1970), and Luttrell et al. (1982) reported no difference between mixtures and the virus alone.

However, Bell and Romine (1986) found a synergistic effect when a low concentration of Bt was combined with two concentrations of *H. armigera* NPV and tested against *H. zea* and *H. virescens* (F.).

Further information is needed on the effects of pathogen dosages and mixtures to better understand their possible use in insect pest management. There is also a need to identify microbial alternatives because of the development of resistance in insect populations to chemical and microbial insecticides currently in use. The objectives of this research were 1) to determine the estimated LC₅₀s of HD-1-S-1980, Javelin, XenTari, and the *A. falcifera* MNPV (AfMNPV) against the *H. zea*, *O. nubilalis*, and *S. frugiperda*; and 2) to examine the effects of XenTari and AfMNPV mixtures on mortality of the corn ear pests .

Materials and Methods

Concentration-mortality Bioassays. Test materials included *B. thuringiensis kurstaki* HD-1-S-1980; Javelin[®], a Sandoz Corporation experimental product, containing an isolate of *B. thuringiensis* subsp. *kurstaki*; XenTari[®], a commercial formulation of *B. thuringiensis* subsp. *aizawai* produced by Abbott Laboratories, North Chicago, IL; and *A. falcifera* MNPV, propagated in *Trichoplusia ni* and provided by P. Vail, USDA-ARS Horticultural Research Laboratory, Fresno, CA. Test insects were neonate *H. zea*, *O. nubilalis*, and *S. frugiperda*. *Helicoverpa zea* and *S. frugiperda* eggs were obtained from USDA-ARS Insect Biology and Population Management Research Laboratory, Tifton, GA, and *O. nubilalis* eggs were obtained from the USDA-ARS Corn Insect Research Unit, Ames, IA.

AfMNPV was propagated in *H. zea* by treating the surface of a laboratory diet (Young et al., 1976) minus antibiotics in 30 ml plastic cups with an aliquot of the originally prepared suspension to provide a concentration of $\approx 10^3$ polyhedral inclusion bodies (PIB) per mm^2 . In each cup, a fourth instar *H. zea* was placed and incubated at 27°C. Dead larvae prior to putrefaction were collected after ≈ 8 days and homogenized in distilled water (DH_2O). The homogenate was filtered through 4 layers of cheese cloth to remove insect parts. The filtrate was centrifuged at 2500 rpm for 30 min, and the resulting pellet was resuspended in DH_2O . To avoid clumping of polyhedra, the suspension was thoroughly mixed in a Tenbroeck homogenizer; the PIBs were counted using a Neubauer hemocytometer.

Stock suspensions were made by adding measured quantities of each test material to a solution of 250 mg of FDC Blue No. 1 coloring and 12.5 ml of 1% Tween 80 per 500 ml of phosphate saline buffer (Dulmage et al., 1971), i.e. blue dye solution. The suspensions were thoroughly mixed in a Tenbroeck homogenizer; subsequent dilutions were made with the blue dye solution. Each dilution was mixed using a vortex for 1 min before the next dilution was made, and all dilutions were mixed the same way prior to their bioassay.

Preliminary assays tested 10 concentrations of half-fold dilutions ranging from 6 to 0.02 mg/ml for the Bt materials and 10^7 to 5×10^2 PIB/ml for AfMNPV to determine the range of concentrations for the LC_{50} bioassays. The LC_{50} bioassays consisted of 6 serial dilutions; the concentrations were selected to approximate from 25 to 75% mortality which varied with the microbial material and test insect. The range of concentrations in mg per ml used for the HD-1-S-1980, Javelin, and XenTari were the following: for *O. nubilalis*,

0.03 to 0.8, 0.01 to 0.2, and 0.03 to 0.8; for *H. zea*, 0.2 to 0.8, 0.01 to 0.1, and 0.03 to 0.4; and for *S. frugiperda*, 2.0 to 7.0, 0.04 to 0.6, and 0.1 to 0.8, respectively. The concentrations of AfMNPV for *H. zea* and *S. frugiperda* ranged from 10^6 to 5×10^4 PIB/ml; for *O. nubilalis* the concentrations ranged from 10^8 to 5×10^6 PIB/ml. In all assays, control larvae were fed the blue dye solution with no pathogens.

Bioassays were conducted using the modified droplet feeding method (Hughes et al. 1986). (This method consists of allowing neonates to feed from droplets containing the pathogens suspended in the blue dye solution; the blue dye solution can be seen through the insect cuticle after ingestion of the suspension.) After visual confirmation the larvae had ingested each treatment concentration, 30 larvae were placed individually in 30 ml plastic diet cups and incubated in the dark at 27°C and ≈ 60 % RH. For *H. zea* and *S. frugiperda*, the diet was the same as used for virus propagation, and a wheat germ diet (Lewis and Lynch, 1969) was used for *O. nubilalis*. Mortality was recorded daily (with the exception of the *S. frugiperda* bioassays) for the Bt materials and AfMNPV. Due to the increased amount of time necessary for mortality to occur from AfMNPV infection, the recording period was extended from 6 days for the Bt materials to 8 days for viral assays of *H. zea* and *S. frugiperda* and 12 days for *O. nubilalis*.

The mortality data was analyzed by Proc Probit procedure to determine an estimate of the LC_{50} , 95% fiducial limits, and slope for each microbial product (SAS Institute 1985). Values from 3 replicates (for *O. nubilalis* and *H. zea*) and 5 replicates (for *S. frugiperda*) were pooled.

Mixture Bioassays. Two microbial agents were used for the bacterial-viral mixture bioassays: XenTari (*Bacillus thuringiensis* subsp. *aisawai*) and *A. falcifera* MNPV (propagated in *H. zea* and purified using the same materials and procedures as the concentration-mortality bioassays). Stock suspensions of both agents were made by adding measured quantities of each test material to the blue dye solution and thoroughly mixing as before. Six serial dilutions of XenTari, 7 half-fold dilutions of AfMNPV and combinations of both were tested against neonate *H. zea*, *O. nubilalis*, and *S. frugiperda*. A total of 56 treatments, 55 microbial treatments and 1 control treatment (blue dye solution) were tested for each replication. The range of concentrations for XenTari (0.02 to 0.2 mg/ml) were the same for all test organisms and was set to provide <50% mortality. The range of concentrations of AfMNPV was different for each test organism: 1.6×10^4 to 10^6 PIB/ml for *H. zea*, 7.8×10^3 to 5×10^5 PIB/ml for *S. frugiperda*, and 7.8×10^5 to 5×10^7 PIB/ml for *O. nubilalis*. Larvae were fed the test solutions using the modified droplet method. Thirty larvae per treatment were transferred singly to a diet cup after ingestion of each test solution and incubated in the dark at 27°C and ≈60% RH. Mortality was recorded after 8 days for *H. zea* and *S. frugiperda* bioassays and after 12 days for *O. nubilalis* bioassays.

The effects of the treatments on percent larval mortality were determined by 5 replications for a total of 150 larvae per treatment. Data of percent mortality were transformed by arcsine transformation and were evaluated by analysis of variance; means were examined by the method of least significant difference (LSD) (SAS Institute 1985).

Results

Concentration-mortality Bioassays. Mortality from the Bt agents for the three insects usually occurred within the first two days after ingestion; only rarely did it extend into the third day. The relative virulence of the Bt materials for *H.zea* and *S. frugiperda* was Javelin > XenTari > HD-1-S-1980 and for *O. nubilalis* was Javelin > XenTari and HD-1-S-1980 (Table 1). Javelin was approximately twice as virulent as XenTari and HD-1-S-1980 for *O. nubilalis* and 5 and 8x more toxic than XenTari and HD-1-S-1980, respectively, for *H. zea*. XenTari and Javelin were much more potent than HD-1-S-1980 for *S. frugiperda*: the estimated LC₅₀ of HD-1-S-1980 (3.10 mg/ml) was 10x and 20x greater than that of XenTari and Javelin, respectively.

Helicoverpa zea was the most susceptible of the test insects to AfMNPV (Table 2): the estimate of LC₅₀ for *H. zea* was 9.5×10^4 PIB/ml compared to 3.1×10^5 PIB/ml for *S. frugiperda* and 4.9×10^7 PIB/ml for *O. nubilalis*. Not only was *O. nubilalis* from ≈ 100 -500x less susceptible to AfMNPV than *S. frugiperda* and *H. zea*, but the time of mortality after viral ingestion was longer, less uniform, and extended over a longer period of time. Mortality for *S. frugiperda* and *H. zea* occurred by the sixth day after ingestion; in *H. zea* mortality began on the fourth day and peaked on the fifth day. In contrast, *O. nubilalis* mortality began on the sixth day and extended to the twelfth day. Control mortality for all bioassays was <10%.

Mixture Bioassays. The effect of the microbial mixtures were similar for the three test insects. There was no significant change in mortality, either synergistic or antagonistic, for mixtures of all concentrations of XenTari with concentrations of AfMNPV causing less than $\approx 50\%$ mortality (Tables 3-5). In

general, however, percent mortalities from concentrations of AfMNPV causing >50% mortality were significantly lowered by the addition of concentrations of Bt with the exception of the lowest concentration of Bt (0.02 mg/ml). The addition of AfMNPV to Bt had no effect on mortality compared to Bt alone.

The percent mortalities from the 7 concentrations of AfMNPV and 6 concentrations of Bt ranged from 11 to 80% and 5 to 49% for *O. nubilalis* (Table 3), from 28 to 96% and 7 to 39% for *H. zea* (Table 4), and from 7 to 58% and 6 to 39% for *S. frugiperda* (Table 5), respectively. Antagonistic effects of the mixtures were noted at 1.3×10^7 and 2.5×10^7 PIB/ml of AfMNPV with 0.04, 0.08, 0.12, and 0.16 mg/ml of Bt for *O. nubilalis*; at 5×10^7 PIB/ml, additions of all Bt concentrations significantly decreased mortality compared with the virus alone (Table 3). Significant decreases were found in *H. zea* mortality at viral concentrations of 1.3×10^5 , 2.5×10^5 , and 5×10^5 PIB/ml with four Bt concentrations of 0.8 to 0.20 mg/ml; in addition, mortality was significantly decreased from that of the virus alone from ingestion of 2.5×10^5 PIB/ml of AfMNPV with 0.04 mg/ml of Bt (Table 4). Two exceptions were found with *H. zea*: mortality was decreased from the virus alone at 3.1×10^4 PIB/ml with additions of 0.04 and 0.12 mg/ml of Bt, and no decrease in mortality was found at the highest viral concentration (10^6 PIB/ml) for *H. zea*. *Spodoptera frugiperda* mortality was only significantly decreased compared with mortality of the virus alone at the highest viral concentration tested (5×10^5 PIB/ml) with 0.16 and 0.20 mg/ml of Bt (Table 5).

