Field Studies of Selected Pathogens for the Control of the Corn Earworm, Heliothis Zea Boddie

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To the Graduate Council:

I am submitting herewith a thesis written by W. Robert Tilyard Jr. entitled "Field Studies of Selected Pathogens for the Control of the Corn Earworm, *Heliothis Zea* Boddie." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Arthur C. Cole, Jr., Major Professor

We have read this thesis and recommend its acceptance:

Charles D. Pless, Arthur W. Jones

Accepted for the Council:

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(Original signatures are on file with official student records.)
November 15, 1972

To the Graduate Council:

I am submitting herewith a thesis written by W. Robert Tilyard, Jr., entitled "Field Studies of Selected Pathogens for the Control of the Corn Earworm, Heliothis Zea Boddie." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology.

We have read this thesis and recommend its acceptance:

[Signatures]

Major Professor

Accepted for the Council:

[Signature]

Vice Chancellor for
Graduate Studies and Research
FIELD STUDIES OF SELECTED PATHOGENS FOR THE CONTROL OF THE
CORN EARWORM HELIOTHIS ZEA BODDIE

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
W. Robert Tilyard, Jr.
December 1972
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I am indebted to Dr. C. J. Southards for generously supplying the nematodes and the use of his lab for this study.

A special thanks is extended to Lynn Torbeck for his statistical analysis of the data results, and to Elizabeth Shull for printing the photographs used.

To my wife, Martha, goes my deepest appreciation for her patience and encouragement throughout the preparation of this thesis.
Tests conducted at The University of Tennessee were designed to compare the control results of the corn earworm by three different pathogens and a standard chemical insecticide. Also, some methods of application were tested. The pathogens tested were a bacterium, Bacillus thuringiensis Berliner, a nuclear polyhedrosis virus specific for Heliothis zea, and the nematode, DD-136.

The treatments were applied under field conditions directly to the ears of corn.

The results indicated that general effectiveness is based on how accessible the pathogens are to the earworm larvae. Best control was accomplished by a treatment using a virus-nematode combination. That treatment and another using the bacillus gave better control results than a standard chemical insecticide.
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CHAPTER I

INTRODUCTION

The corn earworm, *Heliothis* zeae Boddie is a native insect with wide distribution and is considered the most serious pest of sweet corn in North America. *H. zeae* larvae are also serious pests of other important crops, and are sometimes called the tomato fruitworm or the cotton bollworm. The standard method of control has been, and still is, a type of chemical insecticide applied as a dust or a spray preparation on the corn ears. However, chemical insecticides generally possess common undesirable qualities, such as producing possible contamination to adjacent forage crops, being a hazard to honeybees and other beneficial insects, and possessing varying degrees of toxicity to mammals.

However, with the successful use of pathogenic organisms, these problems would be mostly eliminated. The literature indicates that the following three pathogens show promise as possible means of control: (1) a nematode, DD-136, (2) a bacterium, *Bacillus thuringiensis* Berliner, and (3) a nuclear polyhedrosis virus.

The purpose of this study was to further test the suitability of these pathogens for biological control.

A. BIOLOGIES

Before reviewing the literature dealing with this topic it should be helpful to go over briefly some biological background of the host
and of each of its pathogens in order that the nature of the problem might be better understood.

The corn earworm, *Heliothis zea* Boddie

The corn earworm is the destructive larval stage of a moth in the family Noctuidae. The favored host plant of the corn earworm is corn, especially sweet corn. Corn earworms damage corn in several ways. When eggs are laid on the leaves and stalks of young early corn, the hatched larvae migrate to the whorl and feed on the tender, folded leaves. However, earworms do their greatest damage when the ears begin to silk. Larvae desert all other parts of the plant to feed on the silks and the moths prefer to lay their eggs on them. The larvae then feed their way into the ear where they begin eventually eating the kernels. Whenever two earworms come together they fight until one or both are fatally injured. This cannibalistic habit explains why normally only one larva is found in an invaded ear.

Molting five times, the larvae develop to full growth in two to four weeks. Those inhabiting corn ears gnaw their way through the husks, drop to the ground, and pupate in the soil. In the Corn Belt there are usually two or three generations annually, while further south there may be as many as seven generations. Winter is passed in the pupal stage.

There is a certain amount of natural control by predators, parasites, and disease. Some common parasites of both eggs and larvae include the wasp, *Trichogramma minutum* Riley; the ichneumonid,
Campoletis sp., certain braconids, and a fly, Winthemia quadripustulata Fabricius (Pfadt, 1962; Oatman and Platner, 1970). However, the parasitism is not effective enough to be considered for biological control. Natural control by disease is most likely to occur under wet environmental conditions, to both larvae on the plant and pupae in the soil.

