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Margaret Elaine Cox  
*Louisiana State University and Agricultural & Mechanical College*

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STUDIES ON VIRAL, FUNGAL, AND BACTERIAL PATHOGENS FOR THE
SOYBEAN LOOPER, PSEUDOPLUSIA INCLUDENS (WALKER)

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Microbiology

by
Margaret Elaine Cox
B.S., Louisiana State University, 1969
August, 1972
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ABSTRACT

Studies were undertaken to determine the causative agent(s) of epizootics among soybean loopers (Pseudoplusia includens) observed in the late summer of recent years. Bacteria, fungi, and viruses associated with diseased larvae were isolated and characterized.

The microflora of laboratory-reared larvae differed markedly from the microflora of larvae collected from soybean fields. Twenty-four bacterial isolates representing 13 genera were cultured from mid-gut tissues of field-collected loopers. It appeared that the microflora of the soybean looper was dependent upon the microflora of the food ingested. Five bacterial isolates were cultured from mid-guts of laboratory-reared larvae, although seventy-five per cent of the larvae were free of bacteria that could be cultured by the methods employed. Sixty per cent of the bacteria isolated from diseased larvae collected during the 1969 epizootic were potential pathogens from the genera Bacillus and Pseudomonas.

Two fungi were associated with diseased P. includens larvae collected during epizootics. Resting spores and conidia of Entomophthora (T.) gammae were found. Infectivity of this fungus
for soybean loopers could not be established in the present investigation and attempts to culture the pathogen were also unsuccessful. A second fungal isolate was obtained by placing antibiotic-treated resting spores of E. (T.) gammae in a culture of mouse L cells. This fungal culture showed no pathogenicity for soybean loopers by ingestion or through cuticular contact. The fungus was identified by Dr. E. G. Simmons of U. S. Army Natick Laboratories as a species of Rhinocladella.

Viruses were isolated from P. includens larvae during the epizootics of 1970 and 1971. Both of the isolates were nuclear polyhedroses. Target tissues for polyhedral formation were determined to be identical for the isolates - fat body, epidermis, tracheae, and wing bud tissues. By electron microscopy virions were observed in nuclei of mid-gut cells infected with the 1970 isolate. Infection of fat body and tracheal cells was similar for the isolates. Virogenic stroma was observed in fat body cells which gave rise to virions. Virions acquired membranes and coalesced with polyhedral matrices. Polyhedra found in tracheal cells were surrounded by membranes and some had dark staining centers.

Infectivity tests with the 1970 and 1971 viral isolates were conducted to determine the pathogenicity of the viruses for P. includens larvae. LD's of the viral isolates for 3rd, 4th, and 5th instar larvae were obtained.
From these studies it appeared that bacterial, fungal, and viral pathogens were involved in epizootics among larvae of *Pseudoplusia includens* in Louisiana soybean fields.
INTRODUCTION

Epizootics among *Pseudoplusia includens* larvae in Louisiana soybean fields have been noted in late August to September of recent years. Dead larvae were found on the upper part of soybean plants affixed to the undersurface of leaves by their prolegs. Two different types of diseased larvae were collected during the epizootics of 1969, 1970, and 1971: a brown type and a black type. Brown specimens were dry, leathery, and firmly attached to leaves. Larvae of the second type were moist, brittle, and easily crushed. Two weeks after the epizootics had begun, the looper population had been reduced by more than 95%.

Crop damage is extensive in many instances before epizootics among soybean loopers occur. Since chemical insecticides registered for use on soybeans do not effectively control this insect in Louisiana, a biological control agent is desirable. Biological control has many advantages. Pollution of the environment is curtailed since the need for applying chemical insecticides is eliminated. Many biological control agents (especially viruses) are highly specific for the pest insect, thereby posing no danger for nontarget organisms. The
biological agent continually undergoes selection of more virulent strains as the insect develops resistance.

Studies were undertaken to isolate and characterize pathogens associated with diseased larvae collected during epizootics, with the eventual aim of developing a biological insecticide against *Pseudoplusia includens*. 
SELECTED LITERATURE

I. Biology of *Pseudoplusia includens*

Soybeans have become a major economic crop in Louisiana during the last decade. In 1960, 216,000 acres were planted with soybeans as compared to 1,436,000 acres in 1968 and 1,695,000 acres in 1971 (Fielder, Parker, and Penn, 1969; USDA Statistical Reporting Service, 1972). An increase in damage to soybeans caused by *Pseudoplusia includens* has been associated with increased production.

The soybean looper, *Pseudoplusia includens* (Walker) is a Lepidopteran in the family Noctuidae. Its preferred host is soybeans, although it has also been found on peanuts, sweet potatoes, cotton, tomatoes, crucifers, and peas (Hensley, Newsom, and Chapin, 1964). They reported that 90% of the Plusinae larvae found on soybeans in Louisiana were *P. includens*. The larvae of the soybean looper damage crops mainly by extensive leaf defoliation and crop yield can be seriously reduced in late-planted beans. Pod damage often seen in bean fields was assumed to be caused by bollworms, although extensive searches revealed very low numbers of these larvae. In the summer of 1971, soybean looper larvae were observed attacking pods in fields with a high population density of these pests (Jensen, personal communication).
Evidence is fairly convincing that the soybean looper does not overwinter in Louisiana, but it may do so in the lower Rio Grande Valley of Texas and parts of Florida (Newsom, personal communication). Many adults fly across the Gulf of Mexico every summer from Central and South America or Mexico. Mitchell (1968) described the development of these insects reared on cotton, soybeans, and artificial diet. Larvae reared on soybeans required 26.58 days to reach adulthood, while 32.71 days were required for those reared on cotton to emerge as adults. At 80°F the entire life cycle, from egg to adult, required 29.73 days when reared on the artificial diet of Shorey and Hale (1965). Newsom (personal communication) observed that large populations of loopers occur in areas where cotton fields adjoin soybean fields. The peak of the population is reached when the cotton begins to bloom and is thought to be directly correlated to fecundity of adults feeding on cotton nectar.

_P. includens_ is resistant to most chemical insecticides. Organochlorine insecticides satisfactorily controlled these pests in the 1950's as did the organophosphates in the early 1960's (Chalfant, 1969). Recently the larvae have shown resistance to all chemicals except the carbamate, methomyl, which is not registered for use on soybeans (Graves, personal communication). The 1972 Insect Control Guide distributed by the Louisiana State
University Cooperative Extension Service states "none of the insecticides currently registered on soybeans will give effective control" of the soybean looper.

In the late summer of 1966, a disease in epizootic proportions was observed among *Pseudoplusia includens* larvae (Newsom, personal communication). The epizootic developed similarly each year in late August to September, after extensive damage to the crop had occurred (Burleigh, 1972). If the epizootic could be induced several weeks earlier than under normal circumstances, control of *Pseudoplusia includens* could be achieved without using chemical insecticides.

II. Bacterial Flora of Healthy Insects

"The primary reason for considering the microbiota of healthy insects in a work devoted largely to the microbial diseases of insects is the valid assumption that a sound understanding of the abnormal is based on a knowledge of the normal" (Brooks, 1963). Bacterial contaminants of insects can be harbored either externally or internally. Microorganisms carried on the body surface are fortuitous associates and seem to reflect the immediate past history of the insect—its breeding site, rearing chamber, or feeding habits (Brooks, 1963). Only a small number of bacterial species can live on the cuticle of insects (Steinhaus, 1949; Kaidsumi, 1957).
A very diverse bacterial flora is found internally in healthy insects. Microorganisms may be located in the blood, genitalia, spiracles, and tracheal matrix, although the greatest number of organisms are harbored in the gut (Steinhaus, 1949). Mycetocytes are cells located in the abdominal cavity of certain insects (cockroaches, lice, aphids, and ticks) and they harbor symbiotic bacteria, fungi, rickettsiae, and yeast. These symbionts are passed from generation to generation by direct entry into the micropyle of the egg or by the larva eating its way out of the egg which has been either externally or internally contaminated with the symbionts. Symbiotic organisms may furnish vitamins and other essential nutrients to their hosts, although in some instances the host is not dependent upon the microbes it harbors.

Investigators have studied the microflora associated with the guts of many insect species. The anatomy of the gut (either a straight tube or a tube invested with folds, sacs, and specialized regions) influences the nature of the microflora (Brooks, 1963; Patton, 1963). Studies involving the gut microflora of diverse groups of insects have shown that most of the harbored microorganisms are also a constituent of the insect's environment. The mid-gut of larval *Tenebrio molitor* (yellow mealworm) contained *Aerobacter cloacae*, *Bacillus* sp., *Streptococcus* sp., *Hafnia* spp., and two unidentified gram-negative rods. Only the *Hafnia* and
gram-negative rods were not found in the food and environmental samples (Wistreich, Moore, and Chao, 1960). In a study of the microorganisms associated with mosquitoes, a totally different microflora was found in the mid-guts of larvae as compared to adult *Anopheles albimanus* (Weidemann) except for a common *Saccharomyces* sp. No microorganisms were recovered from the guts of 21% of the larvae and 40% of the adults studied (Wistreich and Chao, 1961). A similar pattern was observed among larval and adult *Aedes sierrensis* (Ludlow) (Wistreich and Chao, 1961). It seems evident from these reports that the bacteria found in the guts of mosquitoes differs with each species studied (Ferguson and Micks, 1961; Wistreich and Chao, 1961).