Discussion

An understanding of two factors, the susceptibility of the pest population and the potency of microbial product, is necessary to determine the amount of microbial insecticide that is needed to manage pests in the field (Burgess and Thomson, 1971). Laboratory bioassays are the first step to quantify these two factors for targeted pest species. Our objectives were to determine the relative potency of three Bt products and AfMNPV toward three corn ear pests and effect of Bt-virus mixtures on mortality of these three pests.

There is a paucity of data in the literature generated using the droplet assay method used in this research. Mueller and Harper (1987), however, used this method to assay the HD-1 isolate against *S. frugiperda*; they reported an estimated LC_{50} of 3.11 mg/ml and a slope of 4.72. The HD-1 isolate has formed the basis of the Bt industry for the last 25 years, but has not been recommended for use against *Spodoptera* species because of their low susceptibility to the isolate. Our assay results (LC_{50} of 3.10 mg/ml and a slope of 3.65) for HD-1-S-1980 against *S. frugiperda* were surprisingly similar to those of Mueller and Harper (1987); XenTari and Javelin were shown to be substantially more virulent toward *S. frugiperda* than HD-1-S-1980. Differences among virulence of the Bt products for the other two test insects were not as extreme, but Javelin was more potent for both insects and XenTari more virulent against *H. zea* than the HD-1-S-1980. These results suggest the two commercial products would be excellent alternatives for the HD-1 isolate and useful in situations where more than one of the pests occur simultaneously.

AfMNPV was not as virulent toward *O. nubilalis* as *H. zea* and *S. frugiperda*; however the results are still promising considering the history of

O. nubilalis as a viral host. The European corn borer is unique among the major lepidopteran pests in that a virus has never been isolated from it (Lewis et al., 1977). Due to the high degree of specificity of insect viruses, *O. nubilalis* has been found susceptible to only two other MNPVs. Lewis and Johnson (1982) determined MNPVs isolated from *Autographa californica* (Speyer) (AcMNPV) and *Rachiplusia ou* (Guenée) (RoMNPV) were pathogenic to the European corn borer with RoMNPV $\approx 50\times$ more virulent than AcMNPV. Our results of AfMNPV compare favorably with these two MNPVs in virulence and length of time required for mortality to occur. Future bioassays are required to compare these three MNPVs to determine their actual relative virulences toward *O. nubilalis*.

Research is also necessary to determine the cause for the low susceptibility of *O. nubilalis* to baculoviruses. Lewis et al. (1977) observed AcMNPV inclusion bodies in 8 tissues of the corn borer; fewer tissues infected with AcMNPV were found in *H. zea*. The corn earworm seems to be more susceptible to AcMNPV than the European corn borer (Lewis and Johnson, 1982; Hostetter and Puttler, 1991), therefore the extent of infection may not be the determining factor of virulence. Rather, it may be due to a factor prior to the viral replication process. The pH of fourth and fifth instar midguts of *O. nubilalis* are variable and range from 7.01 to 8.22 (Raun et al., 1966). The midgut pH of lepidopteran larvae is typically very high (Dow, 1986), and the dissolution of the nuclear polyhedrosis polyhedron in Lepidoptera is dependent on this highly alkaline condition of the midgut (Granados and Williams, 1986).

Mixtures of entomopathogens have been suggested as a means to increase the virulence over either pathogen alone. The results of our

bioassays indicated no advantage of combining Bt with AfMNPV: pathogen mixtures had no effect on or reduced mortality of the three corn ear pests when compared with the mortality caused by virus alone, and the addition of AfMNPV had no effect on Bt mortality. Although Bell and Romine (1986) reported examples of synergism with mixtures of sublethal concentrations of Bt (Dipel®) and a multiple nuclear polyhedrosis virus of *H. armigera* (Hübner), as with our results, antagonistic effects predominated with addition of Bt at the highest concentrations of the virus. In actuality, their results showed antagonism from addition of Bt at all concentrations of the virus with the exception of the lowest level, and the synergism found would have been better described as potentiation, i.e. an increased response to one microbial concentration in the presence of a second microbial concentration which by itself causes no mortality (Mueller and Harper, 1987). This special form of synergism was not found in our research. Our research and that conducted by Bell and Romine (1986) are unique in the numbers of concentration mixtures tested with more than one test insect. Different subspecies of Bt , MNPVs, insects, and bioassay methods were used for each with basically the same results.

Reduced mortality from the *Trichoplusia ni* nuclear polyhedrosis virus with the addition of Bt was also found by Chancey et al. (1973). Chancey et al. (1973) suggested that Bt interferes with viral infection in two ways: either larvae do not ingest an adequate dosage of virus due to feeding cessation caused by Bt or Bt changed the midgut to inhibit viral passage into the midgut cells. The bioassay method we utilized, based on a one-time ingestion of pathogens, suggests the latter which implies competition between pathogens for binding sites. *Bacillus thuringiensis* is clearly the fastest-acting pathogen:

the first histopathology of the midgut columnar cells occurs within a 5-10 minutes, and cell lysis and sloughing of cells from the basement membrane occur within 30-60 min (Knowles, 1994). In contrast, viral fusion to the columnar cell membrane happens within 1/4-4 h after ingestion (Granados and Williams, 1986). Thus alternative sites for viral fusion, i.e. columnar cells not affected by Bt, must be present to the virus to initiate infection. It is not clear why the addition of Bt concentrations (causing <50% mortality) produced antagonism at the higher virus concentrations (causing >50% mortality) and not at the lower virus concentrations.

In conclusion, the results of these laboratory assays suggest no additional benefit, in terms of increased mortality of *H. zea*, *S. frugiperda*, and *O. nubilalis*, would be achieved by combining Bt and AfMNPV; on the contrary, reduced efficacy of AfMNPV would be expected. The relative similarities in virulence of Javelin and XenTari for the three pests suggest these Bt products may be more effective in situations where all of the corn ear pests occur; the reduced susceptibility of *O. nubilalis* for AfMNPV also suggests the two Bt products would be more efficacious when the European corn borer is present with the corn earworm. However, the differences in the development cycles and habits of the three insects within growing seasons and between latitudes as well as the characteristics of the pathogens would greatly influence which microbial alternative would be more effective in managing these pests.

Acknowledgements

We thank R. Gunnarson, M. Hildreth, K. Dunbar, A. Laird, M. Nuñez, and M. Beyer for their technical support. This is a joint contribution from USDA,

Agricultural Research Service, and as Journal Paper ____ of the Iowa Agriculture and Home Economics Experiment Station, Ames: Project 3130. Names are necessary to report factually on available data; however, neither the USDA nor Iowa State University guarantees or warrants the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that may be suitable.

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Table 1. Results of bioassays of *Bacillus thuringiensis* products for neonate *Ostrinia nubilalis*, *Helicoverpa zea*, and *Spodoptera frugiperda*

Test Material	LC ₅₀ ^a	95% Fiducial Limits	Slope ± SE
<i>O. nubilalis</i>			
HD-1-S-1980	0.12	0.10 - 0.14	1.60 ± 0.15
XenTari	0.09	0.07 - 0.10	1.97 ± 0.17
Javelin	0.04	0.03 - 0.044	1.71 ± 0.16
<i>H. zea</i>			
HD-1-S-1980	0.32	0.29 - 0.34	4.16 ± 0.32
XenTari	0.19	0.17 - 0.21	2.89 ± 0.29
Javelin	0.04	0.037 - 0.046	3.36 ± 0.31
<i>S. frugiperda</i>			
HD-1-S-198	3.10	2.88 - 3.31	3.65 ± 0.27
XenTari	0.33	0.30 - 0.36	2.61 ± 0.19
Javelin	0.17	0.11 - 0.25	2.06 ± 0.34

^a Estimates of LC₅₀ and 95% FL in mg/ml and calculated from pooled % mortality of 3 replicates for *O. nubilalis* and *H. zea* and 5 replicates for *S. frugiperda*, 30 larvae/rep.

Table 2. Bioassay results of *Anagrapha falcifera* MNPV for neonate *Helicoverpa zea*, *Spodoptera frugiperda*, and *Ostrinia nubilalis*

Test Insect	LC ₅₀ ^a	95% Fiducial Limits	Slope ± SE
<i>H. zea</i>	9.5 x 10 ⁴	8.0 x 10 ⁴ - 1.1 x 10 ⁵	1.81 ± 0.16
<i>S. frugiperda</i>	3.1 x 10 ⁵	2.6 x 10 ⁵ - 3.8 x 10 ⁵	1.17 ± 0.10
<i>O. nubilalis</i>	4.9 x 10 ⁷	3.7 x 10 ⁷ - 6.9 x 10 ⁷	1.22 ± 0.16

^a Estimates of LC₅₀ and 95% FL in PIB/ml and calculated from pooled % mortality of 3 replicates for *O. nubilalis* and *H. zea* and 5 replicates for *S. frugiperda*, 30 larvae/rep.

Table 3 Percent mortality of *Ostrinia nubilalis* from concentrations of *Anagrapha falcifera* MNPV, XenTari (*Bacillus thuringiensis* subsp. *aisawai*), and mixtures of the two pathogens.

Bt mg/ml	AfMNPV (PIB/ml)							
	0	7.8x10 ⁵	1.6x10 ⁶	3.1x10 ⁶	6.3x10 ⁶	1.3x10 ⁷	2.5x10 ⁷	5x10 ⁷
0	2.02	11.38	17.43	26.00	33.51	50.67	64.39	80.00
	eF	bEF	bDE	abcCD	bcC	aB	aB	aA
0.02	4.71	11.52	15.15	14.85	23.47	34.44	49.33	61.25
	dE	bD	cCD	cCD	cC	abcB	abA	bA
0.04	11.59	17.43	16.88	14.35	27.40	20.00	41.01	41.15
	cC	abBC	bBC	cBC	bcB	cBC	bA	dA
0.08	15.58	22.23	18.00	20.00	28.86	21.72	41.15	42.32
	cB	abB	bB	bcB	bcAB	cB	bA	cdA
0.12	26.45	30.07	31.16	36.67	43.33	25.68	33.22	42.00
	bA	abA	abA	abA	abA	cA	bA	cdA
0.16	41.66	42.09	39.93	38.18	54.13	32.76	41.15	51.71
	aAB	aAB	aAB	aAB	aA	bcB	bAB	bcdAB
0.20	49.31	42.00	47.14	38.67	58.05	49.03	45.61	60.09
	aAB	aB	aAB	aB	aA	abAB	abAB	bcA

Means within column followed by different lowercase letters are significantly different; means within row followed by different capital letters are significantly different ($p < .05$; LSD; arcsine transformed); Mean of 5 replicates with 30 larvae/rep.

Table 4 Percent mortality of *Helicoverpa zea* from concentrations of *Anagrapha falcifera* MNPV, XenTari (*Bacillus thuringiensis* subsp. *aisawai*), and combinations of the two pathogens.

