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FACTORS AFFECTING BACULOVIRUS HELIOTHIS - INDUCED MORTALITY IN THE TOBACCO BUDWORM, HELIOTHIS VIRESCENS (F.)

The University of Arizona PH.D. 1982

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FACTORS AFFECTING *BACULOVIRUS HELIOTHIS*-INDUCED MORTALITY IN THE TOBACCO BUDWORM, *HELIOTHIS VIRESCENS* (F.)

by

Michael Fred Potter

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF ENTOMOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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ABSTRACT

Efficacy of Baculovirus heliothis against larvae of the tobacco budworm was studied under laboratory, greenhouse, and field conditions. Dosage-mortality studies using a diet surface inoculation technique resulted in LC_{50} values of less than 2 PIB/mm^2 for 1- to 5-day-old larvae. Onset of mortality was delayed in older larvae, and a greater quantity of inoculum was needed to produce the same mortality level as larvae matured. Length of the incubation period was shortened by increasing the dose.

In laboratory and greenhouse studies, mortality of neonates was enhanced by the addition of commercial feeding stimulants. A cottonseed-base adjuvant was more effective than either virus alone or virus mixed with soybean flour. The value of the bait was particularly apparent when larvae were held for short durations on virus-treated terminals.

Water extracts of fresh and dried garbanzo beans were shown to be highly attractive to tobacco budworm larvae, suggesting their potential for use as a feeding stimulant. Both bean treatments performed as well as the commercial cottonseed adjuvant. Virus-water extracts of garbanzo bean leaves and pods were no more effective in producing larval mortality than virus in water alone.

Although addition of a feeding stimulant significantly extended activity of virus residues on cotton terminals bioassayed

with H. virescens in the laboratory, the combination did not improve efficacy when larvae were allowed to feed on treated plants in the field. It may be that the effect of bait on young larvae was overridden by high temperatures or light intensities in the upper plant canopy.

Time of application studies directed at the egg-stage showed that larvae are capable of ingesting lethal quantities of the pathogen while chewing out of treated eggs. Applications should coincide as closely as possible with egg hatch to maximize infection. Following hatching, there was a consistent decline in effectiveness as treatments were delayed.

No significant effects on longevity or fecundity resulted from the feeding of virus to adults in a sucrose solution. Transovum transmission of virus to progeny was inefficient, regardless of the dose administered.

CHAPTER 1

INTRODUCTION

The tobacco budworm, Heliothis virescens (F.), is a pest of major economic importance throughout the cotton growing areas of the United States and throughout the New World. In the U.S. alone, 1973 cost estimates for control of Heliothis spp. on cotton exceeded $50 million (Ignoffo 1973).

In Arizona, the tobacco budworm is a relatively recent pest, with the first major outbreak occurring in 1972 (Watson 1974). Following scattered reports of budworm infestations in central portions of the state in 1976, cotton yields and production costs in Maricopa, Pinal, and Yuma Counties were severely affected during consecutive 1977-78 growing seasons. The present status of H. virescens on cotton in Arizona is that of a secondary pest which may cause serious problems as a consequence of management practices directed against other pests such as the lygus bug, Lygus hesperus Knight, and pink bollworm, Pectinophora gossypiella (Saunders).

Murray (1972) listed several factors responsible for the rapid rise in pest status of this insect in recent years. He stressed the potential for unrestricted growth by Heliothis spp. following disruption of the naturally occurring predator-parasite complex through intensive, broad-spectrum insecticide applications. The problem has been further compounded by the relative ease with which the tobacco

budworm acquires resistance to previously effective chemicals (Harris et al. 1972). Along with resistance to organochlorine insecticides (Adkisson and Nemec 1967), decreased susceptibility to methyl parathion and other organophosphates has been reported (Harp and Turner 1976, Crowder, Tollefson and Watson 1979). The ability of the tobacco budworm to develop insecticidal resistance has made it the major pest problem in areas such as northern Mexico, where total loss of cotton production since 1975 is attributed to this species (Wolfenbarger, Bodegas and Flores 1981). Resistant populations can presently be controlled only with the newly developed synthetic pyrethroids and some of the newer organophosphates, but development of resistance to these compounds seems inevitable if current use patterns are not changed.

The high cost, deleterious effects on the environment, and development of resistance associated with near-total reliance on synthetic insecticides has created a need for alternative approaches to insect control. The arthropod viruses represent one such alternative. Their specificity, effectiveness, and overall compatibility with other control methods makes them ideally suited for use in integrated pest management (IPM) systems. Federal support for microbial development was evidenced by the 1972 amendment to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) stating that "priority shall be given to develop biologically integrated alternatives for pest control" (Anonymous 1974).

The registration of the Heliothis nuclear polyhedrosis virus (Baculovirus heliothis) in 1975 paved the way for incorporation of the virus into IPM programs. Although early attempts at virus utilization were unsatisfactory due to rapid degradation by ultraviolet radiation (Bullock, Hollingsworth and Hartstack 1970), production-processing techniques have now been modified to produce more stable formulations. Commercial feeding adjuvants have also been developed recently in an attempt to overcome the inherent difficulties associated with a stomach insecticide and a pest which spends most of its life feeding in protected areas.

At present, there are insufficient data on which to base a recommendation for virus control of tobacco budworm in Arizona. The objective of this study was to provide practical information on the virus-insect interaction so that the pathogen may one day be incorporated into Arizona cotton IPM programs.

CHAPTER 2

LITERATURE REVIEW

The Heliothis Complex

The *Heliothis* complex probably constitutes the most important insect group in the world from the standpoint of crop loss. Two species, the tobacco budworm, *Heliothis virescens* (F.), and the bollworm, *H. zea* (Boddie) are extremely damaging pests of cotton throughout the New World. For example, in the United States during 1968, ca. 60% of all insecticides applied to cotton were used specifically for the control of *Heliothis* spp. (Anonymous 1969). In addition to cotton, members of the genus attack a wide variety of economic crops including soybean, tobacco, field and sweet corn, tomato, sorghum and peanut (Kogan et al. 1978). The larvae feed primarily on the flowers and fruits of the host plant, and once having gained entrance into the fruiting structure, become relatively inaccessable to attack by natural enemies or insecticides (Hardwick 1965).

In developing a broad-based IPM program for tobacco budworm in Arizona, it is necessary to determine the population dynamics of the pest, including overwintering, dispersal, mating, and the importance of mortality factors. Comprehensive reviews on these subjects pertaining to *H. virescens* have been presented by Lawrence (1974), Tollefson (1979), Potter (1979), and Rathman (1981).

Baculovirus heliothis - Discovery and Commercialization

Bassi (1835) was the first person to demonstrate that a microorganism could cause disease in animals. His studies with a silkworm-infecting fungus, Beauveria bassiana (Balsamo) led him to suggest that pathogens might be used to control insect pests. The Heliothis nuclear polyhedrosis virus (NPV) was first detected as a "caterpillar wilt" of H. armigera in South Africa (Mally 1891). The causative viral agent, however, was not recognized until 1936 (Parsons 1936), and was reconfirmed later by Stahler (1939) and Steinhaus (1957).

Ignoffo (1973) presented an extensive summary of the historical and technical development of the Heliothis NPV as the first virus approved for use as a commercial pesticide. The energy and expense required to develop an arthropod virus is so great that only those agents showing promise for pest control purposes can be considered. The virus was selected for commercialization because members of the genus Heliothis are severely destructive pests of cultivated and noncultivated crops worldwide (Hardwick 1965). Furthermore, Heliothis resistence to organochlorine, organophosphorus and carbamate insecticides is widespread and increasing (Harris et al. 1972), and the repeated application of these materials has resulted in additional problems of resurgence, secondary pest outbreaks, and environmental pollution. Preliminary laboratory and field experiments also showed the pathogen to be specific to the genus Heliothis, capable of propagation

under laboratory conditions, and nonhazardous to man and other non-target organisms (Ignoffo 1965abc, Ignoffo and Heimpel 1965).