Control includes cultural methods such as pursuing specified agronomic practices, planting resistant varieties of corn, and choosing the right time for planting.

The nematode, DD-136

This nematode belongs to the family Steinernematidae of the superfamily Rhabditoidea. This particular nematode, arbitrarily named DD-36, was discovered inside dead codling moth larvae, Carpocapsa pomonella Linnaeus in 1954 by S. R. Dutky (Dutky, 1956). At one time it was considered a strain of Neoaplectana carpocapsae Weiser but has since been given species status, N. dutkyi Jackson (Turco et al., 1971).

Only the ensheathed second-stage larvae, sometimes referred to in literature as dauerlarvae, are infective. These dauerlarvae are the toughest stage of development, capable of living without food for months and of withstanding adverse environmental conditions. They are about 1 millimeter in length. When ingested with food by the host, they are passed along into the crop and midgut where they exsheathe. Most will then penetrate the wall of the midgut by apparently mechanical means, and enter the hemocoele while the rest remain in the midgut. At this
point the insect usually dies for reasons which were not always understood since this process alone does not adequately explain the quick death of the host (usually within 24 hours). In 1965 Poinar and Thomas isolated a bacterium associated with the nematode which later proved to be the cause of death.

The disease is primarily a bacterial septicemia which must be mechanically injected into the insect's body cavity by the nematode acting as a microsyringe. Once the exsheathed nematodes are within the hemocoele, they release through the anus a number of cells of the bacterium (*Achromobacter nematophilus* Poinar and Thomas) which reside in the lumen of the anterior portion of the nematode's intestine (Poinar, 1967).

Once released within the hemocoele, the bacteria multiply rapidly and cause death of the host insect in 16 to 24 hours at room temperature. The bacteria attack the fatty tissue, Malpighian tubules, silk gland, and muscle tissue of the host, and within 48 hours, most of the cadaver's body contents including midgut epithelium are decomposed. This material, along with the bacteria, is then ingested by the nematode so that the nematode regains much of its mutualistic bacteria. This interesting relationship between the nematode and the bacterium is considered mutualism because it has been found that the nematode cannot form mature gonads (hence, no reproduction) without the bacterium, and the bacterium without the nematode has no way of entering into the hemocoele of the insect. There is another benefit the nematode gains
from the bacterium, and that is the bacterium produces an antagonistic substance (antibiotic) that restricts the invasion of microflora into the insect, thus protecting the cadaver from a harmful decay process, which would interfere with the nematode's development (Poinar and Thomas, 1966).

Meanwhile, the exsheathed nematodes which had entered the hemocoele mature into adult males and females in about four days. After mating occurs, the eggs develop and hatch within the female. The larvae feed on the uterus and then break into the body cavity of the mother nematode, and feed until reaching the second stage of development. At this point they break out of the mother's cuticle and go into the hemocoele of the host insect. Some of the juveniles molt and retain their old cuticle to become daueralarvae, while others continue to mature and go on to reproduce and form a second generation. This process continues until the host's body is filled with infectious nematodes. Each complete life cycle takes from five to eight days, depending on temperature. Eventually, the insect cuticle ruptures, and the new daueralarvae escape into the soil and the surrounding medium. One large host larva may harbor over 100,000 daueralarvae. In many cases the daueralarvae will remain in the host cadaver until free water becomes available. Laboratory tests have shown that daueralarvae can remain viable within a cadaver for up to two months (Dutky, 1956).

With regard to physical characteristics, these neoaplectanids are a remarkably hardy species. Tests show the daueralarvae to be resistant to many popular chemical insecticides such as DDT, chlordane, endrin,
lindane, methoxychlor, and toxaphene. They are also more tolerant of alkaline solutions than of acid, which is not surprising when one considers that the pH of most insect guts is alkaline. Dauerlarvae can survive forced spraying (up to 100 psi) through various size nozzles on conventional insecticidal spray equipment (however, since the dauerlarvae are about 1/25 inches in length, they will clog fine nozzle screens). DD-136 is very sensitive to moisture and will not survive desiccation even for short periods of time. Temperatures above 95°F. will kill a high percentage of the nematodes. On the cold side, they will tolerate 32°F., or lower, as long as they do not actually freeze. These preceding tolerances and more were determined by Schmiege (1963).

To summarize the positive and negative aspects of DD-136 as a commercial insect parasite, Schmiege (1963) mentioned the following:

**Negative Aspects:**
1. moisture requirements
2. dispersal ability (kills the host too quickly)
3. searching ability (none indicated)
4. difficulty in handling

**Positive Aspects:**
1. wide host range (may be too wide)
2. broad temperature tolerances
3. resistance to chemicals
4. ease of application (not killed by high pressure application).