Few studies have been reported on the microflora of the gut of Lepidoptera. Angus (1952) characterized the aerobic bacteria isolated from the mid-gut of the eastern hemlock looper. Organisms were members of the following genera: *Aerobacter*, *Achromobacter*, *Bacillus*, *Bacterium*, *Escherichia*, *Flavobacterium*, *Micrococcus*, and *Paracolobactrum*. He found no specific microflora associated with the insect and concluded that the bacteria isolated from the gut appeared to be those ingested with the food. In studying the microflora from the sugarcane borer mid-gut, Nunez, Hensley, and Colmer (1968) obtained several isolates that were coliforms, the majority of which gave the typical *Aerobacter aerogenes* IMViC pattern. *Streptococcus* sp. and a yeast of the genus *Torulopsis* were also isolated.
III. Biological Control of *Pseudoplusia includens*

A. Parasites

Burleigh (1972) conducted a thorough study of soybean looper populations at three sites devoted to soybean production in southwest Louisiana. He found looper populations to be low during June and July, but increased dramatically in August. Parasitism of *P. includens* by entomophagous insects was observed in 25% of the larvae brought into the laboratory. They were parasitized by the following species: *Apanteles scitulus*, *Mesochorus* sp., *Copidosoma truncatellum*, *Lespesia aletiae*, *Rogas molestus*, *Meteorus autographae*, and *Brachymeria ovata*, with *C. truncatellum* being the predominant parasite.

B. Bacteria

Bacteria are well-known insect pathogens, especially those in the genera *Bacillus* (*B. thuringiensis*, *B. popillae*, *B. finitimus*), *Pseudomonas*, and *Serratia* (Bucher, 1963; Heimpel, 1967; Heimpel and Angus, 1963; Moore, 1972; Slatten and Larson, 1967). *Vibrio apisepticus* was found to be the causative agent of septicemia in honey bees (Barr, 1967). *Serratia* sp. and several *Bacillus* species have been implicated as pathogenic for *P. includens* larvae under both laboratory and field conditions (Holloway, 1971; Hammond, personal communication).
Bacillus thuringiensis is the only bacterium commercially available for insect control in the United States. There are several toxic entities associated with this microorganism (Heimpel, 1967) and commercial products often differ as to the quantities of each toxicant contained in the finished preparation. Laboratory tests have shown the soybean looper to be susceptible to various B. thuringiensis products (Holloway, 1971; Chalfant, 1969), but tests with several commercial preparations failed to give effective control of these larvae in the field (Newsom, personal communication).

C. Fungal Pathogens

Spicaria rileyi is known to be a fungal pathogen of the soybean looper (Gudauskas and Canerday, 1966). Larvae attacked by this fungus become mummified and are covered with white mycelial growth which turns to green as conidia are produced. Burleigh (1972) observed outbreaks of Spicaria in looper populations during the late summer months, but the disease never exerted adequate suppression of the larvae. Spicaria is also a pathogen of the velvetbean caterpillar (Watson, 1916). Velvetbean caterpillars found in soybean fields which are also infested with Pseudoplusia larvae may serve to disperse the fungus throughout the looper population.

The family Entomophthorales contains two genera of fungi that are insect pathogens, Massospora and Entomophthora. Massospora
forms conidia inside its host, while the **Entomophthoraceae** produce conidia on the external surface of the insect. There are 32 alleged species of the **Entomophthoraceae**, in 25 of which the conidial states have not been described (MacLeod and Muller-Kogler, 1970). Identification of these fungi is based primarily on the morphology of the resting spores formed within the insect host. Burleigh (1972) and Holloway (1971) have described species of the **Entomophthorales** found in the soybean looper population. Burleigh described the resting spores of a fungus found in diseased larvae which he called *Massospora* sp. This fungus has subsequently been identified as an *Entomophthora* sp. (MacLeod, personal communication). The fungus isolated by Holloway is pathogenic for the soybean looper by injection into the hemocoel and through contact and/or ingestion (Holloway, personal communication). This species is also pathogenic for the forest tent caterpillar and the eastern hemlock looper when administered by injection (MacLeod, personal communication). Although the conidial state of this *Entomophthora* sp. has been described (Holloway, 1971), the identification of the resting spore stage remains uncertain.

D. Viruses

Virus diseases of insects have been known since the 1800's (Bergold, 1963). There are two categories of insect viruses;
those occluded into a protein matrix or polyhedron and those without such an inclusion body. Polyhedral inclusion bodies vary in size from 0.5-15 u, are visible under the light microscope, and thus characterized the first insect viruses described. Three classes of polyhedrosis viruses have been observed; those that multiply in the cell nucleus (nuclear polyhedroses), those that multiply cytoplasmically (cytoplasmic polyhedroses), and granuloses which form minute granules in cell nuclei which can be detected with difficulty by light microscopy (Stairs, 1968). The virions of the nuclear polyhedroses and granuloses contain DNA and are helical in shape, while the cytoplasmic polyhedrosis are spherical in shape and contain RNA. Poxlike viruses occluded within proteinaceous inclusion bodies have been isolated from Coleopteran and Lepidopteran hosts in recent years (Granados and Roberts, 1970). These viruses multiply cytoplasmically and are similar in many respects to poxviruses of vertebrates. Non-inclusion viral diseases of insects have been described only recently. The sacbrood disease of bees reported in 1913 was thought to be viral in nature, but actual observation of the infectious particles was not reported until 1949 (Vago, 1968). At least 20 non-inclusion virus diseases and 300 inclusion virus diseases of insects have been described (Stairs, 1968).

Viruses of _Pseudoplusia includens_ have been described within the last year (Cox, Larson, and Amborski, 1972; Livingston and
Yearian, 1972). Both of these viruses are nuclear polyhedroses. The nuclear polyhedrosis virus (NPV) described by Livingston and Yearian was isolated from *P. includens* from Guatemala, while the NPV described by Cox, Larson, and Amborski was isolated from an epizootic among *P. includens* populations in Louisiana.
MATERIALS AND METHODS

I. Rearing of *Pseudoplusia includens*

Larvae of *Pseudoplusia includens* were obtained from the Department of Entomology insectary, Louisiana State University. They were reared individually from hatching on pinto bean diet developed by Shorey and Hale (1965) and improved by Burton (1969). The diet contained formaldehyde, sorbic acid, and methyl parahydroxybenzoate which are bacterial and fungal inhibitors. One-ounce plastic jelly cups (Premium Plastics Co., Chicago) sealed with cardboard tops were used as rearing containers. The culture of *P. includens* had been maintained for several years in the laboratory with the introduction of new genes once a year when larvae from the fields were mixed in with the parent stock.

Larvae were held at 30 °C ± 2 from the time they were obtained from the insectary until used for experimentation. After treatment, they were held at room temperature.

II. Collection of specimens of *Pseudoplusia includens* during epizootics.

Diseased *P. includens* larvae were collected from Louisiana soybean fields during the 1969, 1970, and 1971 epizootics. The
first summer, several specimens were placed in any empty jelly
cup used for larval rearing in the laboratory. They were collected
and held in individual containers the next two summers. Collection
sites varied from year to year; in 1969, specimens were collected
near Krotz Springs and Bunkie. In 1970, collections were made
near Batchelor and Bunkie; and 1971, specimens were obtained from
fields near Chambers. Epizootics were in progress at each site
when the specimens were collected. Apparently healthy larvae
were brought to the laboratory in 1970 and 1971 and observed for
symptoms of disease.

Healthy larvae for microbial flora studies were obtained
near Krotz Springs and Bunkie in early July of 1969. No signs
of disease were evident among looper populations in these fields
at the time of collection. Larvae were brought to the laboratory
in a plastic bag filled with soybean leaves.

III. Identification of bacteria comprising the normal flora of
larval tissues.

A. Isolation

The mid-gut flora of larvae reared in the laboratory was
compared to that of larvae from soybean fields. Twelve fifth
and sixth instar laboratory-reared larvae were dissected and the
mid-guts removed and surface-sterilized according to the method of
Nunez, Hensley, and Colmer (1968). The gut tissue was placed in
a screw-cap tube (20 x 125 mm) containing 2.5 ml of sterile brain
heart infusion (BHI) broth and 50-60 glass beads (2 mm in diameter) and mixed on a Vortex mixer for 1 minute. Gut contents were streaked on BHI agar and the resulting microorganisms isolated in pure culture by restreaking on the same medium. The tubes containing the gut homogenate were incubated at 37 C for 24 hours and the contents streaked again on BHI plates; this procedure was repeated at 48 hours. These repeated isolations were made to increase the chance of obtaining microbes present at low concentrations in the gut that might have increased in numbers during the incubation period. Several samples of larval testes and hemolymph were also tested for microbial flora.

Five apparently healthy looper larvae from soybean fields were dissected in a similar manner and the microorganisms isolated. Total bacterial counts from these loopers were made on BHI agar, fungal counts were made on MY agar, and yeast counts were done on MY agar plus Achromycin (50 ug/ml) (Nunez, Hensley, and Colmer, 1968). Colonies were counted with the aid of a Quebec Colony Counter. Fresh frass samples were collected after excretion from larvae, diluted and mixed with 1-2 ml of sterile distilled water, and streaked on BHI agar plates.

Seven diseased larvae and 3 diseased pupae collected from Bunkie and Krotz Springs, Louisiana soybean fields during the 1969 epizootic were analyzed for types of bacteria. After collection, the insects were refrigerated until analyzed. The specimens were dead but fully intact. For isolation of bacteria,
the specimens were surface-sterilized for 2 minutes in 70% ethanol followed by a 1 minute rinse in sterile distilled water. The insects were then placed in sterile petri dishes. A quantity of body fluid was withdrawn aseptically with a disposable 1 cc syringe fitted with a 22 gauge needle. Some of the fluid was placed on BHI agar plates and streaked for isolation. The remainder of the liquid was added to sterile BHI broth, incubated at 37 C and samples plated at 24- and 48-hour intervals.