Bt mg/ml	AfMNPV (PIB/ml)							
	0	1.6x10 ⁴	3.1x10 ⁴	6.3x10 ⁴	1.3x10 ⁵	2.5x10 ⁵	5x10 ⁵	10 ⁶
0	2.33	28.44	44.44	47.93	73.33	87.88	96.00	96.00
	eE	abD	aCD	aC	aB	aAB	aA	aA
0.02	6.67	25.74	37.33	48.16	70.00	80.00	93.33	92.00
	deE	abD	abcCD	aC	abB	abAB	aA	abA
0.04	8.00	22.09	26.67	46.07	63.33	69.33	90.67	89.33
	dE	bD	cD	aC	abcBC	bcB	abA	abA
0.08	12.90	24.11	31.34	54.00	52.00	68.51	74.67	89.14
	cdE	abD	abcd	aC	cC	cB	cB	bA
0.12	19.35	26.00	27.43	46.45	66.66	64.37	82.66	89.33
	bcD	abD	bcD	aC	abcB	cB	bcA	abA
0.16	28.00	33.13	39.10	48.51	57.33	65.19	75.82	91.91
	abF	abEF	abcDEF	aCDE	bcCD	cBC	cB	abA
0.20	38.92	38.00	41.13	48.78	52.00	67.13	84.16	93.33
	aD	aD	abD	aD	cCD	cC	bcB	abA

Means within column followed by different lowercase letters are significantly different; means within row followed by different capital letters are significantly different (p<.05;LSD; arcsine transformed); Mean of 5 replicates with 30 larvae/rep.

Table 5. Percent mortality of *Spodoptera frugiperda* from concentrations of *Anagrapha falcifera* MNPV, XenTari (*Bacillus thuringiensis* subsp. *aisawai*), and combinations of the two pathogens.

Bt mg/ml	AfMNPV (PIB/ml)							
	0	7.8x10 ³	1.6x10 ⁴	3.1x10 ⁴	6.3x10 ⁴	1.3x10 ⁵	2.5x10 ⁵	5x10 ⁵
0	3.40	6.71	9.36	22.34	34.41	31.93	32.67	58.00
	cC	cC	dC	abB	aB	aB	aB	aA
0.02	6.00	7.33	11.33	17.45	30.83	30.59	38.19	46.71
	cD	cCD	cdCD	bBC	aAB	aAB	aA	abA
0.04	7.52	11.55	16.12	14.87	24.10	26.05	33.83	48.00
	cD	cD	bcdCD	bCD	aBC	aBC	aAB	abA
0.08	24.87	11.40	19.33	15.97	31.71	20.00	36.78	46.07
	bBC	cD	abcCD	bCD	aABC	aBCD	aAB	abA
0.12	26.30	17.38	28.33	23.45	26.49	29.19	36.57	50.46
	abBC	bcC	aBC	abBC	aBC	aBC	aAB	abA
0.16	36.26	24.94	26.74	30.23	33.61	36.76	25.59	37.47
	abA	abA	abA	aA	aA	aA	aA	bA
0.20	39.33	41.25	24.35	29.01	31.80	34.18	34.37	37.93
	aA	aA	abB	aAB	aAB	aAB	aAB	bA

Means within column followed by different lowercase letters are significantly different; means within row followed by different capital letters are significantly different ($p < .05$; LSD; arcsine transformed); Mean of 5 replicates with 30 larvae/rep.

**CHAPTER 3: FIELD APPLICATION OF *BACILLUS*
THURINGIENSIS BERLINER AND *ANAGRAPHA FALCIFERA*
MULTIPLE NUCLEAR POLYHEDROSIS VIRUS AGAINST THE
CORN EARWORM, *HELICOVERPA ZEA*
(LEPIDOPTERA: NOCTUIDAE)**

A Paper to be Submitted to Journal of Economic Entomology

Randall L. Pingel and Leslie C. Lewis

ABSTRACT The corn earworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie), is one of the most destructive insects of vegetable crops in the United States. The earworm is particularly harmful in fresh market sweet corn due to loss in grade; 100% of the corn ears in a field may be infested without control. Field evaluations of relative efficacy of applications of two subspecies of *B. thuringiensis* (Bt) and *Anagrapha falcifera* multiple nuclear polyhedrosis virus (AfMNPV) to control artificially infested neonate *H. zea* on the corn ear were made. Also, the compatibility of a starch formulation, a 1:1 mixture of starch (Mirasperse®) and sucrose, when added to the entomopathogens was determined. The applications of the three entomopathogens significantly decreased damage by *H. zea* and increased the percentage of corn ears without damage and of marketable ears; AfMNPV was shown superior to the Bt products in protecting the corn ear. The starch formulation was determined to be compatible with the pathogens, although additional testing with AfMNPV is recommended.

KEY WORDS *Helicoverpa zea*, *Anagrapha falcifera*, nuclear polyhedrosis virus, *Bacillus thuringiensis*, starch encapsulation, maize

Introduction

Integrated pest management (IPM) has been defined as the use of multiple tactics to reduce pest populations to tolerable levels while maintaining a quality environment (Pedigo 1989). The original concept of IPM, developed by Stern et al. (1959), focused on the importance of natural enemies in maintaining acceptable pest densities and the use of selective insecticides only when pest populations rise to dangerous levels. Unfortunately, conventional control of insect pests often employs broad-spectrum insecticides which are also detrimental to the natural enemies; this has led to pest resurgence and outbreaks of secondary pests among other problems (Stern et al. 1959, Metcalf 1982, Pimentel et al. 1992). The problems associated with chemical insecticide misuse have led to the search for alternatives that can be used in crisis situations and are less disruptive of biological control. The use of entomopathogens is one alternative that has been promoted because of their relatively high degree of host specificity, and thus are viewed as compatible with other natural enemies.

Formulations of *Bacillus thuringiensis* Berliner (Bt) have been used for more than two decades as biological insecticides (Hofte & Whiteley 1989); Elcar[®], a commercial product of the *Heliothis* nuclear polyhedrosis virus (NVP), was first marketed in 1976 (Starnes et al. 1993). Although the use of insect pathogens is not a new idea, development of microbial products has been limited (Falcon 1985). While host specificity is an advantage, it can also be viewed as a disadvantage to the development of entomopathogens as commercial products. In particular, insect viruses are usually very host specific which limits their marketability. Also, other viruses have not been commercialized because they have not proven to be more effective than Bt or

Elcar in controlling crop pests (Bohmfalk 1986). Thus to provide more marketable alternatives, continuing developments in microbial control have focused on the search for more virulent forms of entomopathogens with wider host ranges (Payne 1982, Rhodes 1990). A recent discovery of a multiple nuclear polyhedrosis virus (MNPV) of the celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV), has provided encouragement to the possibility of controlling multiple pests because of its relatively broad host range: 31 species of Lepidoptera from 10 families have demonstrated susceptibility to the virus (Hostetter & Puttler 1991). Due to the recent discovery of the virus, nothing is known about its effectiveness in the field.

The development of improved formulations is also viewed as a means to increase the effectiveness of entomopathogens. Encapsulation of Bt within a starch matrix, a technology developed for chemical insecticides but modified for use of insect pathogens, was first tested and found successful in the laboratory as to its palatability and maintaining activity for the European corn borer, *Ostrinia nubilalis* (Hübner) (Dunkle and Shasha 1988). One advantage to this type of formulation is that UV protectants can be easily added. A new process for a sprayable starch encapsulation has been developed which incorporates starch and sucrose into a water dispersible formulation. The starch formulation was effective in maintaining residual activity of Bt on cabbage, but has never been evaluated with baculoviruses or with any entomopathogen on corn (M. R. McGuire, USDA/ARS, Plant Polymer Research, National Center for Agricultural Research, Peoria, IL, personal communication)

The corn earworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie), is one of the most destructive insects of vegetable crops in the United States. The

earworm is particularly harmful in fresh market sweet corn, *Zea mays* var. *saccharata* L., due to loss in grade, and 100% of the corn ears in a field may be infested without control. Past research on control of this pest has evaluated the use of the *Heliothis* NPV (Ignoffo et al. 1965, Hamm et al. 1986) and has also compared the efficacy of *Heliothis* NPV and/or Bt to chemical insecticides (Tanada & Reiner 1962, Young & Hamm 1966, Klostermeyer 1968, Oatman et al. 1970, Janes and Greene 1972, Andreadis 1981, Arnold et al. 1986). In general, the microbial treatments demonstrated an increase in control over untreated corn, but failed to provide the overall control achieved with chemical insecticides. In addition, the efficacy of the virus was limited in multiple pest situations (Ignoffo et al. 1965, Young & Hamm 1966).

AfMNPV has demonstrated a higher virulence toward *H. zea* than *Heliothis* NPV; additional lepidopteran pests that attack the corn ear, *Spodoptera frugiperda* (J. E. Smith) and *Ostrinia nubilalis*, were found susceptible to the virus (Hostetter & Puttler 1991, Pingel and Lewis unpublished data). The objectives of the field study were 1) to evaluate the relative effectiveness of two subspecies of Bt and AfMNPV to control *H. zea* on the corn ear and 2) to determine the compatibility of the starch formulation with the efficacy of the entomopathogens.

Materials and Methods

Field experiments were conducted from 1992-94. In 1992 and 1993, the experiments were conducted on the Iowa State University (ISU) Research

Farm, Ankeny, Iowa; and in 1994, experiments took place at the ISU Johnson Research Farm, Ames, IA. For all years, corn was planted at approximately 56,000 seeds per hectare.

For all experiments, the test materials included: Javelin[®], a Sandoz Corporation experimental product, containing an isolate of *B. thuringiensis kurtsaki*; XenTari[®] (Abbott Laboratories, North Chicago, IL) containing *B. thuringiensis aizawai*; and *A. falcifera* MNPV, a sample of the original isolate from the celery looper propagated in *Trichoplusia ni* (Hübner) provided by P. Vail, USDA-ARS Horticultural Research Laboratory, Fresno, CA. The virus was propagated in *H. zea* by treating the surface of an artificial diet (Young et al. 1976) in 30 ml plastic cups with an aliquot of the originally prepared suspension providing a concentration of $\approx 10^3$ PIB (polyhedral inclusion body) per mm². *Helicoverpa zea* eggs were obtained from USDA-ARS Insect Biology and Population Management Research Laboratory, Tifton, GA. In each cup, a fourth instar *H. zea* was placed and incubated at 27°C in the dark. The dead larvae were homogenized in distilled water (DH₂O), and the homogenate was then filtered through 4 layers of cheese cloth to remove insect parts. The filtrate was centrifuged at 2500 g for 30 min. The resulting pellet was resuspended in distilled water and the PIBs counted in a Neubauer hemocytometer.

A complete randomized block design was utilized for all experiments, but in 1993 and 1994 changes were made in materials and procedures based on the 1992 results and an additional research objective. However, an unexpected change was precipitated by the heavy rainfall of the summer of 1993 which initially delayed planting and later stunted the sweet corn; this

resulted in a switch from sweet to hybrid field corn. Any other materials and procedures for the treatment preparation, application, and evaluation not mentioned for 1993 and 1994 remained the same as in 1992.