The bacterium, *Bacillus thuringiensis* Berliner

Much of the following information was extracted from Heimpel's review of crystalliferous bacteria (Heimpel, 1967).
Like the nematode, this bacterium kills the insect indirectly, except that in this case, death is the result of an intoxication, not an infection.

The host range is rather wide and includes species in Lepidoptera, Hymenoptera, Diptera, and Coleoptera. The lepidopterans, which include many serious economic pests, have been found to be the most susceptible.

There are several toxins involved but the most important toxin is associated with the bipyramidal crystal produced during sporulation. Recent studies indicate that the crystals are nucleotides (Noordink et al., 1967). It was suggested that they may act by becoming incorporated into the nucleic acids and in this manner interfere with the transmission of genetic information during protein synthesis (an interesting parallel with the behavior of a virus).

*B. thuringiensis* belongs to that group of bacteria known as endospore-forming bacteria. It is closely related to *B. cereus* but differs in that *B. cereus* does not form crystals during sporulation.

Death of the insect is the result of a chain of events which shall be briefly mentioned. Preparations of the bacillus usually exist as a mixture of spores and parasporal bodies (crystals). The typical reaction of a susceptible insect such as the silkworm, *Bombyx mori* Linn., is that the alkaline environment of the gut juices induces germination of the spores and dissolves the alkaline-soluble parasporal bodies. In many lepidopterans the gut pH is around 10, whereas the pH of the hemolymph is 7 to 7.2. Once the toxins are released, the gut becomes paralyzed, followed by loss of integument of the midgut. This allows for leakage
of the highly buffered gut juices into the more acidic hemolymph. This change in the pH of the blood then causes general paralysis and death. Death occurs in susceptible insects in 60 to 80 minutes after ingestion. Physiological effects of the bacillus vary according to the susceptibility of the insect.

Standardization of a commercial product using this microorganism has been very difficult indeed. Many varieties and even strains of varieties of *B. thuringiensis* have been discovered. No single test has yet been devised which can separate or classify them. Some of the more reliable tests include serological comparison of the flagellar antigens, and esterase patterns based on gel electrophoresis. However, there have been strains found which gave the same results for these two tests, but different results when tested for relative virulence.

In referring to *B. thuringiensis* preparations, the spore count has been, and still is, used as the official measure of potency. However, the spore content does not correlate with the killing power of the preparation. But since the spore content is directly proportional to the crystal content, this measure is useful in describing the strength of various formulations of the same product. Also, the spore count is the only measurement in use not directly connected to the effect on a single species of insect (*Biotrol Res. Bull.*, 1971). For this reason, the dosages are usually given in terms of spore count and the name of the commercial product.

*B. thuringiensis* was the first bacterium to be produced industrially, and in several countries hundreds of tons per year are being produced.
The spores are highly resistant to heat, shortwave radiation, and toxic chemicals, and they can remain dormant for long periods (up to half a century). Spores prepared as a dust are more stable than spores prepared in a spore-crystal slurry. Various supplements commonly used with insecticides have little effect on Bacillus thuringiensis. This is true for many wetting agents, emulsifiers, adhesives, and fillers. Heimpel (1967) sums up the following advantages and disadvantages to the use of B. thuringiensis as a biological control:

Advantages:
1. apparent specificity for target insects
2. no harm to man or vertebrates
3. harmless to bees except in massive dosages
4. no phytotoxicity found
5. no tolerances required (because of no toxic residues)
6. little influence on the biocoenosis
7. no sign of resistance build-up in insects treated in past
8. good persistence under adverse conditions except to sunlight
9. mixes well with other insecticides and additives
10. may persist as a disease in certain field populations

Disadvantages:
1. specificity of B. thuringiensis will not protect a given plant from all enemies
2. strict timing of application is sometimes necessary
3. in many cases, the effective period is only for two weeks
4. agitation in spray tank often necessary during application, and antifoaming agents may be necessary
5. may be commercially expensive.

Taking these points into consideration, the design of the tests was planned accordingly.

The nuclear polyhedrosis virus of Heliothis zea

The nuclear polyhedrosis viruses of insects are a rather specialized group, differing from the viruses causing diseases of plants or verte-
brates. The term "polyhedrosis" refers to the large protein inclusions of polyhedral shape as opposed to the rounded shapes of the "granulosis" type viruses. The term "nuclear" refers to the location in the host cell in which new viral particles and polyhedra are replicated as opposed to the "cytoplasmic" locations of some other viruses. Nucleopolyhedrosis viruses are specific to the species of insect, i.e., the nuclear polyhedrosis virus of *Heliothis zea* is different from the nuclear polyhedrosis virus of *Trichoplusia ni*.