The number of bacteria, fungi, and yeast present on soybean leaves was determined from leaves collected at Bunkie, Louisiana during the 1969 epizootic. The leaves were weighed and added to distilled water (1:100 W/V). The mixture was shaken vigorously on a Vortex mixer. One-tenth ml aliquots were plated on BHI, MY, and MY-Achromycin agar plates.

Pure cultures of the microorganisms isolated from the mid-guts of laboratory-reared, healthy field-collected, and diseased field-collected *P. includens* were maintained on BHI agar slants. Transfers were made monthly, with the cultures stored at 4 C in the interim.

B. Bacterial identification

No attempt was made to culture anaerobic species from the mid-gut tissue of larvae. The presence of yeast and fungi was noted, but the cultures were not identified.
All bacterial isolates were gram stained and colonial characteristics described from colonies on BHI agar plates. Hanging drop mounts were made from 18-24 hour cultures to determine motility. Motile bacteria were negatively stained with phosphotungstic acid (pH 7.0) and examined for flagellar arrangement by electron microscopy (Pease, 1964). Biochemical characteristics of each isolate were noted on the following media: glucose broth, sucrose broth, mannitol broth, lactose broth, Kligler's iron agar, Simmon's citrate, nutrient gelatin, litmus milk, nitrate broth, MR-VP medium, and tryptone broth. Kligler's iron agar, Simmon's citrate, and MR-VP medium were purchased in dehydrated form from Difco Laboratories; the other media and reagents used were prepared according to the Laboratory Manual for General Bacteriology (fifth edition) (Peltier, Georgi, and Lindgren, 1967). All chemicals employed were reagent grade and obtained from various sources. Cultures were incubated at 34 C for the duration of the test period. Young cultures of gram-positive cocci were examined for cellular arrangement; spore stains were made on the gram-positive rod-shaped bacteria; acid fast stains were performed on non-sporulating gram-positive rods. Other biochemical tests were performed when necessary for final generic identification of the bacteria—reactions on starch, casein, and alginate. Hugh-Leifson's medium with 1% glucose was used to differentiate Streptococcus from Micrococcus (Breed, Murray, and Smith, 1957) and Pseudomonas from Aeromonas (Hugh and Leifson, 1953).
The generic placement of the bacterial isolates was accomplished with the aid of Skerman's key in *Bergey's Manual of Determinative Bacteriology* (seventh edition) (Breed, Murray, and Smith, 1957).

IV. Fungi associated with diseased *Pseudoplusia includens* larvae collected during epizootics

Specimens of diseased larvae were sent to Dr. D. M. MacLeod of the Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada for identification of the fungal organism involved in the epizootics.

Culturing of the fungal pathogen was attempted by plating resting spores from dead larvae on BHI agar and BHI agar enriched with 10% horse serum. Slide cultures and shake cultures were also made with BHI containing 10% horse serum and 0.5% beef extract.

Infectivity tests were conducted with the fungal resting spores. Laboratory-reared larvae of all instars were allowed to crawl over and feed upon diseased specimens. Larger larvae (4th, 5th, and 6th instars) were force-fed and injected with quantities of spores from crude preparations and from preparations purified to a degree by a discontinuous sucrose gradient (20-50% sucrose). After treatment, larvae were fed on pinto bean diet. These experiments were repeated with resting spores that had been held at 4°C for 10 months.
Resting spores purified by the discontinuous sucrose gradient method described above were collected and held at 37°C for 48 hours in an antibiotic bath containing 500 μg/ml each of streptomycin, penicillin, and chloramphenicol. A large inoculum of spores was then added to an actively-growing culture of mouse L cells. The cultures were held at 30°C with 90-100% relative humidity. Observations were made daily with an inverted microscope. The fungal isolate obtained from this experiment could be grown on potato dextrose agar (Difco Laboratories) after initial germination in the tissue culture system and was maintained by successive passages on this medium. Cultures of the isolated fungus were sent to two mycologists for identification, Dr. H. L. Barnett of West Virginia University and Dr. E. G. Simmons of U. S. Army Natick Laboratories. The fungus was tested for pathogenicity to P. includens larvae. Larvae of all sizes were allowed to ingest massive quantities of hyphae and spores while they crawled upon potato dextrose agar plates containing young cultures of the fungus. After 8 hours, the larvae were returned to rearing cups which contained pinto bean diet.

V. Viruses isolated from diseased soybean looper larvae collected during epizootics

A. Isolation

Viruses were isolated from dead larvae collected from the 1970 and 1971 epizootics by macerating diseased specimens and
spreading this material on diet surface or soybean leaves gathered from one of the Louisiana State University laboratory farms. Healthy laboratory-reared loopers were allowed to feed on inoculated diet or soybean leaves. Healthy larvae were also fed leaves gathered from soybean fields in which epizootics were in progress. Larvae which died from these treatments were placed in a clean reagent bottle, covered with deionized water, and allowed to putrefy at room temperature.

B. Virion liberation and infectivity

Virions were released from polyhedra by treating virus suspensions with sodium carbonate as described by Bergold (1963). The virion suspensions were filtered through a 0.45 μM Millipore filter. The filtrate was used to inoculate the surface of diet upon which 3-4th instar larvae were allowed to feed. The absence of bacteria and fungi in the virion suspension was confirmed by plating an aliquot of the suspension on BHI agar plates.

C. Stock cultures

Viral stocks were maintained by feeding 4th instar larvae diet inoculated with polyhedra as described above. Large numbers of inclusion bodies were used for infection. After death larvae were placed in clean reagent bottles and covered with deionized water. Putrefaction was allowed to proceed at room temperature or at 4 C until the stocks were needed.
D. Light microscopic observations

Late 4th instar loopers were infected with virus by allowing them to feed on diet inoculated with a concentrated suspension of polyhedra. Specimens for light microscopy examination were collected before infection and every 24 hours post-infection. The loopers were bisected between the 3-4th abdominal segments. The anterior and posterior ends were fixed in separate test tubes containing Lillies's alcohol-acetic-formalin fluid (formalin-10 ml; glacial acetic acid-5 ml; 70% ethanol-85 ml). Three larvae were prepared in a similar manner at each 24-hour interval. Specimens were fixed for a minimum of 24 hours, dehydrated in 70%, 90%, 95%, and 100% ethanol rinses of 1 hour each, and cleared with 2 rinses of xylene of 1 hour each. Infiltration with paraplast (Curtin Scientific Co.) was carried out at 60 C for 24-72 hours. The specimens were then embedded in fresh paraplast for sectioning.

Sections were cut with a microtome at a thickness of 6 u. The sections were collected on albumin-coated slides (Gray, 1952) and allowed to dry before staining. Hamm's azan technique (Hamm, 1966) was used to stain the sections specifically for detection of nuclear and cytoplasmic polyhedrosis viruses. Cover slips were affixed to the stained sections with permount.

E. Electron microscopic observations

Fourth instar larvae were infected with virus in the same manner as the larvae for light microscopy. Two infected larvae
were dissected in physiological saline solution after 12-hour intervals after infection. Two healthy control larvae were also dissected. Dissections were performed with fine forceps and with the aid of a dissecting microscope. The tracheal matrix, fat body, and gut tissues were removed and fixed immediately with 3% glutaraldehyde (Ladd Research Industries, Inc.) in Sorenson's phosphate buffer (0.01 M, pH 7.3) maintained at 4°C. After 2 hours of fixation, the tissues were washed overnight at 4°C with Sorenson's buffer plus 1% sucrose. If several samples were to be processed at one time, specimens were fixed in glutaraldehyde and kept in buffer until all of the samples were ready for postfixing. One percent osmium tetroxide in Sorenson's buffer was used to postfix the excised tissues for 1½ hours at 4°C. The tissues were dehydrated in a graded series of ethanol and embedded in Spurr low-viscosity embedding resin (Polysciences, Inc.) as described by White (1971).

Sections were cut on an LKB Ultratome with a diamond knife. Silver to silver-gray sections were collected on copper grids without a supporting film. All sections were doubly stained with 1% uranyl acetate in 90% ethanol for 2 minutes and Reynold's lead citrate (Kay, 1965) for 3 minutes.

Carbon replicas of polyhedral inclusion bodies were prepared as described by Pease (1964). Grids of replicas and thin sections were examined with an RCA EMU 3-G electron microscope using an accelerating voltage of 50 KV.
F. Measurements of polyhedral inclusion bodies

Polyhedral inclusion bodies were measured by light microscopy using an oil-immersion objective and a filar ocular micrometer. One-hundred polyhedra of each isolate were measured and an average size obtained.

G. Demonstration of antibacterial substances present in hemolymph of diet-reared larvae

The presence of antibacterial agents in the hemolymph of loopers reared on Burton's diet was demonstrated by the following procedure. Hemolymph was collected from 5th instar surface-sterilized larvae by cutting off the tips of the prolegs. Sterile filter paper discs were used to collect the drops of hemolymph. These discs were placed on petri dishes of BHI agar seeded with a heavy inoculum of the following organisms: *Escherichia coli*, *Bacillus cereus*, *Pseudomonas graveolens*, *Staphylococcus aureus*, and *Streptococcus faecalis*. (The cultures of bacteria were obtained from Dr. J. Larkin, Department of Microbiology, Louisiana State University). Zones of inhibition were measured after a 24-hour incubation period at 34 C. These tests were repeated with larvae that had fed on soybean leaves for 24 hours and 48 hours after removal from the rearing diet.
H. Infectivity tests

Infectivity tests were performed with the viral isolates against *P. includens* larvae to determine the number of polyhedra required to kill larvae of various instars. Virus preparations were made from the virus stocks by sedimenting the material by low-speed centrifugation for 5 minutes. Deionized water was added to the pellet which was strained through 4 layers of cheesecloth. Polyhedra were counted with an Improved Neubauer Hemocytometer and dilutions were made accordingly in deionized water. The 1971 viral stock used for the tests was prepared from tracheal tissue obtained from a soybean looper that was heavily infected with the 1971 isolate.