The original source of Heliothis NPV used in commercial production was isolated in 1961 from 100 diseased bollworms attacking cotton in the Rio Grande Valley, Texas (Ignoffo 1965a). The early mechanics of small-scale virus production were developed at Brownsville, Texas in 1961-62 (Ignoffo 1965b), and eventually led to industrial participation by International Minerals and Chemical Corp. (Viron/H®), Nutrilite Products, Inc. (Biotrol®VHZ), and more recently, Sandoz-Wander, Inc. (Elcar®).

Since the initial stages of development, production of the Heliothis NPV has occurred only in living caterpillars reared on semisynthetic diet (Ignoffo 1965ab). The high cost of producing a commercial insecticide in this manner has stimulated research in tissue-culture and fermentation technology. Established lines of Heliothis cells are currently available for Heliothis NPV replication (Hink and Ignoffo 1970), and a patent was issued to McLaughlin Gormley King Co., South Africa, in 1970 for production of insect viruses (including Heliothis NPV) using fermentation techniques (Wells 1970). However, while some progress has been made in these areas, commercial utilization is not anticipated for several years to come (Falcon 1976).

Hazard to Humans and Non-Target Organisms

Since 1963, and throughout its development for commercial use, the Heliothis NPV was subjected to extensive safety testing.

Ignoffo and Heimpel (1965) demonstrated that the virus was non-toxic to guinea pigs and white rats when administered orally, dermally, or by intravenous or intraperitoneal injection. Between 1965 and 1970, the NPV was tested on 24 plant, 36 invertebrate, 4 avian, 7 piscian, and 6 mammalian species including man. In no instance was toxicity or pathogenicity reported due to virus exposure (Heimpel and Buchanan 1967, Meinecke, McLane and Rehnborg 1970, Ignoffo et al. 1971). Based on this record of safety and performance in the field, the Environmental Protection Agency in 1973 granted Viron/H® an exemption from the requirement of a tolerance on a raw agricultural product (cottonseed).

B. heliothis Morphology and Taxonomy

The virions of Heliothis NPV are imbedded in the matrix of a proteinaceous inclusion body ca. 1μ in diameter. Inclusion bodies (Figure 1) are irregularly-shaped polyhedrons having a crystal-like lattice and rounded edges. The infectious agent, the virions or virus particles, are occluded in the cubic-protein lattice of the inclusion body. They are rod-shaped elongated particles with rounded ends, ca. 336 mμ in length and 62 mμ wide (Greogry, Ignoffo and Shapiro 1969). The genetic material of the virions is DNA (Estes and Ignoffo 1965), and there are roughly 26 virions imbedded within each inclusion body. According to the classification of Wildy (1971), the most appropriate name for the Heliothis NPV is Baculovirus heliothis.

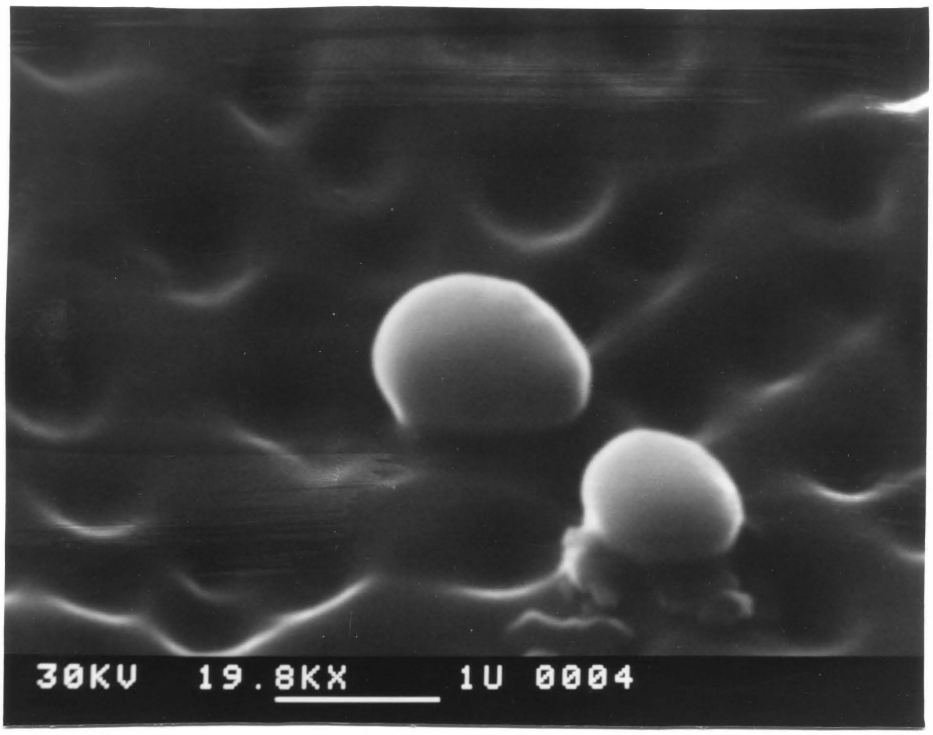

Figure 1. Polyhedral Inclusion Bodies of B. heliothis Shown on the Surface of a Tobacco Budworm Egg (19,800X).

Symptomology

B. heliothis is infective only to lepidopterous larvae of the genus *Heliothis*. Susceptible species include *H. virescens* (F.), *H. zea* (Boddie), *H. armigera* (Hubner), *H. phloxiphaga* (Grote and Robinson), and *H. obtectus* (Ignoffo 1965c). The virus must be ingested to be infective, and following entrance into the midgut, the polyhedral inclusion bodies (PIB) are dissolved within seconds, releasing the intact virus particles. The virions then penetrate through the midgut epithelium, entering the hemolymph. Nearly all tissues of larval *Heliothis* are susceptible, but replication of the virus and formation of inclusion bodies occur only within the nuclei of cells (Steinhaus 1949). As cells and tissues are broken down, the hemolymph becomes cloudy and billions of inclusion bodies are visible in the blood. Eventually, the larva becomes flaccid (Figure 2) and the integument ruptures releasing the inclusion bodies. The cadaver is capable of initiating further infections if polyhedra are ingested by larvae of a susceptible species.

Death usually occurs within 2-9 days of initial infection depending on the dose and larval age at time of viral ingestion (Ignoffo 1965c, 1966a). While temperature is known to have little effect on overall incidence of larval mortality, low temperatures have been shown to retard the onset of virus infection. For example, Ignoffo (1966b) recorded initial mortality at 3 and 21 days for bollworms held at temperatures of 35° and 13°C, respectively. He concluded

Figure 2. Virus-killed Tobacco Budworm Larva.

that timing differences in the onset of mortality were due to temperature effects on bollworm feeding rather than on viral development.

Viral Stability

Early in the development of B. heliothis as a commercial microbial insecticide, it was thought that the protein matrix surrounding the virions would provide considerable protection against field degradation. After observing the loss of most viral activity within 24 h of initial field exposure, however, Bullock (1967) speculated that ultraviolet (UV) radiation may be responsible for inactivation of the virus on cotton foliage. Baculovirus inactivation has since been attributed primarily to sunlight in the UV range of 290 to 320 nm (Bullock et al. 1970). Ignoffo and Batzer (1971) demonstrated that under both natural and artificial sunlight, the extent of inactivation increased with length of exposure, and that ca. 70% of total activity was destroyed within the first 4 hours. Rate of further inactivation was considerably less and a residual activity of ca. 10-15% remained after 8 days of continuous exposure.

In an effort to stabalize B. heliothis against sunlight inactivation, protective adjuvants such as activated carbon, lignin sulfate, and IMC-90001 were incorporated into virus sprays (Ignoffo et al. 1972, Young and Yearian 1974a). Microencapsulation of the virus in these protectants has also extended residual activity, often for a period of up to 1 week (Ignoffo and Batzer 1971, Bull et al. 1976).