Viruses in general, although highly virulent when transmitted immediately to the next host, are easily inactivated by mild heat or desiccation and therefore are not suitable for practical use as insecticides. In the case of the polyhedrosis viruses, the virus rods are encased in a protective protein envelope or inclusion body and remain infective for many years. The polyhedra are approximately one micron in diameter, with many submicroscopic virons enclosed within. These polyhedra are tough in that they are resistant to desiccation, to heat and low temperatures, and to organic solvents. However, they are vulnerable to the ultraviolet irradiation of direct sunlight and may lose up to one-half their potency after two days of direct sunlight (Bullock, 1970).

The nuclear polyhedrosis virus of *Heliothis zea* is of the genus *Borrelina* (Weiser, 1965). Early instar larvae are the most vulnerable to infection while the adults seem to be immune. The polyhedral inclusion bodies (PIB) of the virus must be ingested while the larvae are feeding, and like *B. thuringiensis*, (and DD-136), the action begins
When the polyhedra reach the midgut. The alkaline-soluble protein coat readily dissolves in the midgut juices, and releases the viral rods intact. The virus particles penetrate through the midgut epithelium and begin multiplying in the nuclei of the blood cells, tracheal matrix, fat bodies, and epidermis (Steinhaus, 1963). Shapiro (1971) found an interesting characteristic at this point in that after 96 hours of infection, the effect on the hemolymph was hyperaminoacidemia and hypoproteinemia. An infected larva shows little evidence of illness until shortly before death. The first sign of infection is the characteristic glossy appearance of the integument. Within 2 to 6 hours after this condition is first noted, the insect will die (Biotrol Res. Bull., 1971). After death, the integument remains more or less intact and is filled with dark homogeneous remains of body tissue.

Quantities of polyhedrosis virus are measured in terms of larval equivalents (LE). A larval equivalent is based on the amount of virus contained in one full grown larva, freshly dead of the disease. This amount has been standardized as $6 \times 10^9$ polyhedra or their equivalent in insecticidal activity. Recommended dosages for a crop usually range from 100 to 200 LE per acre. Most research seems to indicate that the higher the dosage the better the control (Chapman and Bell, 1967).

As is true of any insecticide, there are advantages and disadvantages to this pathogen which should be considered. So far, tests indicate the virus to be safe in that no adverse effects have been noted on humans, warm-blooded animals, birds, fish, crustaceans, zooplankton, plants, or
insects other than *Heliothis* sp. Barnes (1970) fed large quantities of the virus to rats but noted no ill effects. He was especially watchful for neoplasia or carcinogenic effects. Another favorable factor is that it may be applied like a chemical insecticide.

Wetting agents show no detrimental effects on the virus and are recommended as a supplement. On the other hand, a disadvantage is the length of time it takes for death to occur after feeding. It usually takes three or four days in which time much feeding can take place. Thompson (1959) found that infection fails to occur after ingestion if the temperature is above 39° C. However, it is doubtful that the earworm could survive that temperature for long. Another problem of possible commercial significance is the typical adherence of the cadavers to the food crop. Finally, the timing of application is critical in that earworms are most vulnerable immediately after they hatch.

**Summary**

The aforementioned pathogens share some common good and bad points to keep in mind when considering the suitability of pathogens for insect control. Generally, they are rather host selective; they are safe; they have not lost their toxicity or infectivity to host insects; they can be applied by conventional equipment; and their cost is already competitive with standard chemical insecticides, while having few of the faults common to such insecticides (Cameron, 1966). For the most part, they all are vulnerable to sunlight and heat to a certain extent. And finally, best results are obtained with liberal dosages, without fear of toxicity.
B. LITERATURE REVIEW

Use of pathogens for biological control of insect pests has steadily gained prominence in the research field; especially since control results have been encouraging and mass-production of some pathogens has become feasible. In spite of all control efforts, the United States is estimated to have corn earworm damage of 75 to 140 million dollars annually (Pfadt, 1962). Even before standard DDT control was being phased off the market, the corn earworms were showing increased resistance (Brazzel, 1964). Other chemical insecticides have generally proven either expensive, too toxic, ineffective, too residuous, or otherwise undesirable.

Though corn earworm damage has been substantially reduced by improved cultural methods including the use of resistant varieties of corn, recent research for better controls has continued. Studies involving biological control by predaceous and parasitizing insects have shown little promise (Gonzalez et al., 1970; Oatman, 1970). Trapping adult moths with blacklight traps also proved inadequate (Graham et al., 1972).

Consideration of pathogens for biological control is but a natural consequence of study in the field of insect pathology. E. A. Steinhaus, who might be considered the "father of insect pathology," speculated on this use of pathogens almost thirty years ago (Steinhaus, 1945) even before writing his noted volumes, *Insect Microbiology* (1947) and *Principles of Insect Pathology* (1949). Progress has been slow, however, and Hurpin (1970) noted that out of more than 100 entomopathogenic
organisms discovered, only five have come into practical application. Among them he listed *Bacillus thuringiensis* and the nuclear polyhedrosis virus of *Heliothis zea*.