Larvae to be used for these experiments were allowed to feed for one day on pinto bean diet without inhibitors usually added to the diet (formaldehyde, sorbic acid, and methylparahydroxybenzoate). Leaf discs (6mm diameter) were cut from soybean leaves (collected from greenhouse plants or soybean fields) and 1 or 2 ul of the appropriate viral dilution was added to each disc. After the droplet had completely dried, the discs were placed into rearing cups that had been lined with paper toweling moistened with deionized water. One larva was put into each cup. Twenty to 40 larvae were fed discs coated with each virus concentration and 20-30 control larvae were fed discs coated with deionized water. The insects were held at room temperature for the duration of the
tests. After 24 hours, larvae that had not consumed the entire disc were discarded. Test larvae were transferred to diet without inhibitors every day for two days. They were then transferred to diet with inhibitors on which they were maintained for the rest of the experiment. Per cent mortality was recorded after the control larvae had pupated.

Data obtained from these experiments were corrected for mortality among the control larvae by Abbott's formula and were plotted on log-dosage probit paper. Eye-fitted curves were drawn.
RESULTS AND DISCUSSION

Microscopic examination of diseased larvae collected during epizootics revealed several microbial pathogens involved in the epizootics. Resting spores and conidia of an *Entomophthora* sp. were observed in some specimens. Dark resting spores were found in many of the black type larvae, while examination of the brown type revealed conidia external to the body. Some specimens showed no symptoms of a fungal disease, but rather contained viral inclusion bodies. Upon closer examination, it was observed that many larvae infected with fungus also contained viral inclusion bodies.

Attempts to transfer the disease complex resulted in death of laboratory-reared *Pseudoplusia includens* larvae. Virus was isolated from all specimens. No evidence of *Entomophthora* infection was observed in any of the experiments.

I. Bacteria isolated from tissues of *Pseudoplusia includens*

The bacterial isolates from the mid-gut tissues of laboratory-reared, healthy field-collected, and diseased field-collected larvae are listed in Table 1. Bacteria were keyed only to genera since many of the isolates were not identical to the species
Table 1: Bacterial isolates from laboratory-reared *P. includens*
larvae and healthy and diseased field-collected larvae

<table>
<thead>
<tr>
<th>Laboratory-reared loopers</th>
<th>Healthy loopers</th>
<th>Diseased loopers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Paracolobactrum</strong></td>
<td><strong>Paracolobactrum</strong></td>
<td><strong>Paracolobactrum</strong></td>
</tr>
<tr>
<td>sp. (II)(^a)</td>
<td>sp. (I, III)</td>
<td>sp. (II)</td>
</tr>
<tr>
<td><strong>Flavobacterium</strong></td>
<td><strong>Flavobacterium</strong></td>
<td><strong>Achromobacter</strong></td>
</tr>
<tr>
<td>sp. (III)</td>
<td>sp. (I, II, III, IV)</td>
<td>sp. (II)</td>
</tr>
<tr>
<td><strong>Alcaligenes</strong></td>
<td><strong>Alcaligenes</strong></td>
<td><strong>Aerobacter</strong></td>
</tr>
<tr>
<td>sp. (II)</td>
<td>sp. (I, II, III, IV)</td>
<td>sp. (I)</td>
</tr>
<tr>
<td><strong>Escherichia</strong></td>
<td><strong>Erwinia</strong></td>
<td><strong>Pseudomonas</strong></td>
</tr>
<tr>
<td>sp. (II)</td>
<td>sp. (I)</td>
<td>sp. (III, IV, VI)</td>
</tr>
<tr>
<td><strong>Xanthomonas</strong></td>
<td><strong>Pseudomonas</strong></td>
<td><strong>Bacillus</strong></td>
</tr>
<tr>
<td>sp. (I)</td>
<td>sp. (I, V)</td>
<td>sp. (III, IV, V)</td>
</tr>
<tr>
<td><strong>Bacillus</strong></td>
<td><strong>Bacillus</strong></td>
<td><strong>Escherichia</strong></td>
</tr>
<tr>
<td>sp. (I)</td>
<td>sp. (I)</td>
<td>sp. (III)</td>
</tr>
<tr>
<td><strong>Corynebacterium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Brevibacterium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Micrococcus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Staphylococcus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Streptococcus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Micrococcus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sp. (I)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Isolates within a genus were assigned different Roman numerals. Bacteria within a genus giving the same reactions on the differential media employed were assigned the same numeral.
described in Bergey's Manual. Isolates within a genus were assigned different Roman numerals. Bacteria within a genus giving the same reactions on the differential media employed were assigned the same numeral.

Only five isolates were found in mid-gut samples from laboratory-reared larvae. Seventy-five per cent of the larvae examined were free of bacteria that could be cultured by the methods employed. Low numbers of organisms cultured from the gut were probably acquired by the larvae during the process of transferring newly hatched insects to diet since bacterial growth was rarely seen on diet surface. Hemolymph and testes from larvae reared on diet were bacteria-free in all 5 samples tested.

Twenty-four bacterial isolates representing 13 genera were cultured from healthy field-collected larvae. The branching bacterium was not identified since the culture was lost upon subsequent attempts to transfer it. Organisms comprising the larval microflora seem to be typical of those found in soil or plant surfaces. Cursory examination of the fungal isolates revealed Aspergillus, Penicillium, and Mucor species. Two species of yeast were cultured but not identified. The majority of the bacterial isolates were small gram-negative rods. Next in prominence were cocci, gram-positive non-sporeformers, and gram-positive sporeformers. The observations are in general agreement with analyses of bacteria present in many different insects.
(Steinhaus, 1949). **Erwinia** was cultured from only one larval gut. There were no indications of the loopers harboring and dispersing members of this genus of plant pathogens. **Brevibacterium** and **Corynebacterium** are soil bacteria that have been cultured from several insect species (Steinhaus, 1949). Lysenko (1959) studied four species of **Brevibacterium** isolated from insect sources, two of which were previously undescribed. **Achromobacter**, **Bacillus**, **Flavobacterium**, **Micrococcus**, **Paracolobactrum**, and **Streptococcus** present in the soybean looper mid-gut have been isolated from mid-gut tissues of other lepidopterous larvae (Angus, 1952; Nunez, Hensley, and Colmer, 1968). Thus the microflora from the mid-gut of *P. includens* larvae consists of gram-negative rods, gram-positive sporeforming rods, gram-positive non-sporeforming rods, and cocci. Since the isolates are of soil or plant origin, it seems that the larval microflora reflects the microflora of the host plant.

The bacterial flora isolated from frass pellets of healthy larvae from the field differed only slightly from the mid-gut microflora. Bacteria isolated from frass were **Achromobacter** sp. (I, II, IV), **Bacillus** sp. (II), **Escherichia** sp. (I), **Paracolobactrum** sp. (I, III), **Psuedomonas** sp. (I, V), and **Staphylococcus** sp. (I). Only the **Bacillus** and **Escherichia** species were not found in the larval mid-gut. Angus (1952) collected frass from larvae of the eastern hemlock looper which had been anesthetized and surface-sterilized in 0.1% Hyamine solution. The larvae were dried and
placed in test tubes on filter paper. After the insects revived
defecation occurred. Frass pellets were collected by removing the
filter paper from the test tubes. Microorganisms were cultured
from the frass in sterile broth. One bacterium, a Micrococcus
species, was isolated from frass collected from 45 insects. A
number of fungi and yeast were present. From the observation
that frass was usually bacteria-free, Angus (1952) suggested that
ingested bacteria do not survive passage through the gut or that
their numbers are greatly reduced. In the present work, six genera
of bacteria were isolated from frass of the soybean looper.
Differences in the two reports may reflect only differences in
technique.

Six genera and 10 species of bacteria were cultured from
diseased larvae collected from soybean fields. Sixty per cent of
the isolates were members of the genera Bacillus and Pseudomonas.
Species of Achromobacter, Aerobacter, Escherichia, and
Paracolobactrum comprised the remaining microflora. Moore (1971,
1972) found two Bacillus species, Pseudomonas fluorescens, and
Serratia marcescens present in healthy and diseased Southern pine
beetles. He concluded that these potential pathogens were not
common gut inhabitants except for Serratia but that they could
have a deleterious effect on the insect as they pass through the
alimentary tract.
Bacteria cultured from diseased pupae were *Paracolobactrum* sp. (II), *Pseudomonas* sp. (II, IV), *Staphylococcus* sp. (II), and *Streptococcus* sp. (V). The cocci associated with diseased pupae were not present in diseased larvae. The effect of metamorphosis on the microflora composition has not been reported for the soybean looper and this observation may reflect only a change in microflora as the insect matures (Steinhaus, 1949).

Total numbers of bacteria, fungi, and yeast in the gut of healthy field-collected loopers were compared to numbers of these organisms from soybean leaves. The data are presented in Table 2. More bacteria, fungi, and yeast were cultured from leaves than from healthy larval mid-gut. Fungal counts were 100-fold greater and yeast counts 1,000-fold greater from leaf surfaces than from the gut. Identification of bacteria from soybean leaves was not attempted, but from observations of colonial morphology it seemed that the majority of bacteria isolated from the gut of healthy loopers was similar to those cultured from leaves. Thus, it appeared that the microflora of the soybean looper is derived from the microflora of the soybean leaves ingested.