Unfortunately, while addition of these materials has increased persistence of the virus in the field, Heliothis control on cotton has rarely been enhanced above the virus alone.

Although UV irradiation has been the primary factor implicated in B. heliothis degradation, the alkaline pH of cotton leaf surfaces may also affect field inactivation of virus to a limited extent. The pH of cotton dew is frequently above 9.0 and may increase to 10.5 during extended periods of dry weather (Falcon 1969). In the laboratory, several researchers (Andrews and Sikorowski 1973; McLeod, Yearian and Young 1977; Young, Yearian and Kim 1977) reported polyhedral swelling in cotton leaf washes and eventual inactivation of polyhedra in dew during evaporation. Few field studies have shown pH to be a significant factor in virus degradation, but indications are that such an effect would most likely occur in cotton-producing areas of low rainfall and flood-type irrigation.

From a practical field standpoint, temperature is known to exert little effect on virus inactivation. MacFarlane and Keeley (1969) demonstrated that B. heliothis can withstand temperatures of 65°C for 2 h without loss of infectivity. At a temperature of 85°C, however, complete inactivation of ploy hedra occurred within 5 minutes, suggesting that polyhedral contaminants of food products could be destroyed by normal cooking or processing.

Degradation of Heliothis NPV was studied on various hosts of H. zea and H. virescens, and found to be most rapid on cotton

(Young and Yearian 1974a). Persistence on tomato was significantly greater than on either cotton or soybean and was presumably due to the pilose nature and curvature of the tomato leaf. In a related study, Young and Yearian (1974b) also found persistence to vary with location on the cotton plant. Virus deposits in more shaded locations such as on square bracts, blooms, and undersurfaces of leaves were more persistent than those on upper leaf surfaces. These authors concluded that since both greater persistence and larval feeding occurs on fruiting structures, more discreet placement of virus on the cotton plant could improve efficacy of treatments.

Application Technology

While there have been numerous attempts to improve the field persistence of virus formulations, application technology designed specifically for microbial insecticides has been sorely lacking (Ignoffo 1970). Since the virus must be ingested to be infective, uniform plant coverage, particularly in the areas of larval feeding, is essential if successful control is to be achieved. Chapman and Ignoffo (1972) showed that doubling the spray volume per acre provided control equal to doubling the dosage of *B. heliothis*. Microdroplet application of the virus in crude cottonseed oil has also proven effective for controlling larval *Heliothis* (Falcon, Sorensen and Akesson 1974).

Baits and Feeding Stimulants

Along with attempts to increase spray coverage, baits or feeding stimulants have been used to enhance the efficacy of virus treatments. Several plant substances have been shown to elicit feeding responses in larval Heliothis, including water extracts of green beans, fresh okra, and corn (Montoya, Ignoffo and McGarr 1966), and crude cottonseed oil (Guerra and Shaver 1968). For control of Heliothis spp. on cotton, Allen and Pate (1966) and Montoya et al. (1966) found combinations of virus and aqueous extracts of fresh corn applied as sprays to be more efficacious than virus alone. In contrast, Stacey, Yearian and Young (1977) reported that virus combined with water extracts of mature seed from corn, cotton, and crimson clover combined with wheast and applied as sprays and dusts did not significantly improve cotton yields over the application of virus alone. McLaughlin, Andrews and Bell (1971) were able to achieve a reduction in bollworm numbers by incorporating B. heliothis into a cottonseed oil (boll weevil) bait. Andrews et al. (1975) also used this bait to provide bollworm control equal to a standard treatment of methyl parathion.

The virus has also been tested on cotton in combination with sugars. Stacey et al. (1977) found that virus sprays with invert sugars provided significantly greater cotton yields than 2 commercial virus preparations applied without adjuvant. They cautioned, however, that there is evidence to suggest that foliar applications of sugar alone may increase cotton yields when plants are under stress.

In addition to those materials which act as either sunlight protectants or phago-stimulants, there have also been multipurpose adjuvants developed which incorporate characteristics of a bait, UV protectant, and evaporation retardant into a single compound. For example, McLaughlin et al. (1971) and Andrews et al. (1975) combined the virus with an adjuvant containing cottonseed oil, invert sugars, Dacagin®, hydroxyethyl cellulose and glycerol to obtain a reduction in H. zea. Ignoffo, Hostetter and Smith (1976) also demonstrated that a multipurpose adjuvant developed by Sandoz Inc. increased efficacy of B. heliothis treatments over application in water alone. In general, the yield increases achieved with these multipurpose adjuvants have been superior to those associated with adjuvants exhibiting a specific characteristic (Young 1978).

Autodissemination of NPV

The unique nature of microbial insecticides encourages other methods for dispersal in the environment besides the reliance on "traditional" spray technology. Knipling (1960) mentioned autodissemination, or the use of insects to spread pathogenic organisms, as one of four ways to utilize insects for their own destruction. Shortly after this, Martignoni and Milstead (1962) demonstrated transovum transmission of the NPV of the alfalfa caterpillar, Colias eurytheme Boisduval, by applying a paste of PIBs to the genital armature of female moths. In laboratory and field cage studies with the cabbage looper, Trichoplusia ni, Elmore and Howland (1964) and later,

Vail and Hall (1969), reported disease transmission to progeny of moths sprayed with, or fed PIBs in a suspension of sugar water. No disease occurred, however, when the eggs were surface sterilized, indicating that transmission was transovum rather than transovarial. Working with H. zea, Hamm and Young (1974) also reported surface contamination of eggs when adults were fed PIBs. They also showed that virus-fed adult males could transmit the infection to their progeny following pairing with untreated females. Finally, the mechanical transmission of B. heliothis was demonstrated by Gard (1972) who showed that bollworm moths could be drawn to a UV light trap, automatically contaminated with a virus dust, and released back into the environment. Contaminated moths reportedly distributed the pathogen (1) to cotton plants when they land and walk on them, (2) to untreated adults during mating, and (3) to the eggs during oviposition.

Field Performance of B. heliothis

Coaker (1958) was the first to use B. heliothis against field populations of the bollworm on cotton in Uganda. Since that time, the virus has been used for control of Heliothis spp. on several commercial crops with varying degrees of success. Ignoffo, Chapman and Martin (1965) were able to effectively control field populations of H. zea and H. virescens with the virus on corn, cotton, and grain sorghum. Allen, Gregory and Brazzel (1966) further showed that a dual program of Heliothis NPV application and preservation of the naturally-occurring predator-parasite complex, could be as

effective as a standard insecticide program for controlling Heliothis in cotton. Conversely, McGarr and Ignoffo (1966) and McGarr (1968) were unable to achieve adequate control with the virus on cotton in Texas. Kinzer et al. (1976) found B. heliothis applied as a foliar spray 3 times/wk as effective as monocrotophos (applied at 5-7 day intervals), but when virus treatments were reduced to 1-2 times/wk, cotton yields were significantly affected.

Working on sweet corn in southern California, Oatman et al. (1970) reported virus dusts to be more effective than Bacillus thuringiensis dusts for control of corn earworm, but repeated applications of virus at short intervals were again necessary to maintain adequate control. On tobacco, Mistric and Smith (1973) found that significant budworm mortality did not commence until the 6th day after virus treatment. They concluded that the amount of foliage consumed during this period made B. heliothis an unacceptable control alternative to TDE or methomyl. These results were consistent with those of an earlier study by Chamberlain and Dutky (1958). Finally, Ignoffo et al. (1978) found B. heliothis to be more effective than either the bacterium B. thuringiensis or the fungus Nomuraea rileyi in controlling populations of H. zea feeding on soybeans.