1992 Field Experiment. The sweet corn variety 'Silver Queen' was planted on May 18, 1992 in 10-m rows. Each of 3 replications contained 5 treatments and an untreated control. The treatments consisted of 3 concentrations of Javelin (1.5 g/l, 3 g/l, and 6g/l); XenTari at 3 g/l; *Anagrapha falcifera* MNPV with a polyhedral count of 10^6 PIB/ml, and a control of DH_2O . Two guard rows separated treatments and replications; at least 4 rows guarded the ends of the plots. Ten plants from the middle of the rows were marked with stakes at both ends. The selection of plants was based on the condition of their silks, i.e. late R1 to early R2 (Ritchie et al. 1992); any plants not meeting this criterion were removed. Treatment applications were made on July 31 and were directed at the silk of the primary ear of each plant using backpack sprayers. Sprayers were calibrated to deliver ≈ 200 ml within 10 sec, and the spraying of 10 plants for all treatments was timed to take 20 sec. Immediately after treatment applications, 5 *H. zea* neonates were placed on the silks of each ear. The larvae were placed on the silks with a camel-hair brush.

The infested ears per treatment/replication were harvested on August 17 at the milk or R3 stage(Ritchie et al. 1992), placed in plastic bags, and returned to the laboratory for grading. The ears were graded for larval damage and indexed by depth of penetration from the ear tip on a scale of 0 - 5 as follows: 0 = no damage; 1 = tip to 0.5 cm penetration; 2 = 0.5 to 1.9 cm; 3 = 1.9 to 3.8 cm; 4 = 3.8 to 6.3 cm; and 5 = more than 6.3 cm penetration

(Douglas and Eckhart 1957). Data were analyzed by analysis of variance with treatment means separated by LSD, $P < 0.05$ (SAS Institute 1985).

1993-94 Field Experiments. A hybrid corn, Garst 8555[®], was planted on May 26. Only one concentration (6 g/l) of the two Bt products was tested, and a starch formulation was added to a suspension of the test materials to provide a 2% solution. The starch formulation consisted of 1:1 water dispersible starch (Mirasperse[®], Staley, Decatur, IL) and sucrose and was provided by M. R. McGuire, ARS/USDA, Plant Polymer Research, National Center for Agricultural Research, Peoria, IL. Thus, 8 total treatments (3 microbials with and without starch) and 2 controls (DH₂O and DH₂O+starch) were randomly assigned to rows within each of 4 replications.

On August 13, the silks of fifteen plants were sprayed and then infested with 3 neonates per ear. Ten ears were randomly selected and evaluated for amount of *H. zea* damage on August 26; the number of live *H. zea* larvae was also counted. This date was based on completion of development of the larvae not on corn maturity.

In 1994, two experiments were conducted in corn planted on May 5 and 19. Treatment applications (≈10 ml of each solution per silk) and infestations were made on July 26 and Aug 3; evaluations of corn ear damage were completed on August 11 and 19, respectively.

The results of the three experiments were combined and analyzed as a factorial design by analysis of variance (SAS Institute 1985). No significant formulation (starch vs. no starch) x treatment interaction was found; thus, means of treatments over formulation were separated using LSD, $P < 0.05$.

Results

1992 Results. Table 1 summarizes the results of the 1992 preliminary experiment. For all microbial treatments, there was significantly less damage than the control, but the percent of damaged ears was not decreased by the treatments of 1.5 g/l of Javelin and 3.0 g/l of XenTari when compared to the control. Javelin, at a concentration of 6g/l, provided the least number of damaged ears (17%) and damage (0.26) as measured on a scale of 1-5 of amount of *H. zea* larval penetration from the ear tip. However, the results from the application of AfMNPV (27% and 0.63, respectively) were not significantly different than Javelin at 6 g/l with both measures of damage.

1993-94 Results. The efficacy of the treatments was measured by four variables: the amount of ear damage measured by depth of larval penetration from the ear tip, the number of live larvae per ear, and the resulting percentage of ears without damage and percentage of marketable ears (ears with <3.8 cm of penetration from ear tip) (Table 2). The three microbial treatments averaged over formulation (starch vs no starch) significantly decreased amount of penetration and increased the percentage of ears without damage and marketable ears when compared to the control treatment; only AfMNPV reduced the number of larvae per ear. Among the microbial treatments, AfMNPV significantly reduced the amount of ear damage and and ear infestation when compared to Javelin and XenTari. However, there was no significant difference among treatments for the percentage of marketable ears, and the virus application only produced a higher percentage of ears without damage compared to XenTari. While no significant treatment by formulation was found, the means for all variables

indicated a negative effect of the starch formulation on the efficacy of AfMNPV and only slight differences in the means for formulation of the Bt products.

Discussion

The three microbial treatments significantly decreased amount of penetration and increased the percentage of ears without damage and marketable ears when compared to the control treatment. Laboratory bioassays to determine estimates of the LC_{50} for the two Bt products showed that Javelin was approximately five times more virulent than XenTari toward *H. zea* (Pingel and Lewis unpublished data). This difference in relative toxicity did not improve control in the field by Javelin, nor did the speed in which the effect of Bt is realized after ingestion improve control compared to AfMNPV. *Bacillus thuringiensis* causes a midgut paralysis in 5-10 min, a general paralysis in 1-7 hr with cessation of feeding, and death in 1-3 days after ingestion by the host (Knowles 1994). Contrastingly, the most virulent of baculoviruses take from 2-8 days to kill their host, and larvae can continue to feed until death. Fuxa (1987) suggested the slow effect of viruses on their hosts may limit their use as microbial insecticides. But, results of our experiments indicated otherwise: applications of AfMNPV reduced damage and produced a higher or similar percentage of ears without damage and of marketable ears when compared to Javelin and XenTari.

AfMNPV showed a statistical improvement in control over the Bt products, but in a practical sense this improvement is questionable. All three products provided approximately 40% of ears without damage and 80% of marketable ears. But, baculoviruses have a distinct advantage over Bt: viruses have the ability to recycle in the environment. There have been few reported cases of

Bt epizootics in nature because of the lack of sporulation and crystal development in infected insects (Tanada and Kaya 1993). On the other hand, epizootics of baculoviruses are common occurrences; thus virus infected larvae, that remain in the silk or crawl into the silk channel and die, release infective viral particles. This inoculum may serve to infect future larvae feeding in the silk channel, and further tests of AfMNPV against natural *H. zea* populations are necessary to evaluate this possible advantage.

The ear protection afforded by the microbial applications in these experiments was comparable to past research which used similar indicators of damage and product quality (Young and Hamm 1966, Klostermyer 1968, Oatman et al. 1970). Previous research testing the efficacy of entomopathogens to control *H. zea*, however, used natural populations of the pest; at high natural infestation levels the control achieved by pathogen applications has been less than satisfactory (Tanada and Reiner 1962, Podgwaite 1985). In our experiments, silks were infested once with three *H. zea* neonates on each silk. It is difficult to assess the population pressure exhibited by this level of infestation, but only one in three ears contained a larva in the control treatment. Further evaluations are necessary in natural infestations of *H. zea* to determine the effect of increased population levels on the efficacy of the AfMNPV and Bt products. In addition, tests on the efficacy of these pathogens are necessary in situations where more than one corn ear pest occurs. *Spodoptera frugiperda* is not susceptible to Elcar, and has demonstrated minimal susceptibility to the HD-1 isolate of *B. thuringiensis kurstaki* (Mueller and Harper 1987). Our laboratory bioassays indicated that *S. frugiperda* was less (but not considerably) susceptible than

H. zea to AfMNPV and the Bt products were from 10-20 times more virulent than HD-1-S-1980 toward *S. frugiperda* (Pingel and Lewis unpublished data). The results of the addition of the starch formulation to suspensions of the entomopathogens suggested a reduction in the efficacy of AfMNPV and no effect on the Bt products.

Protecting the corn ear from *H. zea* larvae is one of the most difficult situations encountered in pest management; it is particularly difficult for entomopathogens to control a crop pest in this type of situation. The difficulty stems from the mode of action of the entomopathogens and the behavior of *H. zea*. Most entomopathogens have to be ingested by the host, and sufficient quantities of the pathogens must be consumed by the host to initiate infection. Feeding by *H. zea* larvae after emergence from the egg on corn silks was deemed as incidental to reaching the tip of the ear; the larvae then move between the silks surrounded by the husk (Barber 1941). Once in the husk channel, the larvae are protected from any insecticide. However, all larvae observed by Barber (1941) did some feeding before reaching the tip of the ear, and the type of corn husk influenced if and where the larvae fed. In loose-husked corn, the larvae crawled into the husk channel for considerable distances before beginning to feed. On the other hand, feeding was often in the silk at the husk tip or just beyond in the adjoining exterior silk in tighter husks. Elcar reduced the number of *H. zea* larvae and ear feeding on a corn hybrid with a tight husk when compared to a loose husk variety (Hamm et al. 1986). Garst 8555 has a medium to short husk channel with a medium to tight husk (personal communication, R. Johnson, ICI, West Des Moines, IA) and 'Silver Queen' has similar ear characteristics. There is a possibility that

these ear characteristics increase larval feeding of the entomopathogens outside of the husk channel and influence control; therefore, the combination of entomopathogens and host plant resistance shows promise.

Maximum protection of the corn ear was not the desired outcome of these experiments, rather the relative efficacy of the three entomopathogens was evaluated. The percentage of ears without damage and marketable ears achieved are considerably lower than those produced by currently recommended insecticides (Arnold et al. 1986), and present grower standards for worm-free ears (>98%) dictate higher control levels. However, we have demonstrated that pathogens are efficacious in controlling *H. zea*; additional research is necessary to test increased concentrations and improvements in formulations.

ACKNOWLEDGEMENTS

We thank J. Dyer, M. Abbas, N. Gallager, and R. Gunnarson for their technical support. This is a joint contribution from USDA, Agricultural Research Service, and as Journal Paper ____ of the Iowa Agriculture and Home Economics Experiment Station, Ames: Project 3130. Names are necessary to report factually on available data; however, neither the USDA nor Iowa State University guarantees or warrants the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that may be suitable.

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Table 1. Damage of sweet corn ears caused by *H. zea* in 1992 field experiment after application of *B. thuringiensis* and AfMNPV

Treatment	% damaged ears ^a	Damage score ^b
Javelin, 6 g/l	17a	0.26a
Javelin, 3 g/l	50b	1.30bc
Javelin, 1.5 g/l	59c	1.50c
XenTari, 3 g/l	57bc	1.33bc
AfMNPV, 10 ⁶ PIB/ml	27ab	0.63ab
Control, DH ₂ O	77c	2.26d

^{ab}Means with common letter do not differ significantly at 5% level (LSD multiple range test). Average of 3 replicates.

^bDamage scored: 0 = no damage; 1=tip-0.5 cm penetration; 2=0.5-1.9 cm; 3=1.9-3.8 cm; 4=3.8-6.3 cm; and 5=over 6.3 cm penetrated.