II. Fungi associated with diseased *Pseudoplusia includens* larvae

A. *Entomophthora* sp.

Diseased specimens sent to Dr. D. M. MacLeod were found to contain conidia and resting spores of an *Entomophthora* species.
Table 2: Total numbers of bacteria, fungi, and yeast per gram of soybean leaves and of gut tissue from healthy soybean loopers

<table>
<thead>
<tr>
<th></th>
<th>total number of microorganisms/gram of soybean leaves</th>
<th>total number of microorganisms/gram gut tissue from healthy loopers</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacteria</td>
<td>9.8 x 10^8</td>
<td>3.7 x 10^8</td>
</tr>
<tr>
<td>fungi</td>
<td>7.2 x 10^8</td>
<td>2.2 x 10^6</td>
</tr>
<tr>
<td>yeast</td>
<td>1.5 x 10^7</td>
<td>1.5 x 10^4</td>
</tr>
</tbody>
</table>
The following descriptions of the fungus are his (MacLeod, personal communication). Dead larvae collected from epizootic areas were of two distinct types, one black and moist, the other brown and leathery. The black type was filled with resting spores or azygospores. These spores were black in mass but varied from dark brown to black when viewed singly. They varied in shape from globose to oval. Globose spores average 47.5 ± 3.9; oval spores average 48.0 ± 5.4 x 42.0 ± 3.1. Spores had a distinct foramen which measured 12.5 μ in diameter. Very fine projections were seen on the surface of the resting spores (Fig. 1). By applying pressure, the spores were ruptured exposing a smooth-walled epispore (Fig. 2). Resting spores produced in P. includens developed from vegetative hyphae (Fig. 3).

Diseased larvae in which conidia were produced were typical of the second type, brown in color and they seemed to contain conidia only. Conidia were uninucleated. They were cylindrical to club-shaped, symmetrical to somewhat elliptical (Fig. 4). They averaged 20.4 ± 2.15 x 8.4 ± 0.87. In general the conidia tapered toward the base to form a rather blunt or truncate papilla.

Resting spores in diseased P. includens larvae were very similar to those described by Weiser in 1965, for which he proposed the name Tarichium gammae. Following the convention of MacLeod and Muller-Kogler (1970), the fungus will be referred to as Entomophthora (Tarichium) gammae in this dissertation. In the original description
Fig. 1: Resting spores observed in a wet mount preparation from a diseased soybean looper. Some spores have a distinct foramen (F), others show projections on the surface. 347 X
Fig. 2: Epispores (E) produced upon rupture of resting spores observed in a wet mount preparation. Fragments of spore coats (SC) may be seen. 293 X
Fig. 3: A wet mount preparation showing development of resting spores from vegetative hyphae. Spores may be seen in various developmental stages. 153 X
Fig 4: A wet mount preparation of conidia of Entomophthora gamme found on the cuticle of diseased larvae of the soybean looper. 204 X
of the species, Weiser (1965) referred to mature azygospores as flattened into a pill-like form. After comparing resting spores from the soybean looper to a slide of E. (T.) gammae prepared by Weiser, Dr. MacLeod and the author concluded that flattened forms were more prevalent in the slide from Weiser than in diseased P. includens larvae. This observation may reflect only the degree of distortion of these spores caused by crowded conditions in the insect host or by dehydration. One other discrepancy existed between Weiser's description and the resting spores from the soybean looper. Measurements of E. (T.) gammae spores averaged 53 x 56 μ; globose resting spores from the soybean looper averaged 47.5 μ, while oval spores averaged 48.0 μ x 42.0 μ. Wuest and Turian (1971) and Koval (1969) have subsequently described E. (T.) gammae from Ocnognya parasita larvae and Pieris brassicae larvae, respectively. Koval reported azygospores as spherical, making no mention of flattened forms. Wuest and Turian described the spores as having the form of a sphere, depressed toward the two poles, with an average diameter of 45 μ.

Infection by fungi of the Entomophthorales proceeds when a conidium falls on the cuticular layer of the host insect. A germ tube is formed which penetrates the cuticle and establishes infection in the hemocoel. Hyphae are produced which later break up into short hyphal bodies. Mycotoxins produced by some entomogenous fungi are powerful enough to cause death of the host.
before tissue invasion occurs (Prasertphon, 1969; Prasertphon and Tanada, 1969). If hyphal penetration occurs, the fat body is the preferred site of infection (Roberts and Yendol, 1971). Conidia are produced by conidiphores borne either internally or externally. The mechanism that triggers resting spore formation is not known. It has been suggested that resting spores are formed near the end of the growing season and that formation may be dependent upon environmental conditions (MacLeod, 1963). Conidia are the infectious forms and resting spores serve to endure weather conditions and germinate the following year (MacLeod, 1963). Conidia are viable for only a short period of time, possibly for only several hours (Roberts and Yendol, 1971).

Attempts to culture the fungal pathogen from soybean loopers failed. BHI agar, BHI agar with 10% horse serum, and BHI agar-10% horse serum-0.5% beef extract media were inoculated with diseased specimens bearing conidia and resting spores. Germination did not occur. Slide cultures prepared with the same media using resting spores as inoculum were kept for periods of two weeks in a chamber at 95-100% relative humidity. Germination did not occur. Shake cultures containing BHI-10% horse serum-0.5% beef extract-500 ug/ml chloramphenicol inoculated with a crude preparation from diseased larvae containing resting spores and conidia produced several types of fungi. When the culture was inoculated on agar plates of the same medium, a Mucor species overgrew the entire plate in 2 days. If the Entomophthora species
had germinated it could not be demonstrated because of contamination by other fungi.

Very few Entomophthora have been successfully cultured in the laboratory. Some of the fungi have fastidious nutritional requirements and egg yolk seems to be the best culture medium available for conidial germination. Germination proceeds by formation of a germ tube into which flows protoplasm from the conidium. Recently Tyrrell and MacLeod (1972) have reported the spontaneous release of protoplasts from conidia of an Entomophthora species germinating in Grace's tissue culture medium. During the first several days of growth, the protoplasts assumed a shape similar to animal cells in culture. Injection of protoplasts produced infection of healthy insects with reproducible results. Whether the protoplast stage exists in an insect host or is an artifact induced by the tissue culture medium is not known.

Resting spore germination has been reported for a few Entomophthora species (MacLeod, 1963). Various physical and chemical treatments have been utilized to induce germination in other species without success (Evlakhova and Woronina, 1964). Conflicting reports exist concerning the sequence of events involved in resting spore germination. It is thought that a stout germ tube protrudes from the spore, but whether it produces conidia or hyphae has not been established (MacLeod, 1963).

It is possible that germination of conidia from diseased soybean loopers could have been observed in the laboratory if
egg yolk medium had been utilized and a freshly sporulating fungal
culture from a dead insect used as inoculum. From available
reports, it seems that resting spores of many of the *Entomophthora*
require one year of quiescence before germination (MacLeod, 1963).
If this observation holds true for *E. (T.) gammae* resting spores
it may offer an explanation for the failure encountered by the
author to induce germination of spores isolated from the soybean
looper.

No mortality was produced by feeding or injecting *P. includens*
larvae of various instars with preparations of resting spores and
conidia. Larvae were placed into petri dishes containing diseased
specimens of the black and brown types for 24 hours. Some
larvae ingested portions of the diseased loopers. None of the
larvae showed symptoms of fungal infection. Healthy larvae were
fed soybean leaves collected from fields where epizootics were in
progress. The larvae died within one week. Upon microscopic
examination of the loopers, the cause of death was ascribed to
viral rather than fungal infection.

Attempts by other workers to infect larvae of Lepidoptera
with conidia from sporulating cultures of entomogenous fungi have
been only minimally successful. Probably infection could be
produced with greater success by intrahemocoelic injection of
protoplasts into healthy insects (Tyrrell and MacLeod, 1972).
If conidial protoplasts had been injected into the soybean looper,
infection may have ensued.
B. Fungal isolate from diseased larvae of the soybean looper

A fungal isolate from soybean loopers was obtained from tissue culture experiments in which antibiotic-treated resting spores were added to actively-growing L cells. The fungus had fastidious nutritional requirements for germination since it would not grow on BHI-10% horse serum-0.5% beef extract medium. Germination was observed only in the tissue culture system. Mycelial growth began 3-4 days after inoculation and proceeded rapidly. In later experiments, hyphae were not produced. Instead, spores gave rise to yeast which formed long chains of cells by budding. Spores which gave rise to the fungal isolate could not be ascertained in the studies because a large inoculum of material prepared from diseased larvae was required to initiate germination. Repeated experiments always yielded this fungal isolate, growing either in the yeast or hyphal phase, with no other microbial contaminants. After germination, the fungus could be grown on various media and was maintained by passage on potato dextrose agar.

Cultures of the isolate were sent to two mycologists for identification. Dr. H. L. Barnett (West Virginia University) described the fungus as having sporulation characteristics much like Sporothrix, but was similar in other respects to Scolecobasidium. Dr. E. G. Simmons (U. S. Army Natick Laboratories) identified the culture as a species of Rhinocladiella, some of which are human pathogens.
Infectivity tests were performed to determine the pathogenicity of the fungus to *P. includens*. No mortality was observed in any of the tests. It made no difference whether the culture was administered by feeding, injection, or by spraying it onto soybean foliage consumed by the larvae.