Compatability with Other Insecticides

The erratic performance of B. heliothis under moderate to heavy Heliothis pressure has prompted investigations aimed at combining the virus with low doses of synthetic insecticides. The application of

microbial agent-insecticide combinations was reviewed by Benz (1971) who reported a range of interactions from antagonism to synergism. Ignoffo and Montoya (1966) found the virus to be compatable with most insecticides and insecticidal adjuvants (DDT, toxaphene, carbaryl, Triton X-100, xylene, etc.), but methyl parathion was shown to adversely affect the infectivity of inclusion bodies. Chapman and Ignoffo (1972) found virus-pyrethrum combinations to increase cotton yields over application of virus alone, but differences were nonsignificant. Luttrell, Yearian, and Young (1979) also looked at virus-chemical insecticide interactions, combining Elcar® with permethrin, methomyl, and EPN- methyl parathion. Both laboratory and field data indicated little added benefit from the combinations, although the efficacy of virus was again reduced by incorporation of methyl parathion. Chlordimeform, a reported synergist of several chemical insecticides (Plapp 1976), was evaluated in combination with B. heliothis and B. thruingiensis by Pieters et al. (1978). These authors found the chlordimeform-microbial agent mixtures to compare favorably with conventional insecticides for control of Heliothis spp. on cotton.

Resistance to B. heliothis

To date, there have been no reported cases of larval resistance to Heliothis NPV infection. Ignoffo and Allen (1972) attempted to select for resistance in laboratory populations of H. zea by maintaining continuous pressure (LD_{50} - LD_{75}) for 20 to 25 generations. No significant changes in LD_{50}, slope, or intercept of

dose-mortality lines were detected when compared with nonselected or natural Heliothis populations. In contrast, Carter and Phillips (1968) demonstrated an 8- to 10-fold increase in resistance to methyl parathion after only 10 generations of selection pressure.

The only example of increased tolerance to a standard dose of virus is that associated with larval maturation. For example, when viewed from the standpoint of age, Allen and Ignoffo (1969) noticed a 250-fold difference in LC_{50} values between 3- and 8-day-old larvae. Less than a two-fold difference was obtained, however, when dose was interpreted on the basis of PIB/mg body weight.

CHAPTER 3

MATERIALS AND METHODS

Source of Insects and B. heliothis Preparation

All lifestages of tobacco budworm used in this study were originally collected as larvae from cotton fields throughout Arizona. New cultures were established each year toward the end of the growing season.

The virus preparation used in all treatments was a commercial WP (Elcar®, Sandoz Inc., San Diego, CA) containing 4.4×10^9 PIB/gram (Figure 3).

Rearing of H. virescens

Budworm adults were held in 3.79 liter wide-mouth glass jars (20-30/jar), the bottom of which was covered with a circular piece of fine mesh plastic screening. Oviposition and resting sites were provided in the form of paper towel strips, ca. 5cm x 10cm, suspended from the lip of the jar. An additional egg sheet was furnished beneath the jar lid. The moths were fed a 5% sucrose solution dispensed from inverted glass vials at the top of each jar. Eggs were surface sterilized with 5% solutions of chlorox and sodium thiosulfate and then rinsed with water (Ignoffo 1963). After drying, the egg sheets were placed in 237 ml waxed cardboard containers (Lily Fountain and Paper Supply, Tuc., AZ) until hatching occurred. Newly-emerged

Figure 3. Commercial Preparation of B. heliothis Used in All Experiments.

larvae were then placed in 30 ml clear plastic cups ca. two-thirds full of a modified lima bean diet (Patana 1969), and sealed with paper lids. Adults and eggs were held in an air-conditioned room (25 ± 2°C) under naturally-occurring daylengths, while larvae were maintained in a large, controlled-temperature rearing room at 28 ± 1°C and 14:10 (L:D) photoperiod. Pupae were held in 237 ml paper cartons capped with screened lids for adult emergence.

Dosage-Mortality Studies

The purpose of this study was to gain a quantitative estimate of B. heliothis activity when directed against tobacco budworm larvae of different ages. Virus in the form of inclusion bodies was presented to 1- to 5-day-old H. virescens using a diet surface inoculation technique (Ignoffo 1965c). Hot liquid lima bean diet was poured into 30 ml plastic cups to a depth of ca. 25.4 mm. After solidification and cooling had occurred, decimal dilutions of virus were dispensed in volumes of 0.1 ml onto the diet surface with an adjustable Eppindorf pipette. A blunt glass rod was used to uniformly distribute the virus over the entire upper surface of the media. An aqueous stock suspension of virus containing 1.32×10^6 PIB/ml was used to make decimal dilutions ranging from 0.164 to 1642 PIB/mm^2 diet surface. To further bracket the LC_{50}, other dilutions were prepared as needed. One larva was placed in each cup after the surface of the media had dried. The cups were then sealed with paper lids, transferred to 30 ± 1°C, and examined over a period of 12 days for larval mortality.

Virus + Feeding Stimulant Studies

Since the virus must be ingested to be effective, any substance which causes a larva to feed preferentially in areas of the spray deposit should enhance infection and the subsequent level of control. A series of 3 experiments was conducted in the laboratory and greenhouse to determine whether the incidence of viral infection could be enhanced by addition of a feeding stimulant. The two commercial stimulants used in these studies were Coax®, a cottonseed-based adjuvant produced by Traders Oil Mill Co., Fort Worth, TX, and Gustol®, a soybean-based product of Sandoz Inc., San Diego, CA. Both materials were applied at a final concentration of 1.2% (1.12 kg/ha).

Leaf Disk Bioassay

Leaf disks (2.54 cm diam), punched from tender leaves of greenhouse cotton plants, were held in individual plastic petri dishes (50 mm diam) on a double layer of filter paper saturated with distilled water. Treatments consisted of aqueous suspensions of virus alone, virus + Gustol®, and virus + Coax®. Virus was diluted and applied at final concentrations of 1.3 and 13 PIB/mm^2 leaf disk. Disks treated with water alone served as controls. Test solutions (50 µl) were spread evenly over the upper surface of the disks with a glass rod. After the material had dried, two laboratory-reared neonate tobacco budworm larvae (less than 12 h old) were placed on the treated surface of each disk and allowed to feed in darkness for 15 h. Larvae were then removed and incubated at 30 ± 1°C in individual 30 ml

plastic cups on lima bean diet. Mortality due to virus infection was recorded for 12 days, and each treatment was replicated 4 times, using 30-40 larvae/replicate. Complete disintegration or "melting" of the larvae was the criterion used to establish cause of death as viral-induced.

Greenhouse Test

Tests were also conducted with greenhouse cotton plants to further determine the effect of feeding stimulants on viral-induced mortality of neonate larvae. Young plants (ca. 0.5 m tall) were sprayed with 2 dosages of virus, 2.35×10^{10} and 1.17×10^{11} PIB/ha, applied alone and in combination with the soybean- and cottonseed-based stimulants. Plants treated with water without virus served as controls. All treatments were applied at dusk with a CO_2-pressurized backpack sprayer (141 liters/ha, 276 kPa). After the plants had dried, ca. 20 neonate larvae were placed randomly on the lush terminals of each plant. Larvae were allowed to feed for 12 h, after which they were removed and held at 30 ± 1°C in individual 30 ml cups on lima bean diet. Treatments were replicated 10 times, and mortality due to virus infection was recorded over a period of 12 days.