Table 2. Corn earworm control from combined results of 1993-94 experiments after application of *Bacillus thuringiensis* and *Anagrapha falcifera* MNPV (AfMNPV) with and without starch formulation

Formulation	Treatments ^a			
	Control	Javelin	XenTari	AfMNPV
Ear Damage ^b				
No starch ^c	2.9	1.9	2.1	1.3
Starch	2.8	2.1	2.0	2.1
Mean	2.9a	2.0b	2.1b	1.7c
# Larvae per Ear				
No starch	0.33	0.33	0.31	0.15
Starch	0.45	0.42	0.38	0.29
Mean	0.39a	0.38a	0.35a	0.22b
% Ears with No Damage				
No starch	13.3	40.0	33.3	59.2
Starch	15.0	35.0	37.5	35.0
Mean	14.2a	37.5bc	35.4b	47.1c
% Marketable Ears				
No starch	60.8	81.7	77.5	90.0
Starch	69.2	80.8	75.0	76.7
Mean	65.0a	81.3b	76.3b	83.3b

^a≈10 ml of Javelin and XenTari at 6g/l and AfMNPV at 10⁶ PIB/ml; starch-sucrose added to form 2% solution.

^bDamage scored: 0 = no damage; 1=tip-0.5 cm penetration; 2=0.5-1.9 cm; 3=1.9-3.8 cm; 4=3.8-6.3 cm; and 5=over 6.3 cm penetrated.

^cMeans of treatments with and without starch calculated from 3 experiments with 4 replicates per experiment; combined means for formulation with common letter do not differ significantly at 5% level (LSD multiple range test).

**CHAPTER 4: FIELD PERSISTENCE OF A MULTIPLE NUCLEAR
POLYHEDROSIS VIRUS OF THE CELERY LOOPER, *ANAGRAPHA
FALCIFERA* (KIRBY) (LEPIDOPTERA: NOCTUIDAE),
ON SWEET CORN SILKS**

A Paper to be Submitted to Environmental Entomology

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ABSTRACT One factor limiting the use of nuclear polyhedrosis viruses as microbial insecticides is their inactivation by sunlight. To protect viruses in the field from sunlight and prolong their activity, various materials have been added to viral sprays to increase the efficacy of applications. Two sunlight protectants, Congo red and a mixture of starch (Mirasperse®) and sucrose, were mixed singly and in combination with a suspension containing 10^7 PIB/ml of the *Anagrapha falcifera* multiple nuclear polyhedrosis virus. The treatments, including the virus without protectants, were sprayed on sweet corn silks in the field. The silks were collected on the day of application (Day 0) and 1,3,6, and 9 days after application. The viral residuals retrieved from the silks were fed to neonate corn earworms, *Helicoverpa zea*, using a modified droplet method and subsequent mortality was recorded. Results showed no significant difference between the virus and virus+starch-sucrose treatments: percent of original activity was maintained for at least 3 days ($\approx 90\%$) and after nine days was 78 and 64%, respectively. The larval mortality from bioassays of residuals of the two treatments containing Congo

red was greatly reduced: mortality on Day 0 from virus+Congo red was 10% and virus+Congo red+starch-sucrose was 33%. These unexpected results are discussed.

KEY WORDS *Anagrapha falcifera*, nuclear polyhedrosis virus, persistence, starch encapsulation, Congo red

The initial concentration of virus and the ability of the virus to remain in the environment are critical factors for success of any virus applied for management of crop pests. The use of baculoviruses as microbial insecticides has been limited due to the lack of field persistence. Viruses are inactivated rapidly on a variety of crops (David et al. 1968, Ignoffo et al. 1973, Ignoffo et al. 1974, Young and Yearian 1974), and therefore frequent applications would have to be made to protect the crops from insect pests. Sunlight is the major factor contributing to the rapid inactivation of viruses in the field (David et al. 1968, Jaques 1972, Smirnoff 1972, Young and Yearian 1974). Ultraviolet radiation (UV), ranging from 290 to 400 nm, is the principle component of sunlight causing inactivation (David 1969, Bullock et al. 1970, Witt and Stairs 1975).

Various materials have been added to protect viruses from UV radiation to increase persistence. The UV absorbing qualities of various dyes have been tested in a laboratory experiment: five were found very effective and Congo red provided complete protection (100% of the original activity remained) after 14 days of exposure (Shapiro 1989, Shapiro and Robertson 1990). A starch encapsulation procedure (Dunkle and Shasha 1988) was also tested in the laboratory with the *Heliothis* NPV: four other UV protectorants were added to the starch, and Congo red provided the best protection (Ignoffo et

al. 1991). A different sprayable starch has been developed: it incorporates a commercially available starch product with sucrose; it has never been evaluated with baculoviruses or with any entomopathogen on corn (M. R. McGuire, USDA/ARS, Plant Polymer Research, National Center for Agricultural Research, Peoria, IL, personal communication)

In 1985, a new multiple nuclear polyhedrosis virus (MNPV) was isolated from the celery looper, *Anagrapha falcifera* (Kirby); the discovery has provided encouragement for the use of a virus in multiple pest situations because of its broad host range (Hostetter and Puttler 1991). The residual activity of this virus has not been evaluated in the laboratory or field. Residual activity is often not measured, but with it the reliability and cost-effectiveness of control programs utilizing entomopathogens can be improved (Morris 1971, Pinnock and Brand 1981). The objectives of this research were to measure the residual activity of *A. falcifera* MNPV (AfMNPV) and to determine the effect of two UV protectorants on viral persistence on sweet corn silks.

Materials and Methods

Field Persistence Experiments. The MNPV was a sample of the original isolate from the celery looper propagated in *Trichoplusia ni* (Hübner) (P. Vail, USDA/ARS Horticultural Research Laboratory, Fresno, CA). For this study, the virus was propagated in *H. zea* by treating the surface of a laboratory diet (Young et al. 1976) in 30 ml plastic cups with an aliquot of the originally prepared suspension providing a concentration of $\approx 10^3$ PIB (polyhedral inclusion body) per mm². *Helicoverpa zea* eggs were obtained from USDA-ARS Insect Biology and Population Management Research Laboratory,

Tifton, GA. In each cup, a fourth instar *H. zea* was placed and incubated at 27°C in the dark. The dead, virus-infected larvae were homogenized in distilled water, and the homogenate was then filtered through 4 layers of cheese cloth to remove insect parts. The filtrate was centrifuged at 2500 g for 30 min. The resulting pellet was resuspended in distilled water and the PIBs were counted in a Neubauer hemocytometer.

A viral suspension containing 7.5×10^8 PIB/ml was used for all experiments and diluted to 10^7 PIB/ml for all treatments within each experiment. Two sunlight protectorants, Congo red (Sigma, St. Louis, MO) and a 1:1 mixture of water dispersible starch (Mirasperse®, Staley, Decatur, IL) and sucrose, were added singly and in combination to the viral suspension to form 1 and 2% solutions of each material, respectively. The starch formulation was provided by M. R. McGuire, ARS/USDA, Plant Polymer Research, National Center for Agricultural Research, Peoria, IL.

Three experiments were conducted on two varieties of sweet corn, Crystal N' Gold®(CG) and Silver Queen®(SQ) from July 19 to August 11, 1994. The corn was hand-planted on May 5 (SQ), 12 (CG), and 19 (SQ) at approximately 56,000 seeds per hectare in 10-m rows. A randomized block design was used, and each treatment was replicated 3 (CG) or 4 (SQ) times. Two guard rows separated treatments and replications; at least 4 rows guarded the ends of the plots.

Thirty-five plants within two rows were marked with spray paint on the day prior to treatment applications. Plants were selected on the basis of the condition of their silks, i.e. late R1 to early R2 (Ritchie et al. 1992). On July 19 (CG) and 23 and August 2 (SQ), the silk of each marked plant was sprayed with a single application of ≈ 5 ml of each treatment with a 1/4-l spray bottle;

this was equivalent to 2.2×10^{12} PIB per hectare. Six silks per treatment were collected immediately after spraying on the day of application and 1, 3, 6, and 9 days after application. The silks were placed in plastic bags and frozen at -15°C . Samples of the field spray treatments of the 3rd experiment were also frozen for subsequent bioassay of their activity.

Silks were processed for residual tests using modifications of a procedure developed by Ignoffo et al. (1973). The frozen silks were thawed and cut into 4-mm pieces into 1.25-l blender jars containing a blue dye solution of 0.25 g of FDC blue dye #1 and 12.5 ml of a 1% solution of Tween 80[®] per 500 ml of a phosphate saline buffer (Dulmage et al. 1971). Three silks per 100 ml were stirred to thoroughly soak and blended on low speed for 3 min in a Waring[®] commercial blender. The blended mixture was filtered through 4 layers of cheese cloth; the cheese cloth was twisted to remove the remaining liquid. The resulting filtrates were stored in a refrigerator for 2 or 3 days at 4°C for subsequent bioassay.

Prior to being assayed for determination of residual activity, the filtrates were removed from cold storage and left in ambient temperatures for ≈ 1 hr. Each filtrate was mixed in a vortex for 1 min and then fed to neonate *H. zea* using a droplet assay (Hughes et al. 1986). *Helicoverpa zea* eggs were obtained from USDA-ARS Insect Biology and Population Management Research Laboratory, Tifton, GA. Upon drinking, 30 larvae per treatment per day per replication were placed individually in diet cups (containing the same diet as used for virus propagation) and maintained in the dark at 27°C and $\approx 60\%$ RH. The filtrates were bioassayed by replication, and 2 replications were completed per day. Experimental controls consisted of neonates fed the blue dye solution with and without noncontaminated (no virus applied)

silks of the two sweet corn varieties. Larval mortality was recorded 8 days later. An average percent original activity remaining (OAR) was calculated from % mortality data for each treatment per day posttreatment based on 100% activity on Day 0 (day of application). Data were analyzed by analysis of variance; only the difference between virus and virus+starch-sucrose was compared using a contrast (SAS Institute 1985).

Samples of the field spray treatments were also bioassayed against neonate *H. zea*. Each treatment sample was diluted from the original 10^7 PIB/ml to 5×10^5 PIB/ml from which 4 half-fold dilutions were made. A total of 5 levels of concentrations were tested for each treatment with 2 replications per treatment. The same procedures were followed as in the residual assays. The mortality data was analyzed by Proc Probit procedure to determine an estimate of the LC_{50} and 95% fiducial limits for the virus and virus+starch-sucrose treatments (SAS Institute 1985). The pH of each treatment was also measured at 18°C using a Fischer Scientific Accumet, Model 915.

Persistence Follow-up Experiments. A series of 3 experiments were conducted to determine the cause of low residual activity exhibited by the treatments containing Congo red; the bioassay procedures and test insect were the same as used in the field persistence experiments.

The 1st experiment was designed to test whether Congo red, a combination of Congo red and blue dye solution, or some factor unique to the AfMNPV combined with Congo red caused a similar reduction in percent mortality. The bioassays consisted of five treatments: a 1% solution of Congo red and the blue dye solution, and a combination of the two were added to a

suspension of DH₂O and AfMNPV; only Congo red and the blue dye solution were added to *Autographa californica* MNPV (AcMNPV). AcMNPV was propagated in *Agrotis ipsilon* (Hufnagel) and purified using the same procedures used to propagate and purify AfMNPV. Viral concentrations for treatments containing AfMNPV and AcMNPV were 2.5×10^5 and 5×10^6 PIB/ml, respectively.