Early work with the bacillus involved lab work such as the study of toxicity of the parasporal bodies (Angus, 1956), susceptibility of certain insect pests (Hall, et al., 1958), and physiological effects (Heimpel, 1959). With the discovery of the varieties and strains of *B. thuringiensis*, much effort was concentrated in this area and Heimpel (1967) attempted to bring it all up to date in his review of crystalliferous bacteria. More recent literature indicates that work on the bacillus, its strains and its toxins, is expanding. Companies which sell preparations of the bacillus for insecticidal use continue to search for a strain with both a wide host range and a high degree of toxicity to insects.

Some of these basic trends in research are true also for the nuclear polyhedrosis virus. Here, moreover, much of the work has been done in connection with the corn earworm since one of the nuclear polyhedrosis viruses discovered so far is species specific for *H. zea*. Nuclear polyhedrosis viruses of corn earworms and cabbage loopers (*Trichoplusia ni* Hubner) have been field tested with varying degrees of success (Young and Hamm, 1966; Woodall and Ditman, 1967; Chapman and Bell, 1967).

The nematode, DD-136, must be considered a true pathogen since it fits the definition of a microorganism which causes disease, although it might be argued that it is simply a vector of the disease (caused by the symbiotic bacterium). Schmiege (1963) found promising results with
use of the neoaplectanid against certain forest insect pests. The nematode has also been reportedly successful against the banded cucumber beetle larva, white-fringed beetle larva, cabbage worm, corn borer, and cabbage root worm (Creighton et al., 1968; Harlan et al., 1971; Welch and Briand, 1961). On the other hand, poor results were obtained against the Japanese beetle, soil-inhabiting pasture grubs, Colorado potato beetle, artichoke plume moth, and the corn earworm (Reed and Carrie, 1967; Tanada and Reiner, 1962; Welch and Briand, 1961).

Some comparative studies of the pathogens within a single test have been done with conflicting results. Greighton et al., (1971) obtained good results with the bacillus but poor results with the nuclear polyhedrosis virus on the tomato fruitworm (H. zea). Tanada and Reiner (1961) obtained excellent results with the nuclear polyhedrosis virus (compared favorably with 5 percent DDT), nearly as good with the bacillus, but poor control with the nematodes as mentioned earlier. Anderson (1963) reported complete failure with both the bacillus and the virus. And Oatman et al. (1970) found the virus to be superior to the bacillus but used only one preparation of the bacillus in only one experiment.

The purpose of the following experiments was to help clarify the following points:

1. is there a significance in the method of application
2. can nematodes be used in conjunction with the virus
3. is there a pathogenic control which can compete with a standard accepted chemical control
4. are control results enhanced by late evening treatment.
CHAPTER II

METHODS AND MATERIALS

The field tests were conducted during the summers of 1971 and 1972 on corn grown on the Agriculture Campus of The University of Tennessee in Knoxville. The field is situated in bottomland close to Fort Loudoun Lake. The corn earworm population for the tests depended on natural infestation by the moths in this region. (Judging from the results, this natural infestation was substantial, with 76 percent of the control ears being infested in the first test and 84 percent in the second test.)

The nuclear polyhedrosis virus used was a commercial preparation known as Biotrol VHZ. This particular formulation was a concentrated wettable powder which contained five larval equivalents per gram \( (30 \times 10^9 \text{ PIB/gm.}) \). Its recommended dosage was 50 to 100 LE per acre, repeated as required.

The bacillus used was of three commercial preparations which have been approved for insecticidal use by the U. S. Food and Drug Administration:

1. Dipel dust, \( 25 \times 10^6 \) spores/mg.
2. Thuricide HPC aqueous concentration, \( 6 \times 10^6 \) spores/mg.
   with one ml. of the suspension weighing one gram
3. Thuricide HP-90 M dust, \( 3.75 \times 10^5 \) spores/mg.
The nematodes were propagated in the laboratory by using corn earworm cadavers as hosts. However, the process yielded just enough for one treatment (approximately 1,200,000 dauerlarvae).

The chemical insecticide used was Sevin-80S, a carbamate which is primarily a stomach poison. It is a wettable powder and has been used as a standard insecticide on sweet corn in California (Oatman et al., 1970). The "80S" means that it contains 80 percent active ingredients by weight, and its recommended dosage is 1.5 lb. ai/acre.

The treatments were conducted on 50 foot rows of corn. Each treatment was replicated four times in the randomized complete block design. There were untreated guard rows on the outside margins of the field. Total area of the field covered approximately 4500 square feet, which is a little more than one-tenth of an acre. Dosages and concentrations were figured accordingly.