Duration of Exposure

A final experiment was conducted to determine the effect of incorporation of a bait on mortality of neonates, exposed for different lengths of time on virus-treated surfaces. Larvae were allowed

to feed 3, 6, 18, or 30 h on cotton terminals treated with water alone, water + virus, or water + virus + Coax®. Virus was applied at rates of 2.35×10^{10} and 2.35×10^{11} PIB/ha and Coax® at 1.12 kg/ha with a backpack sprayer. After the plants had dried, treated terminals were placed in microcages (Figure 4) consisting of 2 clear plastic tumblers (237 and 296 ml) stacked together to form a reservoir for water below the inner tumbler. Terminal stems were inserted into the water through a small hole drilled in the bottom of the inner cup. A perforated plastic lid was used to cover the cages, providing ventilation and minimizing excess moisture within the chamber. Ten neonate larvae were then introduced onto each terminal. The microcages were held in darkness at 30 ± 1°C, and at the conclusion of each exposure period, larvae were transferred to lima bean diet for determination of virus infection. Treatments were replicated 3 times with 40-60 larvae/treatment/feeding period/replicate.

Garbanzo Bean as a Feeding Stimulant

Following the discovery by Watson (unpublished) that small commercial plantings of garbanzo bean *Cicer arietinum* L., in Yuma County were a highly attractive early-season host of *H. virescens*, a study was designed to investigate the feeding response of larvae to different parts of the garbanzo bean plant. We also hoped to evaluate garbanzo bean plant extracts as potential feeding stimulants for use with the *Heliothis* NPV.

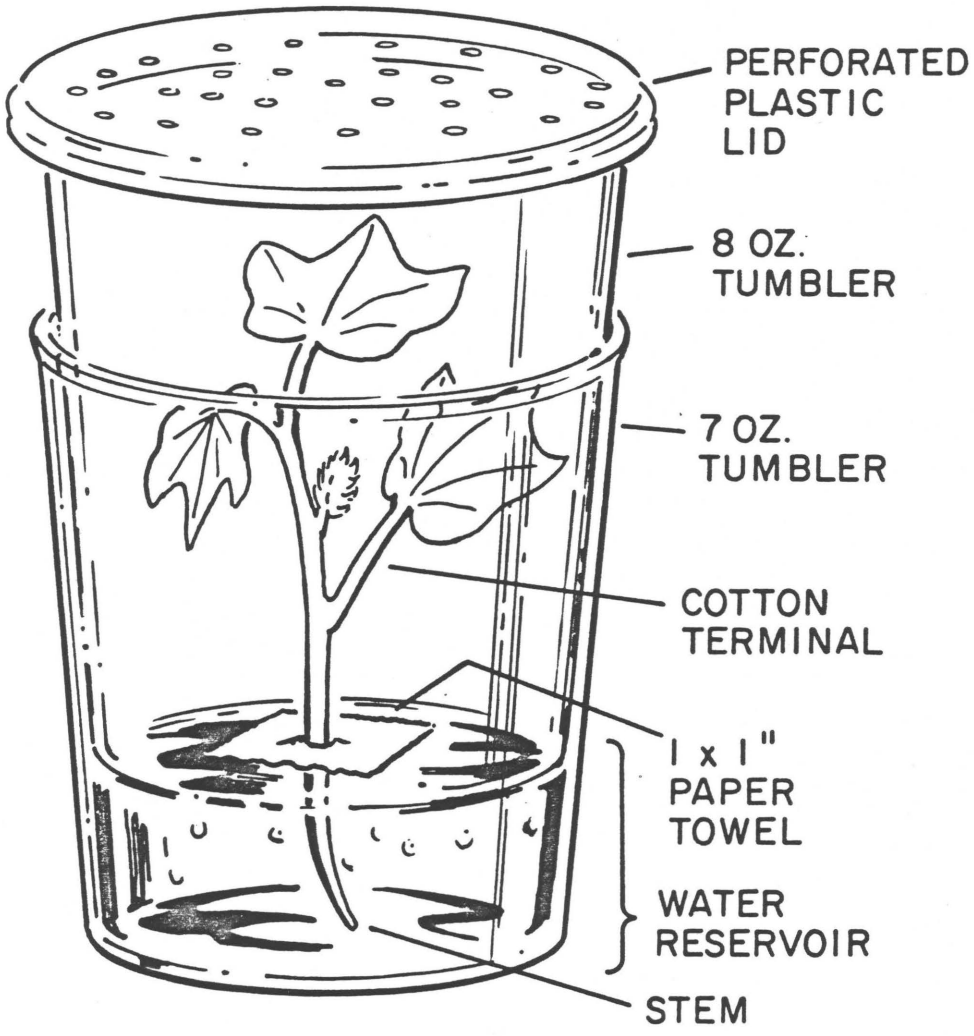

Figure 4. Microcages Used for Bioassay of Treated Terminals.

Two methods were used to determine the activity of the bean extracts. In the first (Ignoffo et al. 1976), laboratory-reared tobacco budworm larvae were released onto 3.0 cm diam cotton leaf disks, the central portion being treated with a combination of virus and one of the potential feeding stimulants. Treatments were confined to the center of each disk by dispensing 10 µl of material through a 1.4 cm diam hole in the middle of a plastic template. Water extracts of leaves, pods, and both fresh and dried beans (0.25 g/ml) were prepared immediately before each test. The commercial cottonseed-base stimulant, Coax®, was also evaluated, as were solutions of NPV in water alone and a water control. Virus used in all treatments was applied at a final concentration of 8.2 PIB's/mm^2 leaf disk.

After the suspensions had dried, disks were placed in individual plastic petri dishes (50 mm diam) on a double layer of filter paper saturated with distilled water. One 3rd-instar larva (19.1 ± 3.2 mg) was released onto each disk and allowed to feed in darkness for 14 h at 29 ± 1°C. Larvae were then removed and held for 12 days in 30 ml plastic cups on synthetic diet for virus mortality.

Remaining areas of both the entire disk and the inner treated portion (cut from the center with a smaller cork borer) were measured with an area meter (Hayashi Denko Co., Type AAM-5) at the conclusion of the feeding period (Figure 5). Values were then subtracted from disk areas measured before feeding to determine consumption per larva. Each treatment was replicated 4 times, using 25 3rd-instars per replicate.

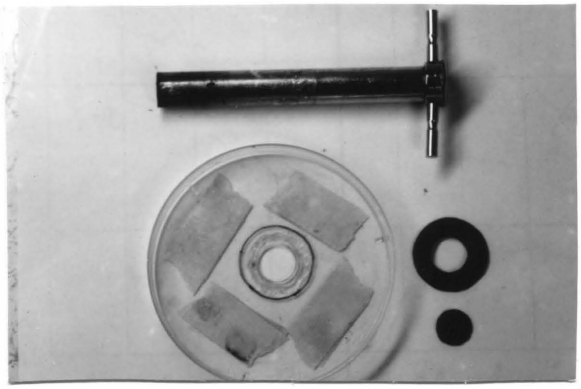

Figure 5. Summary of Bioassay Procedure (Ignoffo et al. 1976) Showing Cork Borer, PLastic Template, and Large Leaf Disk with Inner (Treated) Portion Removed.

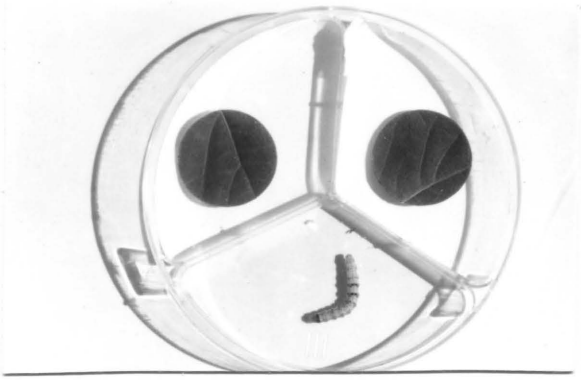

Figure 6. Feeding Stimulant Preference Test (Guerra and Shaver 1968).