III. Viruses isolated from diseased *Pseudoplusia includens* larvae

Viral pathogens were isolated from diseased loopers collected during the 1970 and 1971 epizootics. Very few viral inclusions were evident in some squash mounts prepared from dead larvae. Healthy larvae infected with a suspension prepared from dead specimens died within one week and light microscopy examination showed the body cavity to be filled with polyhedra. Since bacteria were also present, Koch's postulates were satisfied to prove that polyhedra were the infectious agents. Virions were liberated from polyhedra with a solution of sodium carbonate and the resulting suspension was filtered through a Millipore filter. An aliquot inoculated on BHI agar showed that the filtrate was free of any microorganisms that were culturable on the medium. Healthy larvae were allowed to feed on diet surface which had been inoculated with the filtrate. All sixty of the larvae died within a week. Polyhedra were observed in all specimens.
A. Light microscopic observations

Loopers infected with both viral isolates were embedded in paraffin, sectioned, and examined by light microscopy. Tissues in which polyhedra developed were noted.

Both viral isolates proved to be nuclear polyhedrosis viruses, developing in cell nuclei. Tissues in which infection progressed were the same for each isolate. Figure 5 depicts uninfected fat body tissue in which nuclei are small and spherical in shape. Polyhedra developed in the nuclei of fat body (Fig. 6), tracheal matrix (Fig. 7), epidermis (Fig. 8), and wing bud tissues (Fig. 9). Nuclei of these cells were irregular in shape, exhibited hypertrophy, and were filled with polyhedra. Infection was not observed in Malpighian tubules, nuscle, or hemocytes. Mid-gut tissue contained dark-staining areas and was very granular in appearance, but polyhedral formation was not evident.

The histopathology of the viral isolates of the soybean looper is similar to that reported for other Lepidopterous larvae (Adams and Wilcox, 1968; Hunter and Hall, 1968; Mathad, Splittstoesser, and McEwen, 1968; Stairs, 1968). Polyhedra were first evident after 3 days of infection in larvae fed the 1970 isolate and after 5 days of infection in larvae fed the 1971 isolate. These differences may reflect a dose response.
Fig. 5: A section of fat body tissue from healthy loopers. Nuclei (N) are small and round. 1050 X
Fig. 6: Tissue section of fat body cells infected with the soybean looper virus. Nuclei (N) are greatly enlarged and filled with polyhedra (arrow). 1050 X
Fig. 7: Section of infected tracheal matrix. Polyhedra (arrow) may be seen in the nuclei. Uninfected muscle may be seen at the bottom of the micrograph. 1050 X
Fig. 8: Section of epidermal tissue exhibiting hypertrophied nuclei filled with polyhedra. Cuticle (C) may be seen in the upper left-hand corner of the micrograph. 1050 X
Fig. 9: Section of infected cells of developing wing bud tissue. Polyhedra are evident in nuclei of these cells. 1050 X
B. Electron microscopic observations

1. 1970 viral isolate

Viral inclusion bodies isolated from diseased larvae from the 1970 epizootic were sedimented by centrifugation into a pellet which was prepared for thin sectioning and electron microscopic examination. Figures 10 and 11 show polyhedral inclusion bodies (PIB's) with enclosed virions demonstrable by this procedure. Virions are of the singly-embedded type (Tompkins, Adams, and Heimpel, 1969). Holes observed in the polyhedron in Fig. 11 indicate partial degradation of the polyhedral protein. One-hundred PIB's were measured by light microscopy using a filar ocular micrometer and an average size of 0.95 μ per polyhedron was obtained. Virions measured from thin sectioned polyhedra averaged 200 μ x 32 μ.

Carbon replicas of the viral isolate showed polyhedra with several types of surface characteristics. Some polyhedra presented a fairly smooth surface (Plate 1, Fig. 1 and 3); others were rough and composed of several layers (Plate 1, Fig. 2, 3, and 4). Virions evident on the surface of some polyhedra appeared to be inserted randomly into the protein matrix in various orientations (Plate 1, Fig. 2, 3, and 4).

Nuclear polyhedrosis viruses have been shown to infect mid-gut cells without polyhedral formation (Cunningham, 1971). Virions are released from the polyhedral matrix by the alkalinity
Fig. 10: Electron micrograph of a thin section prepared from a pellet of the 1970 viral isolate. Virions (V) may be observed within the polyhedral matrix (P). Contaminating bacteria (B) may be seen in the micrograph. 80,500 X
Fig. 11: Electron micrograph of a thin section prepared from a pellet of the 1970 viral isolate. A free virion (FV) may be seen as well as virions occluded into the polyhedron. Holes in the polyhedral matrix (arrow) indicate partial degradation of the polyhedron. Bacteria (B) may be seen. 73,180 X
Plate 1: Electron micrographs of carbon replicas of the 1970 viral isolate. Surface characteristics of the polyhedra are varied. Virions may be observed on the surface of some polyhedra.

Fig. 1: 35,000 X
Fig. 2: 27,500 X
Fig. 3: 33,000 X
Fig. 4: 30,000 X
of the insect gut. It is thought that they traverse the gut epithelium and multiply in the nuclei of gut cells. Newly formed virions are released into the hemocoel or extracellular spaces (Harrap, 1970; Summers, 1971) and travel to the target tissues in which they replicate and produce polyhedra. Larvae infected with the 1970 isolate showed virions in gut cells within 60 hours after infection. Plate 2 represents several stages of virus replication in mid-gut nuclei. Virions without envelopes (Bergold, 1958) may be seen in Figure 1 of this plate. Figures 2 and 3 show virus particles surrounded by intimate and outer membranes. One of the virions in Fig. 4 is in the process of acquiring the outer or developmental membrane (Bergold, 1958).

An extensive network of rough endoplasmic reticulum may be seen very near the nuclear membrane (Plate 2, Fig. 1). Endoplasmic reticulum was often observed close to the nuclear membrane of infected cells, while a similar observation was not made upon examination of uninfected cells. Empty membrane vesicles in Fig. 2, 3, and 4 (Plate 2) probably represent an excess of membrane fragments produced during the infection process.

Plates 3 and 4 are micrographs of gut nuclei at 72 hours post-infection. Forms seen in Plate 3 (Fig. 1 and 2) and Plate 4 (Fig. 1) probably represent aberrant virions. None of the structures have acquired membranes. It has been suggested by Harrap (1970) that virions without envelopes are incapable of
Plate 2: Electron micrographs of a thin sections showing virions in gut cells at 60 hours post-infection.

Fig. 1: Virions (V) without envelopes. Rough endoplasmic reticulum (RER) may be seen at the nuclear membrane (NM). 31,470 X

Fig. 2: Virions with a complete complement of membranes (EV). Empty membrane vesicles (MV) may be seen. 35,000 X

Fig. 3: Enveloped virions and empty membrane vesicles may be observed. 35,480 X

Fig. 4: A virion may be seen in the process of acquiring an outer membrane (arrow). 36,670 X
Plate 3: Electron micrographs of thin sections of nuclei of mid-gut cells 72 hours post-infection.
Fig. 1: Aberrant virion structures (AV) may be seen. 44,000 X

Fig. 2: Aberrant virion structures (AV). A matrix similar to a polyhedron without viral inclusions may be seen (arrow). 42,430 X
Plate 4: Electron micrographs of thin sections of nuclei of mid-gut cells 72 hours post-infections.  
Fig. 1: Aberrant virion structures (AV) may be seen within the nuclear membrane (NM). 39,650 X 

Fig. 2: Empty membrane vesicles (MV) and an unevolved virion (V) may be seen. An arrow points to a structure which may represent initiation of a polyhedral matrix. 44,690 X
infecting cells. Enveloped virus particles non-occluded in polyhedra spread infection to other tissues (Harrap, 1970). Spherical membrane structures can be seen in Plate 3 (Fig. 1) and Plate 4 (Fig. 2). Structures in Plate 3 (Fig. 2) and Plate 4 (Fig. 2) may represent abortive attempts to form polyhedral matrices in the nuclei. Mitochondria and rough endoplasmic reticulum are in close proximity to the nucelar membrane (Plate 4, Fig. 1).

Infection of fat body cells with virus of the soybean looper proceeds in a fashion typical of other nuclear polyhedrosis viruses (Adams and Wilcox, 1968; Bird and Whalen, 1954; Tompkins, Adams, and Hempel, 1969). Naked virions are formed from virogenic stroma (Cunningham, 1971). Virions of the soybean looper virus are produced in a similar manner (Fig. 12). The mechanism of membrane formation around virions is not known. Summers (1971) suggested that virions may acquire membranes from infoldings of the nuclear membrane or as the particles traverse other cellular membranes. Polyhedral formation proceeds as virus particles coalesce with a protein matrix and the process was observed with the soybean looper virus (Fig. 13). Summers and Arnott (1969) hypothesized that virions must have a small amount of polyhedral protein surrounding them prior to polyhedral occlusion. Virus particles lacking the protein are never occluded.
Fig. 12: Electron micrograph of a thin section showing virion formation from virogenic stroma (VS). Some virions without envelopes are in close proximity to the nuclear membrane (NM). 57,500 X
Fig. 13: Electron micrograph of a thin section showing polyhedral formation in the nucleus of a fat body cell. Virions with a full complement of membranes may be seen coalescing with polyhedral protein. 60,380 X
The morphology of the 1970 isolate was strikingly different in the nuclei of the tracheal matrix from what was observed in fat body cells. Polyhedra were surrounded by a membrane-like structure and some had dark staining centers (Fig. 14 and 16). Membrane profiles have been observed in nuclei of tracheole cells of the cabbage looper infected with an NPV (Summers and Arnott, 1969). Membrane profiles described by these authors were associated with fibrous material which appeared to represent initial stages of polyhedral formation. Mature polyhedra were only partially enclosed in membranes. This observation is in contrast with polyhedra observed in tracheal cells of the soybean looper which were entirely surrounded by membranes (Fig. 14). Fibrous material was observed also in tracheal cells from the soybean looper infected with the 1970 isolate (Fig. 15).