Treatments were initiated when it appeared that a majority of the ears had at least one inch of silk visible. Any ear with silk showing was then treated and tagged so that all ears were treated with the same number of applications for the evaluation. Applications were repeated either once or twice with a two or four day span between.

Both treatments and harvesting were done during the month of August in both experiments. When the ears were considered to be mature but not over mature (about a week or less after applying the last set of treatments), they were picked, shucked, and evaluated on the same day.
Evaluation (see Figures 1 through 5) was originally based on a damage scale rating of from 1 to 5 as explained below:

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<thead>
<tr>
<th>Rating</th>
<th>Amount of Damage</th>
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<tr>
<td>1</td>
<td>No damage, but may include damage to silk</td>
</tr>
<tr>
<td>2</td>
<td>Very slight to light (less than one inch)</td>
</tr>
<tr>
<td>3</td>
<td>Medium (one inch to two inches)</td>
</tr>
<tr>
<td>4</td>
<td>Heavy (two to three inches)</td>
</tr>
<tr>
<td>5</td>
<td>Very heavy (three or more inches)</td>
</tr>
</tbody>
</table>

The data were recorded using this scale. These data were later consolidated into a more practical form as done by Oatman et al., (1970):

<table>
<thead>
<tr>
<th>Old Scale Equivalent</th>
<th>Rating</th>
<th>Amount of Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Perfect</td>
<td>No damage</td>
</tr>
<tr>
<td>2 and 3</td>
<td>Marketable</td>
<td>Less than 25 percent of ear is damaged (2&quot; or less)</td>
</tr>
<tr>
<td>4 and 5</td>
<td>Unmarketable</td>
<td>More than 25 percent of ear is damaged</td>
</tr>
</tbody>
</table>

As an additional measure of effective control, the sum total of both living and dead larvae per ear was recorded. This is considered a valid measurement since the presence of earworms, alive or dead, depreciates the value of an ear as does the amount of damage.

Summer, 1971

This test was designed to find the best method of application, and to compare two commercial preparations of the bacillus.
Figure 1. On the damage rating scale, these ears were rated as "1." Although they may not appear to be perfect there is no evidence of corn earworm damage.
Figure 2. Damage slight, representative of a "2" rating. Marketable.

Figure 3. Damage medium, representative of a "3" rating. Marketable.
Figure 4. Damage heavy, representative of a "4" rating. Unmarketable.

Figure 5. Damage very heavy, representative of a "5" rating. Unmarketable.
The treatments were as follows:

1. Control
2. Thuricide HPC, applied as a spray
3. Thuricide HPC, applied by syringe
4. Thuricide HPC, applied by squeeze bottle
5. Dipel Dust, applied as a dust.

All aqueous treatments were applied at the rate of approximately one milliliter per ear. Thuricide HPC, which has $6 \times 10^9$ spores per gram, was mixed with water at a rate of 20 ml. per 200 ml. mixture. This figured to $6 \times 10^8$ spores per ear per application. Of course, this figure represents an ideal situation since varying amounts of runoff from each ear occurred.

Treatment 2 was sprayed on the silk of each ear with an insecticide spray gun. Treatment 3 was inoculated into the ear through the husk with a syringe. Treatment 4 was squirited down the silk channel by using a plastic squeeze bottle with a nozzle. Treatment 5 was applied thoroughly onto the silk by using a stiff shaving brush.

All treatments were applied in the morning and were accomplished within a single day. Treatments were applied only twice, with the second one applied after a two day interval. Exactly one week following the second or last treatment the ears were picked and evaluated.

Summer, 1972

A laboratory test was conducted to determine if the nematode could be mixed with the virus without harm being done to the nematodes. Three
jars containing 60 ml. each of water were set up at room temperature. The first jar was the control with nematodes only (about 10,000). The second jar contained the same number of nematodes plus .5 gram of the virus preparation, Biotrol NPV. The third jar contained the same number of nematodes plus 1 gram of the virus preparation.

<table>
<thead>
<tr>
<th>Jar</th>
<th>Water</th>
<th>Nematodes</th>
<th>Virus PIB per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>60 ml.</td>
<td>10,000</td>
<td>None</td>
</tr>
<tr>
<td>#2</td>
<td>60 ml.</td>
<td>10,000</td>
<td>$2.5 \times 10^8$</td>
</tr>
<tr>
<td>#3</td>
<td>60 ml.</td>
<td>10,000</td>
<td>$.50 \times 10^8$</td>
</tr>
</tbody>
</table>

The concentrations of the virus approximated those which were used per ear in the field ($8.0 \times 10^7$ PIB per ml.). The jars were then checked 1, 3, and 7 days later. After one day, in all three jars, the nematodes appeared equally vigorous which meant that they could be mixed in the field for a treatment. After three days, again the survival rate seemed equal for the three jars, except that an unidentified fungus was growing in jar #3. After one week, at which time observations were discontinued, jars #1 and #2 still had approximately the same number of living nematodes (about 50 percent in both cases). Jar #3 had large populations of several unidentified protozoans and fungi, and all but a few nematodes were dead. It is realized that these results cannot be considered conclusive since there was no replication in the test's design, and the viability of the virus was not checked.