The effect of the components of the blue dye solution added singly to a suspension of AfMNPV, DH₂O, and Congo red (1% solution) was tested in a 2nd experiment. The treatments were prepared by making half-fold dilutions of a virus-Congo red 2% solution by adding DH₂O, buffer, blue dye solution, and blue dye and Tween 80 in DH₂O. The resulting 5 treatments contained a 1% solution of Congo red with 2.5×10^5 PIB/ml.

A 3rd experiment tested combinations of the components of the blue dye solution: the five treatments consisted of DH₂O, Tween 80+buffer, blue dye+buffer, blue dye+Tween 80 in DH₂O, and blue dye solution added to a virus-Congo red 2% solution. The treatments were prepared the same as the 2nd experiment with each resulting in a 1% solution of Congo red containing 2.5×10^5 PIB/ml. Data from the 2nd and 3rd experiments were analyzed by analysis of variance; if a significant treatment effect was found, treatment means were separated by LSD, $P < 0.05$ (SAS Institute 1985).

Results and Discussion

The *Anagrapha falcifera* MNPV, without any sunscreens, exhibited a high level of residual activity against *H. zea*: percent mortality from the combined results of the three experiments ranged from 82.8 on Day 0 (day of application) to 64.5 after nine days (Table 1). There was a gradual loss of

activity after the first day, but after nine days 78% of the original activity (calculated from % mortality on Day 0) remained on the corn silks (Fig. 1). This was unexpected considering previous research showed rapid inactivation of nonprotected baculoviruses in the field. In particular, Ignoffo et al. (1974) found <50% after one day and only 8% after six days of the original activity of *Heliothis* NPV remaining on corn silks. There are several plausible explanations for the relatively high levels of AfMNPV persistence in the field.

The type of baculovirus (MNPV) and the chosen test insect (*H. zea*) for the bioassays could have functioned together to produce an appearance of viral stability. As the name implies, the polyhedra of MNPV contain multiple packages of enveloped nucleocapsids; there are at least 2 or more nucleocapsids within the packages which greatly increase the likelihood of successful infection (Payne 1982). Bioassay results have shown AfMNPV to be relatively virulent toward *H. zea*, i.e. $\approx 30\times$ more virulent than *Autographa californica* MNPV (Hostetter and Puttler 1991). While the mechanism of polyhedral breakdown and subsequent inactivation of virions from UV light is not understood, it is safe to assume the polyhedra do not dissolve nor are all the virions inactivated at once. Rather, the polyhedral degradation process is gradual and those virions situated close to the polyhedral surface are the first to be inactivated. Therefore while AfMNPV was affected by UV radiation, sufficient active virions remained to cause infection in a susceptible host. Also, Witt and Stairs (1975) indicated the response of virions to UV radiation may differ, and some virions may be more susceptible or resistant than others. It is possible that AfMNPV either, because of the number of virions

per polyhedron, has a greater chance of possessing resistant virions or has a larger proportion of resistant virions than other viruses that have been tested for persistence in the field.

A partially purified viral preparation was used in the present research. David et al. (1968) first suggested that the dried insect fluids and tissues present in partially purified viral preparations protected the virus from UV degradation. Past research has shown differences in persistence of partially purified versus purified preparations of baculoviruses, but results have not been consistent. A purified granulosis virus of *Pieris brassicae* L. rapidly lost its ability to cause death (David 1965) whereas a crude preparation maintained some of its activity for 16 weeks (David and Gardiner 1966). In a laboratory study, David et al. (1971) found insect hemolymph provided a screening action which excluded some UV radiation. Partially purified viral preparations of first to fourth instar gypsy moths, *Lymantria dispar* (L.), also increased virus-caused mortality after exposure to UV radiation when compared to the purified gypsy moth MNPV; however the addition of tissues from fifth- or sixth-stage larvae had little effect (Shapiro 1984). Partially purified suspensions of nuclear polyhedrosis viruses have also been rapidly inactivated when exposed to UV light in the laboratory (Smirnoff 1972) and in the field (Young and Yearian 1974).

Shading virus from UV radiation has been shown to maintain viral activity (Ignoffo et al. 1991), thus the placement of virus within the plant canopy is a critical aspect of persistence. Measurements of sunlight penetrating 120-cm tall cornfield canopy showed that about 50-90% penetrated to the first 30 cm and only about 20% to 60 cm (Dunkle and Shasha 1989). Viral applications on corn silks would therefore be exposed to reduced UV radiation and

maintain their activity for a longer period of time. On the contrary, applications of four multiple nuclear polyhedrosis viruses to sorghum heads lost from 60-80% of their activity within 2 days (Young and McNew 1994). In the only other evaluation of viral persistence on corn silks, however, viral deposits lost >50% of their activity one day after application (Ignoffo et al. 1973). The loss of activity could be explained by shorter plants and increased plant and row spacing which would decrease shading, but this information was not provided.

The addition of the starch-sucrose formulation provided no increase in viral persistence: the percent mortality caused by viral residuals for the virus+starch-sucrose treatment decreased from 85.6 on Day 0 to 54.2 after nine days (Table 1) with 64% of the residual activity remaining after the exposure period (Fig. 1); no significant difference ($Pr > F$, 0.9564) existed between the virus alone or the virus+starch-sucrose. The starch-sucrose formulation used in this experiment has not been tested with baculoviruses or on corn (M. R. McGuire, personal communication). However, other starch formulations have not maintained residual activity when added alone to entomopathogens. *Bacillus thuringiensis* encapsulated in a starch matrix (Dunkle and Shasha 1988) lost all of its spore viability after 12 days, and <1% of its original activity remained after only four days (Dunkle and Shasha 1988). Ignoffo et al. (1991) evaluated a similar starch formulation added to a suspension of purified *H. zea* NPV, and after 24 hr of exposure to simulated sunlight, only 6% residual activity remained. McGuire et al. (1994) found that a different starch encapsulation of Bt showed <20% residual activity after 12 days. However, the primary purpose of starch formulations is to disperse

both entomopathogens and UV protectorants (Dunkle and Shasha 1989). It is yet to be determined whether with baculoviruses the additional cost of this benefit is justified.

The addition of Congo red as a UV sunscreen has been shown to be effective in extending the residual activity of baculoviruses in the laboratory (Shapiro 1989, Shapiro and Robertson 1990, Ignoffo et al. 1991). In our experiment, the residual activity of the two treatments containing Congo red was 32.5 and 9.6% on the day of application (Day 0) for virus+starch-sucrose+Congo red and virus+Congo red, respectively (Table 1). Similar results were found when serial dilutions of the originally sprayed Congo red treatments were bioassayed against *H. zea* (Table 2). The percent mortalities resulting from the highest concentration (5×10^5 PIB/ml) of the virus+Congo red+starch-sucrose and virus+Congo red were 26.6 and 6.7, respectively (Table 2). In both, the addition of starch-sucrose product lessened the negative effect of Congo red on the bioassays. The estimated LC_{50} s for virus and virus+starch-sucrose treatments applied to the corn silks were 1.3×10^5 and 1.2×10^5 PIB/ml; their 95% fiducial limits of 1.1×10^5 - 1.5×10^5 and 9.6×10^4 - 1.5×10^5 PIB/ml, respectively. No LC_{50} was calculated for either of the Congo red treatments due to the low mortality of the highest concentration of virus.

Due to the low bioassayed activity of the Congo red treatments, it was originally thought Congo red was in some way directly affecting the bioassay results. McGuire et al. (1991) found reduced viral activity in *Melanoplus sanguinipes* (F.) from a granular entomopoxvirus formulation containing Congo red; it was suggested that Congo red could be interfering with the viral infection process. Dunkle and Shasha (1989) provided another possible

explanation of reduced activity of *B. thuringiensis* combined with Congo red: Congo red was changed by sunlight to a form that was less palatable and reduced larval feeding resulted.

A series of three experiments were designed to determine the cause of low residual activity of viral treatments containing Congo red. In the first experiment, the direct effect of Congo red and whether its effect was unique to AfMNPV were tested. An examination of the mean percent mortality for AfMNPV and AcMNPV showed no decrease in mortality from the addition of Congo red when compared to the blue dye: percent mortalities from the addition of Congo red and blue dye to AfMNPV and AcMNPV in DH₂O were 81 versus 74 and 100 versus 90, respectively. The mixture of AfMNPV+Congo red+blue dye solution showed a similar drop in mortality to 17% as found in the previous bioassays. Thus, the results indicated that Congo red was not directly affecting the bioassay results, rather decreased mortality was caused by one or more of the components of the blue dye solution acting with Congo red.

The components of the blue dye solution (blue dye, Tween 80, and phosphate saline buffer) added singly and in combinations to AfMNPV and Congo red were tested in the 2nd and 3rd experiments. In both experiments, AfMNPV+Congo red and AfMNPV+Congo red+blue dye solution served as positive and negative controls for comparisons with the other treatments. The percent mean mortalities resulting from their ingestion were similar for both experiments: 79 and 78% for AfMNPV+Congo red and 30 and 18% for AfMNPV+Congo red+blue dye solution in the 2nd and 3rd experiments respectively (Table 3). Mean mortality was significantly lower than AfMNPV+Congo red with the addition of blue dye (65%), Tween 80 (52%),

blue dye+Tween 80 (42%), and blue dye+buffer (34%); all produced significantly higher mean percent mortalities, with the exception of blue dye+buffer, than AfMNPV+Congo red+blue dye solution. Only the addition of Tween 80+buffer to AfMNPV+Congo red showed a mean mortality (10%) significantly lower than all other treatments excluding the AfMNPV+Congo red+blue dye solution. Therefore, an indirect effect of Congo red when added to the blue dye solution was causing the lowered percent mortalities found in the residual and treatment bioassays. In addition, all combinations of the components and the blue dye and Tween 80 added singly caused varying degrees of lessened mortality.

As stated previously of the possible effect of Congo red, the blue dye solution with Congo red may be similarly interfering with the infection process or causing a decrease in palatability. Relative to the infection process, the mixture may be causing viral polyhedra to clump and thus lowering the chance of ingesting the polyhedra (Tween 80 is used for increasing solubility, but in combination with Congo red may be having the opposite effect). It may also inhibit the breakdown of the polyhedra and subsequent release of virions by coating viral polyhedra, changing the pH of larval midgut or inhibiting midgut proteases. To determine if one of these or a combination was responsible, was beyond the scope of these experiments. However, the pHs of the treatments suggested that the midgut pH would not be affected: the pH of the virus alone (7.1) was similar to the virus+Congo red (6.8); the addition of starch resulted in pHs of 3.1 and 4.4 respectively for virus+starch and virus+starch+Congo red (Table 2). It was observed, during feeding of Congo red treatments, that the larvae took longer to ingest the

treatments. This suggested some reduction in palitability, but only larvae that ingested the treatments were selected. Thus, the mechanism affecting the bioassay results remains unclear.