To determine whether larvae were capable of discriminating between materials when given a choice, selected treatments were again screened using the cotton leaf disk method of Guerra and Shaver (1968). In this bioassay, larvae were exposed to a pair of disks (2.54 cm diam) treated with a water extract of dried beans (0.25 g/ml), Coax® (.012 g/ml), or a water control. Virus was excluded from all treatments. The test solutions (50 µl) were spread evenly over the entire upper surface of the leaf disks with a glass rod and allowed to dry. Two disks receiving different treatments were then placed in opposite sections of a 100 x 15 mm quadranted petri dish on filter paper saturated with distilled water (Figure 6). One laboratory-reared 4th-instar larva (132 ± 18 mg) was introduced into the quadrant between the disks and allowed to feed in darkness for 12 h at 29 ± 1°C.

Total consumption from each disk was determined by measuring areas before and after larval feeding, and the percentage of disks fed upon in each treatment was recorded 3 and 12 h after larval release. Results were obtained from 4 tests, each treatment comparison being replicated 25 times per test with 1 larva/replicate.

Field Studies

Viral Persistence and Effect of Stimulant

A major problem with *B. heliothis* in the past has been a lack of persistence in the field. This was attributed largely to inactivation of the pathogen by UV irradiation, but the alkaline pH of cotton dew may also exert some influence in this region. The

objective of this experiment, therefore, was to study persistence of the virus on irrigated cotton and determine whether larval control could be enhanced by addition of a feeding stimulant.

Tests were conducted at the University of Arizona Campbell Ave. Expt. Farm in Tucson on a 0.4 ha block of Deltapine-61 short staple cotton. Each plot consisted of 2 rows, 15.2 m long, with a 102-cm spacing. Sprays were applied at the rate of 141 liters/ha and 276 kPa (15 gal/A, 40 psi) with a CO_2-pressurized backpack sprayer.

Test 1. For this test, plots were treated at 0545 h on June 16, 1981 when the cotton was 46 cm in height. Aqueous suspensions of virus were applied at rates of 3.1×10^{11}, 6.2×10^{11}, and 1.24×10^{12} PIB/ha alone and in combination with the feeding stimulant, Coax®. Terminals were then collected (12/plot) at 0, 1, 2, 4, and 6 days posttreatment and returned to the laboratory for bioassay with neonate tobacco budworm. The terminals were again placed in plastic microcages (296 ml), and neonate tobacco budworm (5-7/cup) were placed randomly onto the terminals and allowed to feed 48 h at 28 ± 1°C. Larvae were then transferred to cups of lima bean diet and held 10 additional days for virus mortality. Treatments were replicated 3 times using 40-60 larvae/formulation/time period/replicate.

Test 2. This test was initiated on August 22, 1981 to further evaluate residual activity and field efficacy of virus treatments

directed against young larvae. Larvae were introduced onto the tender foliage of cotton terminals at 0, 1, 2, and 4 days following an initial treatment of plots with virus in water alone (1.24×10^{12} PIB/ha) or in combination with a feeding stimulant. Two neonatant larvae were inoculated per terminal (50 terminals/plot) between 0500 and 0630 h. After a 48 h feeding period, plants were inspected and recovered larvae were transferred to lima bean diet and maintained as in the previous test. Larval mortality was calculated from 3 replicates, using 30-40 larvae/formulation/time period/replicate. A blanket application of Diazinon (1.12 kg/ha) was applied 2 days before virus treatment in order to minimize predator interference.

Importance of Timing B. heliothis Applications

In an effort to maximize viral control of tobacco budworm, studies were conducted to evaluate the effectiveness of spray applications directed against different lifestages.

Effect On Egg-Stage

To determine the efficacy of virus applied to Heliothis eggs in the field, small paper strips (ca. 1 cm^2) supporting 4-6 eggs each, were pinned onto terminals of field cotton and then sprayed. Eggs representing 3 different stages of maturity (newly-laid, 1-day, and 2-day-old) were sprayed at the same time with 3 virus doses, 3.1×10^{11}, 6.2×10^{11}, and 1.24×10^{12} PIB/ha. The aqueous suspensions of virus were applied at a rate of 141 liters/ha and 276 kPa with a backpack

sprayer. Eggs were then carefully monitored, so that just prior to hatch, the respective samples could be returned to the laboratory for determination of larval mortality. Eggs were incubated at 27 ± 1°C in 572 ml cardboard cups equipped with plastic lids. Emerging larvae were held in individual 30 ml cups on artificial diet, and incidence of viral infection was recorded for 12 days. Mortality determinations were based on an average of 3 replications, with 38-60 larvae/dose/egg-age/replicate.

Effect on Established Larval Populations

To further understand the effect which timing of spray application has on viral control of established larval populations, additional studies were conducted in the greenhouse and in the field. For the greenhouse experiment, potted cotton plants ca. 38 cm in height (3-4 squares each) were inoculated with 5 neonate tobacco budworms per plant. Virus was then applied alone (9.9×10^{11} PIB/ha) or in combination with the feeding stimulant (Coax®), 0, 1, 2, and 4 days after the initial larval release. Cotton plants sprayed with water served as controls. Larvae were allowed to feed on the treated plants 48 h, after which the plants were inspected and as many larvae as possible were transferred to individual 30 ml cups of lima bean diet. Cups were maintained at 30 ± 1°C and mortality was recorded for 12 days. Initial larval inoculations were made on 10 plants/treatment/time period.

A similar experiment was conducted on a 0.4 ha block of Deltapine-61 short staple cotton. Plots consisted of 2 rows, 15.2 m long, with a 102-cm spacing. On July 18, 1981, lush terminals were inoculated with neonate tobacco budworm, 2 larvae/terminal, ca. 80 terminals/plot. Larval introductions were made between 0500 and 0700 h. Four timed spray applications of virus alone (1.24×10^{12} PIB/ha) and in combination with Coax® were made at 0, 1, 2, and 4 days following the initial release of larvae on the plants. Control plots received treatment with water alone. The larvae were allowed to feed an additional 48 h after treatments were applied, and were then removed and held on artificial diet as described in the previous experiment. Mortality determinations were based on 3 replicates of 25-35 recovered larvae/formulation/spray time/replicate. Data on larval instar and feeding site at time of recovery were also recorded. Sprays were again applied at a rate of 141 liters/ha and 276 kPa, and a blanket application of Diazinon (1.12 kg/ha) was applied 2 days before virus treatment to reduce larval consumption by predators.

Effect of B. heliothis on Adults and Progeny

An important method of dissemination of insect pathogens is by the movement of healthy carriers and infected hosts (Tanada 1964). A series of experiments was designed to determine whether *B. heliothis* could be transmitted to progeny of adult tobacco budworm when moths were fed PIBs in a sweet solution. We reasoned that if virus could be readily transmitted to progeny via the adults; moths

could spread the pathogen throughout a population after being drawn to a sugar or molasses line in the field (Figure 7).

An ititial experiment was conducted to determine whether ingestion of polyhedra directly affected the longevity or fecundity of adults, or subsequent hatching of eggs. Pairs of moths (1 ♂and 1♀) were held together in cages consisting of 0.95 liter waxed paper cartons covered with cheesecloth as an oviposition substrate. Virus was fed to newly-emerged moths (< 1 day old) in a 5% sucrose solution at a concentration of 1.0×10^8 PIB/ml. The virus-sucrose mixture was offered to moths in glass vials throughout the entire experiment. Control moths were fed 5% sucrose solution alone.

Adults were held in a temperature-controlled room (26 ± 2°C, 14:10 L:D diurnal cycle) and cages were inspected daily for adult mortality and egg deposition. Viability of eggs was determined on 3 100-egg subsamples from each pair of moths. Sixteen replications with one pair of moths (♂ and ♀)/replicate were tested.