The field test was designed to make comparisons of effective control by all three pathogens and the chemical insecticide. Since all three pathogens are vulnerable to direct sunlight, all applications were
made after 6:00 p.m. E.D.S.T. Also, a wetting agent, Tween 20, was used in all treatments at a concentration of 1:2000.

The treatments were as follows:

1. Nematodes plus virus: 2000 nematodes; $8.0 \times 10^7$ polyhedra per ear
2. Biotrol NPV: $8.0 \times 10^7$ polyhedra per ear
3. Thuricide 90 M Dust
4. Thuricide HPC: $6 \times 10^8$ spores per ear
5. Sevin 80S: 40 g/liter
6. Control.

All liquid treatments were applied with a graduated pipette at the rate of 1 ml. per ear. The dust was applied with a stiff shaving brush. Dosages were based on the recommended amounts given by the manufacturers of the products, i.e., virus at 200 LE per acre, Sevin at 1.5 lbs. ai/acre, and Thuricide HPC at 2 quarts per acre.

There were three applications of each treatment made at four day intervals. Due to the short time available to apply the treatments (from 6:00 p.m. until darkness), it took two days to complete each round of applications. It also took two days to harvest and evaluate the ears, beginning six days after the last treatment.
CHAPTER III

RESULTS AND DISCUSSION

Summer, 1971

Figure 6 shows the percentages based on the 1 to 5 damage rating scale. The application by syringe (treatment 3) gave 74 percent perfect ears, far better than any other treatment.

On the other treatments, the consistently high percentages at a 1 rating can be explained in that they include those ears which were not naturally infested in the first place. There were no unmarketable ears in treatment 3 (Table I) while all other treatments suffered at least a 10 percent loss. The effectiveness of the syringe application is also implied by its having the lowest number of larvae per ten ears.

A statistical analysis based on Duncan's Multiple Range Test (Table II) again shows the treatment by syringe to be significantly better at the 5 percent level. The syringe method of application undoubtedly had the effect of placing more of the pathogen inside the ear. This probably resulted in its being more easily ingested by the earworm larvae and therefore giving the best control. With this in mind, the field test in 1972 was designed to include a wetting agent in the hope that more pathogen, via the silk channel, might end up on the inside of the ear.
Figure 6. Percentages of damages per treatment according to the 1 to 5 damage rating scale, Summer, 1971.
TABLE I

EFFECTIVENESS OF TWO COMMERCIAL SPORE PREPARATIONS AND METHODS OF APPLICATION OF BACILLUS

<table>
<thead>
<tr>
<th>Treatment Number</th>
<th>Preparation</th>
<th>Application by</th>
<th>Percentage of Ears With</th>
<th>Number Larvae Per 10 Ears</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No Damage</td>
<td>Damage Marketable</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>--</td>
<td>24</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>Thuricide HPC</td>
<td>Spray</td>
<td>18</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>Thuricide HPC</td>
<td>Syringe</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>Thuricide HPC</td>
<td>Bottle</td>
<td>27</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>Dipel Dust</td>
<td>Brush</td>
<td>27</td>
<td>58</td>
</tr>
</tbody>
</table>
TABLE II

STATISTICAL ANALYSIS OF SUMMER, 1971 TREATMENT RESULTS

<table>
<thead>
<tr>
<th>Treatment Number</th>
<th>Preparation</th>
<th>Application By</th>
<th>Mean Percent Sound Ears</th>
<th>5 Percent Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Thuricide HPC</td>
<td>Syringe</td>
<td>74</td>
<td>a</td>
</tr>
<tr>
<td>5</td>
<td>Dipel Dust</td>
<td>Brush</td>
<td>27</td>
<td>b</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>---</td>
<td>24</td>
<td>b</td>
</tr>
<tr>
<td>2</td>
<td>Thuricide HPC</td>
<td>Spray</td>
<td>17</td>
<td>b</td>
</tr>
<tr>
<td>4</td>
<td>Thuricide HPC</td>
<td>Bottle</td>
<td>4</td>
<td>c</td>
</tr>
</tbody>
</table>

*aAny two means followed by the same letter are not significantly different at the .05 level of probability.*
Summer, 1972

Generally better control results were attained in 1972 (Figure 7). Natural infestation was a high 84 percent as indicated by the control, treatment 6. The nematode-virus combination gave the highest percentage of perfect ears, 0 percent unmarketable ears, and the lowest number of larvae per ten ears. The bacillus dust treatment gave the poorest results in all categories which seems to further substantiate the conclusions of the 1971 results (see Table III).