Dark staining centers of some polyhedra from tracheal cells of the looper were irregular and varied considerably in detail (Fig. 16). The significance of the observed variation and irregularity is not clear. Staining with 1% uranyl acetate (aqueous) instead of 1% uranyl acetate (alcoholic) was suggested by M. D. Summers (personal communication) did not resolve the situation. It is apparent that one of the staining reagents complexed with some of the polyhedra in an unusual manner. This type of observation has not been reported previously. The
Fig. 14: Electron micrograph of a thin section showing polyhedra in the nucleus of a tracheal cell. Membranes surround the polyhedra which exhibit dark staining centers. Non-occluded long virus rods may be seen. 67,700 X
Fig. 15: Electron micrograph of a thin section showing fibrous material observed in the nucleus of an infected tracheal cell. Such structures may represent initial stages of polyhedral formation. 46,433 X
Fig. 16: Electron micrograph of a thin section showing polyhedra found in infected tracheal matrix. Dark staining centers are irregular and vary in detail from one polyhedron to another. Virus rods may be seen in association with virogenic stroma (VS). 12,000 X
staining is differential, in that the majority of polyhedra may be darkly stained in one nucleus and adjacent nuclei (in the same field) have no polyhedra of this type.

Long rods were associated with polyhedral development in tracheal cells of the looper (Fig. 14). Livingston and Yearian (1972) reported similar structures in fat body nuclei of P. includens, but offered no explanation for their presence. In the present study these rods were observed only in nuclei of the tracheal matrix. Such rods are known to be associated with insect granulosis diseases. Smith and Brown (1965) studied the rods in fat body nuclei infected with granulosis virus and in negatively stained preparations from diseased cabbage loopers. They suggested that these structures may represent an alternate replicative mechanism in which long rods break into shorter lengths which are then embedded into the protein crystal.

Branching of the rods was observed in granuloses-infected cells. The authors suggested this might represent an irregular disturbance of the replicating mechanism, causing dislocations to occur in the core or causing overproduction of protein or the inclusion of abnormal or foreign macromolecules. In the present work branching of the rods was not observed in nuclei of infected cells of the soybean looper. Whether or not the rods observed in the looper are similar in nature to those associated with granuloses is speculative.
2. 1971 viral isolate

The NPV isolated during the 1971 epizootic was found to be similar in many respects to the 1970 isolate. Measurements of 100 polyhedra by light microscopy revealed an average diameter of 0.99 μ with a range of 0.56 μ to 1.58 μ.

Pellets of virus were obtained in a similar manner as described for the 1970 isolate. Thin sections revealed some oddly-shaped inclusion bodies, many having darkened centers (Fig. 17). Other polyhedra were typical of the 1970 isolate.

Virions were not observed in mid-gut tissue although many thin sections were examined. After 132 hours of infection an extensive network of endoplasmic reticulum was observed in close proximity to the nuclear membrane in mid-gut cells (Fig. 18). Gut tissues were probably infected by the viral isolate even though virions were not observed. A very large does of virus must be given to insects to observe gut infection by electron microscopy (Cunningham, 1971).

Fat body was a primary target tissue for viral replication and inclusion body formation. Chromatin material was arranged in discrete clumps prior to virus production. Virions were formed from virogenic stroma (Fig. 19). After acquiring the full complement of membranes, virus particles were occluded into polyhedral matrices (Fig. 20). Polyhedra in Fig. 21 appeared atypically small and only a few mature polyhedra were observed
Fig. 17: Electron micrograph of a thin section of a pellet of the 1971 viral isolate. Some polyhedra have dark staining centers. Partial degradation is evident in one of the polyhedra (arrow). 56,900 X
Fig. 18: Electron micrograph of a thin section of a mid-gut cells 132 hours post-infection. An extensive network of endoplasmic reticulum (ER) is in close proximity to the nuclear membrane (NM). A mitochondrion (M) is associated with the endoplasmic reticulum. 71,300 X
Fig. 19: Electron micrograph of a thin section of a fat body cell showing virion formation from virogenic stroma (VS). 44,000 X
Fig. 20: Electron micrograph of a thin section of a fat body cell showing the process of viral occlusion. Virions may be seen associated with virogenic stroma (VS). 61,000 X
Fig. 21: Electron micrograph of a thin section showing polyhedra within the nucleus of a fat body cell. Polyhedra are atypically small with few viral occlusions. Membrane vesicles (MV) are evident. 25,780 X
within the nucleus. This was in contrast with the majority of infected cells (Fig. 22). This observation might represent an abortive infection or might indicate that there are two different NPV's infecting the same tissue.

Tracheal infection was similar to the patter observed with the 1970 isolate. Inclusion bodies were surrounded by membranes and dark centered polyhedra were evident (Fig. 23 and 24).

From electron microscopic examination of tissues infected with either viral isolate, it was not clear whether or not the isolates represented (1) the same virus, (2) different viruses, or whether (3) both isolates contained two NPV's, one with a tropism for the tracheal matrix, the other with a tropism for fat body tissue. No reports have described nuclear polyhedrosis viruses exhibiting different characteristics in various tissues. The only recognized strains of insect viruses are those which have different inclusion body shapes (Cunningham, 1970).

Heimpel and Adams (1966) described a mixed infection of cabbage loopers with two NPV's. Polyhedra of one isolate developed in nuclei of gut columnar cells while the other isolate did not. The mixed infection was observed in larvae collected from New York State, California, South Carolina, Texas, and Virginia. Many Lepidoptera are infected with several types of viruses simultaneously (Ignoffo, 1968; Lowe and Paschke, 1968). A mixture of viruses may act synergistically, antagonistically, or
Fig. 22: Electron micrograph of a thin section of a fat body nucleus showing typical polyhedra. 32,514 X
Fig. 23: Electron micrograph of a thin section showing polyhedra formed within the nucleus of a tracheal cell. Membranes surrounding the polyhedra are prominent. 55,000 X
Fig. 24: Electron micrograph of a thin section showing polyhedra formed within the nucleus of a tracheal cell. Some PIB's have darkened centers. Non-occluded virus rods may be observed. 17,333 X
without interaction to cause death of the insect (Lowe and Paschke, 1968; Stairs, 1968). The viruses react with each other at the cellular level so that adjacent nuclei may be infected with different viruses. Either virus can occupy all susceptible sites if given a temporal advantage, but double infection in a single cell has never been observed (Lowe and Paschke, 1968; Stairs, 1968).

C. Demonstration of antibacterial substances in hemolymph of diet-reared Pseudoplusia includens larvae

Formaldehyde, sorbic acid, and methylparahydroxybenzoate are incorporated into the pinto bean diet of Burton (1969) to prevent growth of fungi and bacteria. It was assumed by the author that these chemicals were present in hemolymph from larvae reared on diet and would interfere with attempts to infect larvae with microbial pathogens. The following experiment was performed to test this hypothesis.

Filter paper discs were saturated with hemolymph collected from diet-reared larvae. Discs were placed on agar medium in petri plates heavily inoculated with bacteria. Zones of inhibition were measured after 24 hours of incubation. Hemolymph was collected from larvae feeding on soybean leaves for 24 hours after removal from diet and tested in a similar manner. Zones of inhibition were observed around hemolymph of loopers feeding on diet. Zones of inhibition around Streptococcus faecalis and
**Staphylococcus aureus** were 13 mm and 15 mm in diameter, respectively. **Pseudomonas graveolens** and **Escherichia coli** were less susceptible to the antibacterial substances, with zones of inhibition measuring 9 mm each. No inhibition of **Bacillus cereus** was observed. These observations help to explain why so few bacteria were isolated from mid-gut tissues of larvae reared on diet (Results and Discussion, part I). Hemolymph from larvae taken from artificial diet and fed soybean leaves for 24 hours did not contain the antibacterial substances. Enhanced growth was observed around the hemolymph-coated discs, no doubt due to nutrients present in the hemolymph. It appeared that larvae rapidly excreted or destroyed the antibacterial substances which were ingested with the artificial diet.

For LD\textsubscript{50} determinations of the viral isolates larvae were removed from diet containing formaldehyde, sorbic acid, and methylparahydroxybenzoate for at least 24 hours prior to experimentation and placed on the same diet without the antimicrobial substances. Viral activity can be 3–4 times lower when placed on freshly-made diet with formaldehyde than on diet without formaldehyde (Ignoffo and Garcia, 1968; David, Ellaby, and Taylor, 1972).

**D. Infectivity tests**

LD\textsubscript{50} determinations were performed to assess the infectivity of the viral isolates for loopers of various instars. There are
many methods of determining LD_{50}'s with inclusion body viruses, most of which involve the addition of virus to diet upon which test larvae are reared (Ignoffo, 1964; Chauthani, 1968; Stairs, 1965). LC_{50}'s can be obtained by feeding foliage inoculated with various concentrations of virus to larvae (Bird, 1969; Cunningham, 1970). The method of determining infectivity in this study was devised by the author to eliminate any virus inactivation caused by inhibitors present in the diet and to obtain a value not dependent upon the amount of food consumed by each larva. Only four concentrations of the 1970 isolate were used against larvae; seven concentrations of the 1971 isolate were employed. The first isolate had been kept at 4 C in an unpurified state for 5-6 months before use. The 1971 isolate was prepared from larvae that had died of the disease one week prior to its use. The virus had been maintained at room temperature.