To determine whether virus-fed moths are capable of transmitting infection to their progeny during oviposition, 2 pair of ♂and ♀ moths were held together in previously described 0.95 liter cages and offered virus-sucrose suspensions containing 5.0×10^7, 1.0×10^8, and 5.0×10^8 PIB/ml. Prior to feeding, the newly-emerged moths had been held together in 3.79 liter glass jars and starved 48 h. Unfed moths were then offered the various virus-sucrose mixtures for 24 h, after which the vials were removed and replaced with sucrose

Figure 7. Tobacco Budworm Moth Shown Feeding on a Virus + Sweet Bait-treated Cotton Leaf.

alone for the duration of the test. Following the commencement of oviposition, eggsheets were harvested for 5 consecutive days and incubated at 30 ± 1°C in 572 ml waxed cardboard cups. Emerging larvae were observed for 12 days, and mortality determinations were based on 6 replications per treatment with 30-50 larvae/oviposition day/ replicate.

A final experiment was conducted to determine whether moths, confined on cotton leaves treated with virus in either a sugar or molasses bait, are capable of transmitting infection to their progeny. Terminals from greenhouse-grown cotton plants were sprayed with virus at rates of 5.0×10^7, 1.0×10^8, and 2.0×10^8 PIB/ml in 10% mixtures of sucrose or molasses (blackstrap, feed-grade). Terminals treated with water alone served as controls. All sprays were applied with a hand-held atomizer to the point just before run-off (ca. 2.5 ml/terminal). After the leaves had dried, treated terminals were placed in 296 ml plastic microcages equipped with a cheesecloth top for ventilation. One pair of female moths previously starved 24 h was then held overnight (ca. 12 h) in each cage beneath an outdoor ramp. Adults were transferred the following morning to 0.95 liter cages equipped with sucrose-filled feeder vials and cheesecloth for oviposition. Eggs were harvested during the first 4 days of oviposition and held for larval emergence as in the previous experiment. Prior to exposure to treated leaves, the female moths were held 48 h in 3.79 liter jars with males to encourage mating and subsequent commencement of oviposition as soon as possible following

xposure to treated terminals. Determination of virus transmission as based on 5 replications per treatment (10♀ moths total), with 0-50 larvae/oviposition day/replicate.

Statistical Analysis of Data

For the dosage-mortality studies, data were analyzed sing probit-analysis (Finney 1952). When working with percent ortalities, analysis of variance (ANOVA) was performed on the arcsin ransformation of the means. The Student-Newman-Keuls multiple range est (SNK) was used in most of the experiments to separate means of he transformed data. Values were then transformed back to percent- ıges for presentation in the tables. Both the ANOVA and SNK analyses /ere performed with the Statistical Package for the Social Sciences (SPSS) computer program. Chi-square analysis was used to detect ;ignificant differences in the second garbanzo bean feeding preference :xperiment, and data pertaining to the influence of virus on moth ongevity and fecundity were analyzed using Student's t-test. In all :ases, significance was determined at the 0.05 level of probability.

CHAPTER 4

RESULTS AND DISCUSSION

Dosage-Mortality Studies

The results of exposing different aged tobacco budworm to known quantities of virus are tabulated in Table 1 and plotted in Figure 8. While the dose required to produce 50 percent kill was less than 2 PIB/mm^2 diet surface for all age groups, a greater quantity of virus was generally required to produce the same level of mortality as larvae matured. This was particularly evident from the LC_{70} and LC_{90} values which were ca. 11 and 25-fold greater for 5-day-old larvae than for neonates. Only a 4-fold difference was observed between the LC_{30}'s of 1- and 5-day-old larvae. The decrease in susceptibility to virus as larvae mature may be due in part to an increased body weight which could serve to dilute a constant virus dose (Ignoffo 1966a). The somewhat flatter slope associated with dosage-mortality lines of older larvae (Table 1) may also be an indication of maturation resistance. According to Ben-Shaked and Harpaz (1966), maturation resistance may develop shortly before or during prepupation and could be due to changes in cell susceptibility from metamorphosis or presence of a viral inhibitor. The similarity in dosage-mortality response of 4- and 5-day-old larvae was not anticipated, considering the difference in mean body weight between the two groups. It may be that the

Table 1. Calculated Number of PIBs/mm^2 of Diet Required to Produce 30, 50, 70, and 90 Percent Mortality of *H. virescens* Larvae of Different Ages.

Larval Age (days)	Avg. Body Weight (mg)	Percent Mortality				
		30	50	70	90	Slope
1[a]	-	0.1	0.2	0.5	1.5	1.54
2	0.7	0.2	0.4	1.0	3.2	1.44
3	1.6	0.1	0.4	1.3	7.9	0.98
4	4.3	0.6	1.9	5.7	28.2	1.09
5	12.1	0.4	1.5	5.4	36.7	0.91

[a]neonates

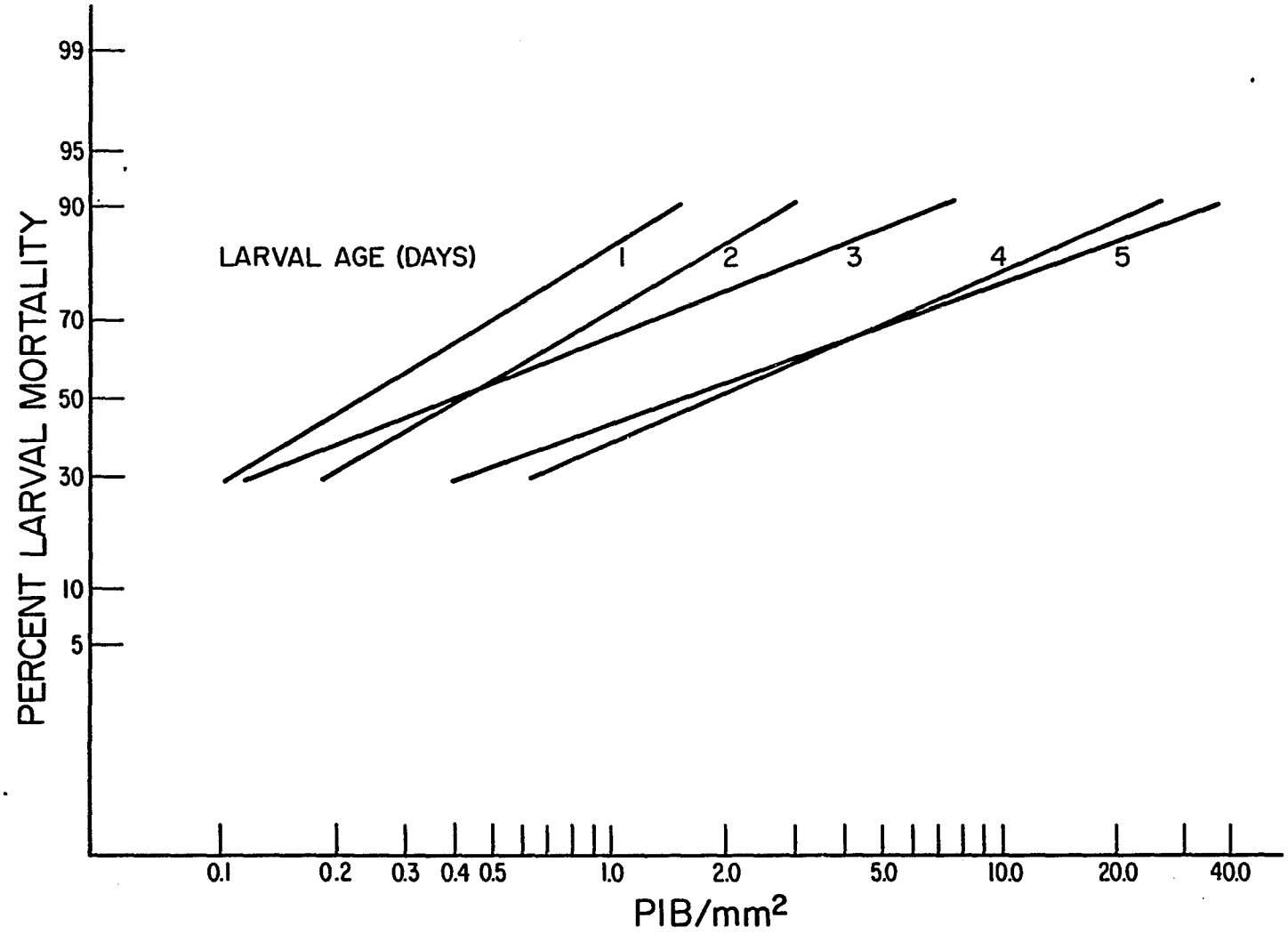

Figure 8. Dose-mortality Lines for 1- to 5-day-old H. virescens Larvae.