Statistical analysis of the data (Table IV) indicates that the nematode-virus combination gave the best control and was significantly better than the chemical insecticide Sevin. It must be added that when the ears were being picked, there were dead insects found on the silks of only those ears treated by Sevin. These included at least two beneficial beetles, the spotted lady beetle, *Coleomegilla maculata* (De Geer), and the nine-spotted lady beetle, *Coccinella novemnota* (Herbst). Both of these beetles are considered beneficial in that both the larval and adult stages feed on aphids, small earworm larvae, and earworm eggs. This might be considered one advantage of species-specific pathogens.
Figure 7. Percentages of damage per treatment according to the 1 to 5 damage rating scale, Summer, 1972.
TABLE III
EFFECTIVENESS OF THREE PATHOGENS AND A CHEMICAL INSECTICIDE, AND A COMPARISON BETWEEN TWO COMMERCIAL SPORE PREPARATIONS OF BACILLUS

<table>
<thead>
<tr>
<th>Treatment Number</th>
<th>Preparation</th>
<th>Percentage of Ears With No Damage</th>
<th>Damage Marketable</th>
<th>Damage Unmarketable</th>
<th>Number Larvae Per 10 Ears</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virus and Nematodes</td>
<td>86</td>
<td>14</td>
<td>0</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>Virus</td>
<td>53</td>
<td>44</td>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>Bacillus, Dust</td>
<td>23</td>
<td>42</td>
<td>35</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus, Slurry</td>
<td>76</td>
<td>22</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>Chemical Insecticide</td>
<td>62</td>
<td>29</td>
<td>9</td>
<td>3.9</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>16</td>
<td>58</td>
<td>26</td>
<td>6.0</td>
</tr>
</tbody>
</table>
### TABLE IV

**STATISTICAL ANALYSIS OF SUMMER, 1972 TREATMENT RESULTS**

<table>
<thead>
<tr>
<th>Treatment Number</th>
<th>Preparation</th>
<th>Mean Percent Sound Ears</th>
<th>5 Percent Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Virus and Nematodes</td>
<td>90</td>
<td>a</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus, Slurry</td>
<td>76</td>
<td>ab</td>
</tr>
<tr>
<td>5</td>
<td>Chemical Insecticide</td>
<td>67</td>
<td>b</td>
</tr>
<tr>
<td>2</td>
<td>Virus</td>
<td>56</td>
<td>b</td>
</tr>
<tr>
<td>3</td>
<td>Bacillus, Dust</td>
<td>25</td>
<td>c</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>15</td>
<td>c</td>
</tr>
</tbody>
</table>

*a* Any two means followed by the same letter are not significantly different at the .05 level of probability.
CHAPTER IV

SUMMARY AND CONCLUSIONS

More tests are needed before the significance of these results can be determined. It has not been proven that a wetting agent is needed, nor that late evening applications give best results, although this was implied by the tests. It is not known whether the nematode-virus combination gave the best control because of a cumulative or a synergistic effect. Prior research showed poor control by nematodes alone but now the possibility exists that they can be used to good advantage when mixed with another control.

Also, evaluation of results may differ according to which is considered more important, highest percentage of perfect ears or lowest percentage of unmarketable ears. There were definite points indicated by the test results, however, and they are as follows:

1. Application by syringe gave the best results with the bacillus.
2. The bacillus was not effective when applied as a dust formulation.
3. The addition of a wetting agent and late evening applications may enhance the effectiveness of pathogens.
4. A biological control surpassed the control by a standard chemical insecticide.
5. A nematode-virus combination produced the most significant control of the corn earworm.

In evaluating the results of these tests, it should be remembered that the individual-ear treatment was thorough and heavy; therefore, control of earworms may be poorer with the lower dosages and less thorough coverage normally used on commercial crops.
LIST OF REFERENCES
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VITA

W. Robert Tilyard, Jr. was born in Baltimore, Maryland, on February 9, 1943, the son of Walter Robert Tilyard and Doris Barber Tilyard. He attended elementary school in Winston-Salem, North Carolina, and graduated in 1961 from Greensboro Senior High in nearby Greensboro, North Carolina. He attended the University of North Carolina at Chapel Hill for one year prior to enlistment in the U. S. A. F. After honorable discharge in 1967 he entered Guilford College in Greensboro, North Carolina, where he received a Bachelor of Science degree in biology in August, 1970. The following September he began study toward a Master of Science degree in Entomology. In September 1971, he accepted a Graduate Teaching Assistantship which he retained until receiving his degree in December 1972.

He is married to the former Martha Jane Barefoot of Greensboro, North Carolina, and they have one son, Richard Sterling.