Comparisons can be made of the % mortality caused by viral residuals of the virus and virus+starch treatments to bioassay results of % mortality of serial dilutions of the same field spray treatments (Table 4). The data provided an estimate of the amount of virus recovered: mortality from residuals on the day of application (84%) was similar to the mortality achieved (89%) from a concentration of 5×10^5 PIB/ml. The data also provided an estimate of the amount of viral loss over the nine days of the experiment. Mortality from viral residuals on Day 9 (59%) fell within the range of mortalities, 48 and 71% of the concentrations of 1.3×10^5 and 2.5×10^5 PIB/ml, respectively. Therefore in terms of equivalency of activity (not actual numbers of polyhedra), there was a loss of 50 to 75%.

In conclusion, partially purified *Anagrapha falcifera* multiple nuclear polyhedrosis virus without sunscreens maintained residual activity at $\approx 80\%$ on corn silks for 9 days. The high degree of persistence was unexpected, but very encouraging relative to management of corn ear pests, and in particular, the corn earworm. Previous research has shown rapid inactivation of baculoviruses in the field which leads to more frequent applications and/or a considerable increase in the initial spray concentration to provide adequate pest control. However, our results indicate otherwise--that AfMNPV could be used similarly to chemical insecticides, with applications every 3-5 days during ear development, to protect the corn ear.

Acknowledgements

We thank R. Gunnarson, J. Dyer, K. Dunbar, D. Bruck, and R. Rose for their technical support. This is a joint contribution from USDA, Agricultural Research Service, and as Journal Paper____ of the Iowa Agriculture and Home Economics Experiment Station, Ames: Project 3130. Names are necessary to report factually on available data; however, neither the USDA nor Iowa State University guarantees or warrants the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that may be suitable.

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Table 1. Residual activity bioassay results measured by % mortality of neonate *H. zea*

Days posttreatment	Treatments ^a			
	Virus (V)	V+St	V+St+Cr	V+Cr
0	82.8 ± 3.2 ^b	85.6 ± 2.7	32.5 ± 4.5	9.6 ± 3.0
1	83.4 ± 2.9	79.9 ± 3.7	39.1 ± 6.3	7.5 ± 1.9
3	70.4 ± 5.3	75.1 ± 4.3	25.4 ± 4.9	5.3 ± 2.0
6	64.8 ± 3.7	64.6 ± 6.0	22.6 ± 4.3	4.9 ± 1.2
9	64.5 ± 3.9	54.2 ± 3.9	17.0 ± 3.0	6.0 ± 1.3
Control ^c	1.5			

^aTreatments consisted of a solution of AfMNPV tested alone and added to starch formulation (St), Congo red (Cr), and a combination of the two additives. All treatments contained 10⁷ PIB/ml.

^bAverage % mortality and standard error from 3 experiments with 3 or 4 replicates per experiment.

^cAverage of % control mortality from neonates fed blue dye mixture minus virus with and without sweet corn silks.

Table 2. Percent mortality, LC₅₀ and 95% fiducial limits resulting from bioassays of serial dilutions of the treatments originally applied to corn silks

PIB/ml	Treatments ^a			
	Virus (V)	V+St	V+St+Cr	V+Cr
5x10 ⁵	93.2	85.0	26.6	6.7
2.5x10 ⁵	75.0	66.6	16.7	5.0
1.3x10 ⁵	41.7	53.3	11.7	6.7
6.3x10 ⁴	28.3	38.3	10.0	8.3
3.1x10 ⁴	11.7	15.0	3.0	6.7
LC ₅₀	1.3x10 ⁵	1.2x10 ⁵	---	---
95% Fiducial Limits	1.1x10 ⁵ - 1.5x10 ⁵	9.6x10 ⁴ - 1.5x10 ⁵	---	---
Control ^c	5.0			
pH	7.1	3.1	4.4	6.8

^aTreatments consisted of a solution of AfMNPV tested alone and added to starch formulation (St), Congo red (Cr), and a combination of the two additives. All treatments contained 10⁷ PIB/ml.

^bAverage % mortality of neonate *Helicoverpa zea* from 2 replicates.

^cAverage of % control mortality from neonate *H. zea* fed blue dye solution minus virus with and without sweet corn silks.

Table 3. Results of 2nd and 3rd experiments to determine the cause of low residual activity of viral treatments containing Congo red

2nd Exp		3rd Exp	
Trt ^a	% mortality ^b	Trt	% mortality ^c
V+Cr	79a	V+Cr	78a
V+Cr+Bu	80a	V+Cr+BD+T80	42b
V+Cr+BD	65b	V+Cr+T80+Bu	10d
V+Cr+T80	52b	V+Cr+BD+Bu	34bc
V+Cr+BDS	30c	V+Cr+BDS	18cd

^aV=AfMNPV (2.5x10⁵ PIB/ml), Cr=Congo red, Bu=buffer, BD=blue dye #1, T80=Tween 80, and BDS=blue dye solution.

^{bc}Means of 3 replications; those with common letter do not differ significantly at 5% level, LSD multiple range test.

Table 4. Comparison of % mortality caused by viral residuals to % mortality from a serial dilutions of the field spray treatments

Days posttreatment	% mortality ^a	% mortality ^b	PIB/ml
0	84	89	5x10 ⁵
1	82	71	2.5x10 ⁵
3	73	48	1.3x10 ⁵
6	65	33	6.3x10 ⁴
9	59	13	3.1x10 ⁴

^aAverage % mortality of neonate *H. zea* from bioassays for virus and virus+starch for residual activity.

^bAverage % mortality of neonate *H. zea* from bioassays of 5 half-fold dilutions of virus and virus+starch spray treatments. Combination of 2 replicates per treatment.

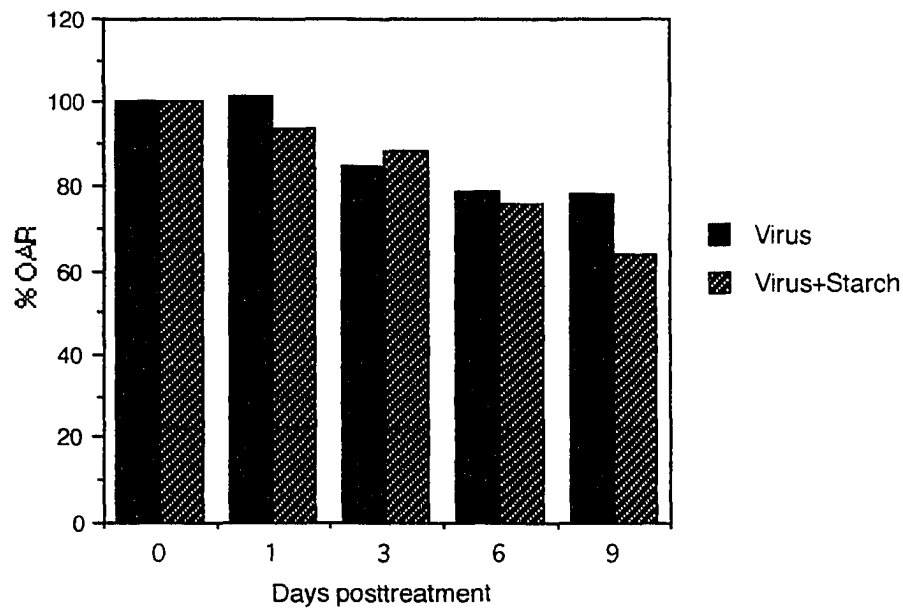


Figure 1. Mean percent of original activity remaining (OAR) after treatment application to corn silks. Treatments contained 10^7 PIB/ml. Average of 3 experiments with 3 or 4 replications. No significant difference ($Pr > F$, 0.8870) existed between the virus alone and the virus+starch-sucrose.

CHAPTER 5: CONCLUSION

The negative consequences from chemical pesticide applications include human pesticide poisonings, toxic effects on domestic animals and wildlife, contaminated food products, and surface and groundwater contamination. Of particular interest in insect pest management, misuse of chemical pesticides causes the destruction of natural enemies, development of resistance in pest populations, and outbreaks of secondary pests. Growing public concern over the health and environmental effects of chemical pesticides and their reduced effectiveness as management tools has led entomologists to consider alternative ways to manage crop pests such as the use of entomopathogens, i.e. microbial control.

Entomopathogens, in contrast to broad spectrum chemical insecticides, are relatively host specific, harmless to beneficial organisms, and nonpolluting; however, they are not without their constraints to development and utilization. The host specificity of microbials is a distinct advantage, because of the safety of their use to beneficial insects and other nontarget organisms, but it is also a disadvantage. Insect viruses are generally very host specific and often only cause disease in insects from which they were isolated. This high degree of host specificity limits the market for microbial products and decreases their usefulness in multiple pest situations. Most entomopathogens are instable in the environment and rapidly lose their insecticidal activity. Ultraviolet radiation (UV), ranging from 290 to 400 nm, has been determined as the principle component of sunlight causing inactivation. The lack of persistence in the environment is complicated by the fact that most pathogens must be ingested; therefore, enough material must be placed before a susceptible insect and remain viable long enough for the

insect to ingest a lethal dose. These constraints, however, can be overcome through the discovery of new strains of pathogens and improvements in formulation. This doctoral research involved the basic steps in the development of microbial insecticides--investigation of the virulence, host range, and persistence in the environment of bacterial and viral alternatives.

Laboratory bioassays are the first step to quantify the susceptibility of the pest population and the potency of a microbial alternative. Three *Bacillus thuringiensis* Berliner products (HD-1-S-1980, Javelin®, and XenTari®) and *Anagrapha falcifera* multiple nuclear polyhedrosis virus (AfMNPV) were tested in the laboratory against three corn ear pests: the corn earworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie), the European corn borer, *Ostrinia nubilalis* (Hübner), and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). The results of the bioassays suggested that XenTari and Javelin, due to their relative similarities in virulence for the three pests, would be excellent microbial alternatives and useful in situations where pests occur simultaneously. The virulence of AfMNPV was more variable than the Bt products: the European corn borer was substantially less susceptible to the virus than the corn earworm and fall armyworm. But, these results are still promising considering the limited number of baculoviruses that have been shown to be pathogenic to the corn borer.

Mixtures of pathogens have been suggested as a means to increase the virulence over either pathogen alone. Laboratory bioassays tested the effect of mixtures of two entomopathogens, XenTari and AfMNPV, on the mortality of the three corn ear pests. In general, the various combinations of microbials either had no effect on mortality when compared to either pathogen alone, or the addition of Bt to AfMNPV resulted in lower mortality

than the virus alone. The antagonistic effects of microbial mixtures were found for viral concentrations causing greater than 50% mortality. These results are disappointing because they suggest that no benefit from Bt-virus mixtures, in terms of increased virulence, would be achieved by their use against a pest that is less susceptible to the virus.