Data from the experiments are presented in Table 3. Log-dosage probit curves represent the data graphically (Fig. 25 and 26). Later instar larvae were found to be less susceptible to the isolates. This observation substantiates reports of other investigators (Bird, 1969; Ignoffo, 1964). Such an increase in dosage required for mortality could represent virus dilution due to increasing size of the larvae (Ignoffo, 1966) or the development caused by larval maturation (Stairs, 1965).
Table 3: LD$_{50}$'s of 1970 and 1971 viral isolates obtained with 3rd, 4th, and 5th instar *Pseudopusia includens* larvae

<table>
<thead>
<tr>
<th>Instar</th>
<th>1970 isolate$^a$</th>
<th>1971 isolate$^b$</th>
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<tbody>
<tr>
<td>third</td>
<td>365$^c$</td>
<td>2,700</td>
</tr>
<tr>
<td>fourth</td>
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<td>2,650</td>
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<tr>
<td>fifth</td>
<td>1,650</td>
<td>9,750</td>
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$^a$20-30 test larvae/group

$^b$30-40 test larvae/group

$^c$LD$_{50}$'s were determined graphically.
Fig. 25: Log-dosage probit curves for 3rd and 5th instar *P. includens* treated with the 1970 viral isolate.
Fig. 26: Log-dosage probit curves for 3rd, 4th, and 5th instar *P. includens* treated with the 1971 viral isolate.
LD$_{50}$'s obtained with the 1971 viral isolate were greater than those obtained with the 1970 isolate. The 1971 isolate used for these tests was derived from tracheal tissue and may account for differences in infectivity observed.

Examination of Fig. 25 and 26 shows higher virus concentrations did not give greater mortality in some cases. Two explanations for these data can be offered: (1) loopers exhibited much heterogenicity in response and (2) a level of resistance was present among the looper population used for experimentation.

Data for the 1971 isolate were more consistent possibly because more virus concentrations were tested and more larvae were represented per group.

Infectivity studies conducted by other authors with viruses against various insects cannot be directly compared with results obtained with the soybean looper since there were differences in technique. Ignoffo (1964) determined an LD$_{50}$ of 237.2 polyhedra/ul of diet for 3-day old cabbage loopers with an NPV isolated from the insect. Diet employed contained chlorotetracycline and formalin to inhibit growth of microorganisms. The same author (Ignoffo, 1965) reported an LD$_{50}$ of 32.0 PIB's/mm$^2$ diet surface obtained with 3-4 day-old Heliothis zea larvae with an NPV isolated from naturally infected H. zea. Fifty per cent mortality was observed among 4th instar larvae of the greater wax moth after
consumption of diet cubes treated with $9.96 \times 10^5$ polyhedra specific for this insect (Stairs, 1965). An $\text{LC}_{50}$ obtained with eastern hemlock loopers fed foliage dipped in various concentrations of its homologous NPV was reported as $10^{4.8}$ by Cunningham (1970a). The $\text{LD}_{50}$'s obtained for the soybean looper and the two viral isolates indicate considerable pathogenicity of the isolates under laboratory conditions.

Resistance to viral diseases has been proposed by various authors. David and Gardiner (1960) reported that resistance to a virus disease had developed by *Pieris brassicae* which had been laboratory-reared for 9 years and survived a severe attack of virus. Resistance was based on comparisons of $\text{LD}_{50}$'s with a freshly caught wild stock of *P. brassicae*. The suggestion of a latent virus infection in the new stock was neither disproved nor substantiated. Martignoni and Schmid (1961) studied resistance to virus in natural populations of Lepidoptera collected from various geographical locations. Cultures of *P. rapae* from different locations behaved in an identical fashion, while some heterogeneity in response was seen among *Phryganidia californica* larvae. Sawflies (*Neodiprion sertifer*) have been effectively controlled since the accidental introduction of a nuclear polyhedrosis virus in 1931 with no evidence of increased resistance (Bird, 1955). In general, if differences of susceptibility exist between cultures of an insect, larvae
collected from the wild are more susceptible to virus diseases than laboratory-reared stock (Sidor, 1959). Therefore, in evaluating the infectivity of viruses to laboratory-reared insects, one must consider that continual selection occurs in the laboratory for the healthiest individuals, those that pupate and emerge first, and adults with the greatest fecundity. Many other factors influence the survival of insects collected from the wild. They are always in contact with pathogenic microorganisms, nematodes, and entomogenous parasites. Adverse weather conditions, population density, and food scarcity can make a population more susceptible to organisms that may not be pathogenic under other conditions. Therefore care must be taken in extrapolating laboratory data to field conditions.

Epizootics among insects caused by entomogenous fungi have been described by many authors (MacLeod, 1963; Roberts and Yendol, 1971; Yendol and Paschke, 1967). Efforts to produce epizootics among pest species at will have for the most part failed (Cameron, 1971; Latch, 1965; Roberts and Yendol, 1971; Jacques, Stultz, and Huston, 1968). Roberts and Yendol (1971) have described the study of epizootiology as interactions among the pathogen, host, and environment, all of which are changing at the same time. Some fungi tend to become less virulent when cultured outside of the host (Roberts, 1967; West and Briggs, 1968), but virulence can usually be recovered by passage through a host insect. Conidia
of most **Entomophthora** are viable for only short periods of time. Infectivity must then be retained by resting spores which germinate under appropriate conditions. Population density of the host insect is critical for some entomogenous fungi, but not for all (Roberts and Yendol, 1971). Environmental conditions are demanding for reproducible control of insects by fungi (Cameron, 1963; MacLeod and Soper, 1965; Roberts, 1967). Temperature, moisture, light, air currents, host density, pathogen density, host activity, and stress are a few of environmental parameters considered important in the establishment and spread of fungal pathogens (MacLeod and Soper, 1965).

If fungi are to be used for biological control, methods of mass production and storage of infectious entities must be developed. Since the majority of entomogenous fungi are inhibited by commercial insecticides and fungicides integrated control programs with chemicals show little promise at the present time (Roberts and Yendol, 1971). In 1890, Kansas farmers attempted to use the fungus **Beauveria bassiana** for chinch bug control. The following excerpt is from Roberts and Yendol's discussion (1971) of the attempt.

"The dramatic reductions in chinch bug populations which had been induced by the fungus in the same locality a few years earlier without the aid of man could not be reproduced. This apparently led to two conclusions . . . First, the mere
presence of a microbial control agent is not adequate to ensure
death of exposed insects, i. e. the environment is a factor of
paramount importance in disease induction. And second, since man
has no control over the environment, there is little hope for
microbes, particularly fungi, as dependable microbial control
agents. The recognition of the importance of microclimates and
the possibility of increasing the virulence of the fungus have,
to some extent, modified this negative feeling. Nevertheless
almost 80 years later, these points are still very much in
evidence when microbial control with fungi is discussed."

Viruses show considerable promise as biological control
agents. Cotton bollworms, budworms, Great Basin and forest tent
caterpillars, cabbage loopers, gypsy moths, wattle bagworms,
African and cosmopolitan armyworms, cotton leafworms, and
imported cabbage worms are among the many insect species that
have been controlled by their respective viruses (Ignoffo, 1968;
Stairs, 1971). Six species of sawflies have been controlled for
many years by viruses in Canadian forests. Transovarial
transmission of virus occurs in three of these species and
epizootics can be initiated when 10% of the eggs are infected
(Bird, 1961).

Insect viruses are relatively stable under environmental
conditions. The NPV of _Trichoplusia ni_ (cabbage looper) has been
shown to remain infective after several years in the soil and to
initiate epizootics year after year (Jacques, 1970). Ultraviolet irradiation is detrimental to insect viruses (Morris, 1971). Jacques (1971) has studied protectants of virus residues and reported that polyhedra suspended in 1% India ink and 3% egg albumin retained 90-96% of the original activity after 17 days in field tests. Microencapsulation of an NPV failed to give protection against sunlight (Ignoffo and Batzer, 1971). A non-occluded virus, Chilo iridescent virus, retained infectivity for boll weevils after 6 days in the field when incorporated into a bait (McLaughlin, Scott, and Bell, 1972).

Production of Heliothis NPV was reported to be commercially feasible (Ignoffo, 1964). Six species of Heliothis larvae are susceptible to the virus giving it a broad market potential (Ignoffo, 1968). The virus is compatible with chemical insecticides, surfactants, and fungicides (Ignoffo, 1968; Ignoffo, Chapman, and Martin, 1965).

Insect viruses are generally highly specific for the host species. In rare instances, infectivity of a virus can be demonstrated for insects in different families and orders (Ignoffo, 1968). Vertebrates have been treated with 28 different insect viruses without any evidence of toxicity, pathogenicity, or allergenicity (Ignoffo, 1968). Extensive testing has been performed with the NPV's of cotton bollworms and cabbage loopers to assure their safety to plants, animals, and beneficial insects (Greer, Ignoffo, and Anderson, 1971; Hostetter, 1971).
A commercial preparation of the *Heliothis* NPV, VIRON/H™, has recently obtained an experimental label for use on cotton insects. This is the first viral pesticide registered for use in the United States. Registration for many more viral pesticides will undoubtedly be sought in the next several years, now that a protocol for evaluating insect viruses has been established.
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VITA

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Candidate: Margaret Elaine Cox

Major Field: Microbiology

Title of Thesis: Studies on viral, fungal, and bacterial pathogens for the soybean looper, *Pseudoplusia includens* (Walker)

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination:

July 17, 1972