Field applications of the three entomopathogens, Javelin, XenTari, and AfMNPV, were made to test their relative efficacy to manage the corn earworm on the corn ear. After pathogen application, three neonate corn earworms were placed on corn silks, and the efficacy of the treatments was measured by the amount of ear damage (measured by depth of larval penetration from the ear tip), the number of live larvae per ear, the resulting percentage of ears without damage and percentage of marketable ears. All microbials significantly decreased damage by *H. zea* and increased the percent of corn ears without damage and marketable ears when compared to no treatment. AfMNPV significantly reduced the amount of ear infestation and damage when compared to Javelin and XenTari. This was somewhat surprising considering the differences in the speed of action between Bt and viruses. Bt is a fast-acting pathogen that kills the host within 24 hr; on the other hand, the most virulent of viruses take from 2-8 days to kill their hosts. It has been suggested the slow effect of viruses on their hosts may limit their use as microbial insecticides. But in this research where AfMNPV provided as good or better corn earworm control than the Bt products, speed of action was not a factor. The laboratory bioassays showed Javelin was four times more virulent than XenTari toward the corn earworm; however, the difference in relative toxicity did not improve control in the field by Javelin when

compared with XenTari. Thus, laboratory bioassays can only provide relative estimates of virulence which can not be relied upon to predict efficacy in the field.

This research was designed to compare the efficacy of AfMNPV with that of the Bt products, rather than provide maximum protection of the corn ear. The percentage of ears without damage and marketable ears achieved were considerably lower than those produced by currently recommended insecticides; present grower standards for worm-free ears dictate higher control levels. To improve protection of the corn ear, additional research is necessary to test increased concentrations and improvements in formulation.

Formulations of microbials must maintain or increase the efficacy of the pathogen. A starch-sucrose formulation was tested for its compatibility with the entomopathogens as a part of the field efficacy research. The primary purpose of this formulation is to increase persistence of microbials; it had been previously used with Bt on cabbage, but had never been evaluated with baculoviruses or with any entomopathogen on corn. Statistical analysis of the results led to the conclusion the starch-sucrose formulation did not affect the efficacy of the pathogens; however, an examination of the means of the treatments between the microbial products with and without the addition of starch-sucrose suggested a reduction in the efficacy of AfMNPV and little effect on the Bt products. Thus, additional testing with starch-sucrose formulations of AfMNPV is recommended.

In another series of experiments two sunlight protectants, Congo red and the starch-sucrose formulation, were mixed singly and in combination with AfMNPV to evaluate their effect on the field persistence of the virus on corn silks. Bioassay results for viral residuals (active virus remaining on silk)

showed no significant differences in persistence between the virus alone and virus and starch-sucrose; the virus alone maintained approximately 80% of its activity after 9 days. This was unexpected considering previous research showed rapid inactivation of nonprotected baculoviruses in the field. The addition of Congo red negatively affected results of the bioassays: very little residual activity was found from the viral treatments containing Congo red on the day the treatments were applied. The mechanism affecting the bioassay results remains unclear.

This dissertation presents data on the relative virulence and field efficacy of a virus and Bt products and persistence of the virus on corn silks. There remains aspects of applied research that have to be investigated before AfMNPV, Javelin, and XenTari are considered as possible alternatives in managing natural populations of the corn earworm. In the efficacy research, natural populations of the corn earworm were not used, rather silks were infested once with three corn earworm neonates on each silk after application of the pathogens. It is difficult to assess the population pressure exhibited by this level of infestation, but the control achieved by pathogen applications has been less than satisfactory at high corn earworm infestations. The correct timing of applications is critical to the success of any insecticide application. This is particularly important for microbial insecticides because most pathogens have to be ingested by larvae to cause disease and as larvae mature they become less susceptible to entomopathogens; therefore, microbial insecticides must be present prior to egg hatch.

In addition, tests on the efficacy of these pathogens are necessary in situations where more than one corn ear pest occurs. The differences in the

development cycles and habits of the three corn ear pests within growing seasons and between latitudes increases the complexity of research into the use of entomopathogens. The concentrations of pathogens necessary to control these pests and the timing of application will be critical areas of study. The characteristics of the pathogens also can influence which microbial alternative would be more effective in managing these pests. For example, baculoviruses have a distinct advantage over Bt in that viruses have the ability to recycle in the environment. Thus, larvae infected with a virus, prior to or early in ear development, can serve as a source of inoculum to infect future larvae feeding on the corn ear.

The ultimate determinant of the success of any insecticide is whether it is used by growers. Growers have come to expect products that control many pests and provide an 'instant death' at a low cost. Marginally effective microbial insecticides will not be seriously considered as alternatives for chemical insecticides. The entomopathogens tested in this doctoral research have potential to be used as microbial insecticides which can be integrated into a program of selective use of chemical insecticides, host-plant resistance, predators and parasites. To realize their full potential, a greater emphasis must be placed on research to develop predictable, efficacious, and cost-effective microbial alternatives.

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ACKNOWLEDGEMENTS

I would like to express my deep appreciation to my major professor, Dr. Leslie Lewis, for his interest, encouragement, and guidance provided throughout my graduate studies. I want to especially thank him for enabling me through his support to pursue my dissertation project and degree program.

I would also like to thank and recognize the other members of my graduate committee: Drs. John Obrycki, Henry Taber, David Williams, and Richard Wilson.

A special recognition is extended to my mother, Irmgard, and my two aunts, Elsie and Lillie. They have always supported my educational pursuits and life goals.

**APPENDIX A: RESULTS BY EXPERIMENT
OF EFFICACY RESEARCH**

Average amount of ear damage caused by *H. zea* in 1993, first 1994, and second 1994 field experiments after application of *B. thuringiensis* and AfMNPV treatments and controls

Formulation	Treatments ^a			
	Control	Javelin	XenTari	AfMNPV
1993				
No starch	3.08 ^b	0.75	1.20	0.95
Starch	2.80	1.83	1.30	2.00
Mean ^c	2.94a	1.29b	1.25b	1.48b
1st 1994				
No starch	3.13	2.63	2.30	1.38
Starch	2.80	2.15	2.33	2.03
Mean	2.96a	2.39b	2.31b	1.70b
2nd 1994				
No starch	2.60	2.40	2.67	1.45
Starch	2.80	2.45	2.38	2.15
Mean	2.69a	2.43a	2.59a	1.80b

^aConcentrations of the two *B. t.* products were 6 g/l; AfMNPV concentration was 10⁶ PIB/ml.

^bMeans of treatments calculated from 4 replicates. Damage scored: 0 = no damage; 1=tip-0.5 cm penetration; 2=0.5-1.9 cm; 3=1.9-3.8 cm; 4=3.8-6.3 cm; and 5=over 6.3 cm penetrated.

^cMeans with common letter do not differ significantly at 5% level (LSD multiple range test). Average for formulation of 4 replicates.

Mean percentage of ears without *H. zea* damage in 1993, first 1994, and second 1994 field experiments after application of *B. thuringiensis* and AfMNPV treatments and controls

Formulation	Treatments ^a			
	Control	Javelin	XenTari	AfMNPV
1993				
No starch	10.0 ^b	77.5	65.0	67.5
Starch	15.0	52.5	60.0	40.0
Mean ^c	12.5a	65.0b	62.5b	53.8b
1st 1994				
No starch	7.5	17.5	22.5	55.0
Starch	12.5	27.5	25.0	32.5
Mean	10.0a	22.5a	23.8a	43.8b
2nd 1994				
No starch	22.5	25.0	12.5	55
Starch	17.5	25.0	27.5	32.5
Mean	20.0a	25.0a	20.0a	43.8b

^aConcentrations of the two *B. t.* products were 6 g/l; AfMNPV concentration was 10⁶ PIB/ml.

^bMeans of treatments calculated from 4 replicates.

^cMeans of formulation with common letter do not differ significantly at 5% level (LSD multiple range test). Average of 4 replicates.

**APPENDIX B: RESULTS BY EXPERIMENT
OF PERSISTENCE RESEARCH**

Residual activity results measured by % mortality of neonate *H.zea* for 1st experiment

Days posttreatment	Treatments ^a			
	Virus (V)	V + St	V +St + Cr	V + Cr
0	91.0 \pm 2.0	84.3 \pm 4.3	30.0 \pm 6.8	4.3 \pm 4.3
1	82.0 \pm 4.2	80.0 \pm 4.1	50.0 \pm 6.8	5.3 \pm 3.9
3	78.3 \pm 2.3	79.0 \pm 9.2	20.0 \pm 12.5	0.0
6	71.0 \pm 11.6	58.0 \pm 16.5	18.7 \pm 7.2	1.0 \pm 1.0
9	66.7 \pm 6.9	66.7 \pm 4.9	9.0 \pm 4.9	2.0 \pm 1.0

^aTreatments consisted of a solution of AfMNPV tested alone and added to starch formulation (St), Congo red (Cr), and a combination of the two additives. All treatments contained 10^7 PIB/ml of the virus.

^bAverage % mortality and standard error from 3 replicates.

Residual activity results measured by % mortality of neonate *H.zea* for 2nd experiment

Days posttreatment	Treatments ^a			
	Virus (V)	V + St	V +St + Cr	V + Cr
0	88.0 \pm 2.8	91.8 \pm 4.4	46.8 \pm 4.9	4.0 \pm 2.1
1	92.3 \pm 3.5	88.3 \pm 5.0	54.3 \pm 6.7	12.5 \pm 2.8
3	85.8 \pm 1.7	84.0 \pm 5.1	39.3 \pm 4.2	4.8 \pm 1.7
6	68.3 \pm 2.8	79.3 \pm 3.5	37.5 \pm 2.8	7.8 \pm 0.8
9	71.5 \pm 7.6	56.5 \pm 5.5	20.8 \pm 6.4	5.0 \pm 2.7

^aTreatments consisted of a solution of AfMNPV tested alone and added to starch formulation (St), Congo red (Cr), and a combination of the two additives. All treatments contained 10^7 PIB/ml of the virus.

^bAverage % mortality and standard error from 4 replicates.

Residual activity results measured by % mortality of neonate *H. zea* for 3rd experiment

Days posttreatment	Treatments ^a			
	Virus (V)	V + St	V +St + Cr	V + Cr
0	71.5 ± 4.2	80.5 ± 3.8	20.3 ± 4.2	19.3 ± 4.7
1	75.5 ± 2.5	71.5 ± 6.8	15.8 ± 2.9	4.3 ± 1.7
3	49.0 ± 3.1	63.3 ± 4.9	15.5 ± 4.8	10.0 ± 4.3
6	56.8 ± 3.8	55.0 ± 8.4	10.8 ± 2.9	5.0 ± 2.2
9	55.8 ± 3.6	42.5 ± 2.8	19.3 ± 2.9	10.0 ± 1.2

^aTreatments consisted of a solution of AfMNPV tested alone and added to starch formulation (St), Congo red (Cr), and a combination of the two additives. All treatments contained 10^7 PIB/ml of the virus.

^bAverage % mortality and standard error from 4 replicates.