5-day-old larvae ingested a greater quantity of inoculum through more intensive feeding over the diet surface.

These estimates of virulence differ somewhat from an earlier study by Ignoffo (1965c) in which he reported LD_{50} values of 16-30 PIB/mm^2 for 3-day-old H. zea weighing 5 mg. While it is true that neonate H. virescens larvae were reported by Ignoffo (1966c) to be 30-50% more susceptible than H. zea, our data are in close agreement with a more recent study by Stacey, Young and Yearian (1977), also involving H. zea. It is likely that more accurate estimates of potency can be achieved with the more recent commercial virus preparations.

In addition to the decrease in overall mortality associated with larval maturation, the onset of mortality was also delayed in older larvae (Figure 9). The fact that more time is required for infections to kill older larvae is of particular importance to the management of pest populations. Unlike infections caused by the bacterium, Bacillus thuringiensis, in which larvae cease feeding shortly after ingestion of the pathogen, virus-infected individuals continue to feed and damage crops up to within 1 day of death. Although diseased larvae generally feed at a slower rate than healthy larvae (Chapman and Ignoffo 1972), they still may have a significant impact on crop yields. Willingness to accept a control agent which kills in this manner is especially unlikely in a crop such as tobacco, where foliage loss from extended periods of feeding cannot be tolerated (Mistric and Smith 1973). One method of reducing the damage caused by this prolonged feeding period is to direct treatments against young

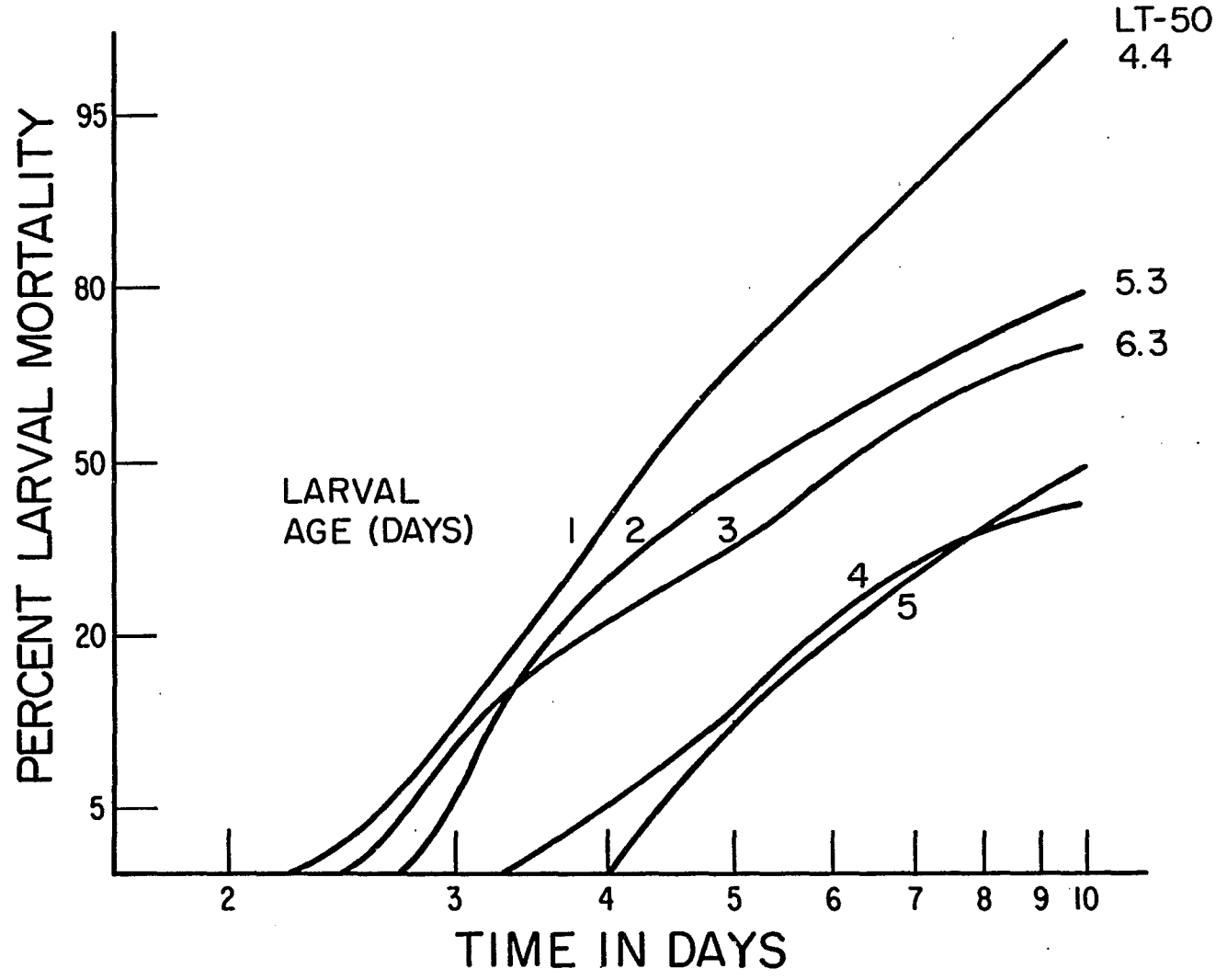

Figure 9. Time-mortality Curves for 1- to 5-day-old H. virescens Larvae Fed Diet Surface-treated with 1.65 PIB/mm^2.

larvae. For example, nearly 70% of all tobacco budworm exposed to 1.65 PIB/mm^2 as neonates died within the 1st or 2nd instar (Table 2). In contrast, ca. 77% of those larvae exposed as 5-day-old budworms succumbed to the infection during the 4th or 5th-instar. Greater boll damage of cotton associated with virus treatment of larvae beyond the 2nd-instar has been reported by Stacey et al. (1977).

Another method for shortening the incubation period of the virus is to increase the dose. The average percent mortality of neonate tobacco budworm exposed to 5 virus concentrations is presented in Figure 10. It can be seen that as the dose was increased, the duration of the incubation period was decreased. This relationship is expressed as a steady increase in LT_{50} values (time required to produce 50% mortality) at lower concentrations. The minimum incubation period for virus mortality was ca. 2 days. Significant reductions in LT_{50} below the 2.7 day value associated with a dose of 165 PIB/mm^2 of diet were not possible, even as the dose was increased to 1642 PIB/mm^2 (ca. 8200x the LC_{50} dose for neonates). While cost factors may prohibit the application of heavy virus doses in the field, attempts to increase the quantity of polyhedra ingested through the utilization of baits, selective placement of spray deposits, and increased persistence deserve considerable attention.

Table 2. Percentage of 1- to 5-day-old H. virescens Larvae Dead at Each Instar after Exposure to Diet Surfaces Treated with 1.65 PIB/mm^2.

Larval Age[a] (Days)	Larval Instar				
	I	II	III	IV	V
1	29.6	38.8	24.5	5.1	2.0
2	21.0	41.0	24.0	10.0	4.0
3	5.6	24.0	53.5	15.5	1.4
4	0.0	2.2	60.0	26.7	11.1
5	0.0	0.0	23.1	50.0	26.9

[a]Larval age at initial exposure to virus.

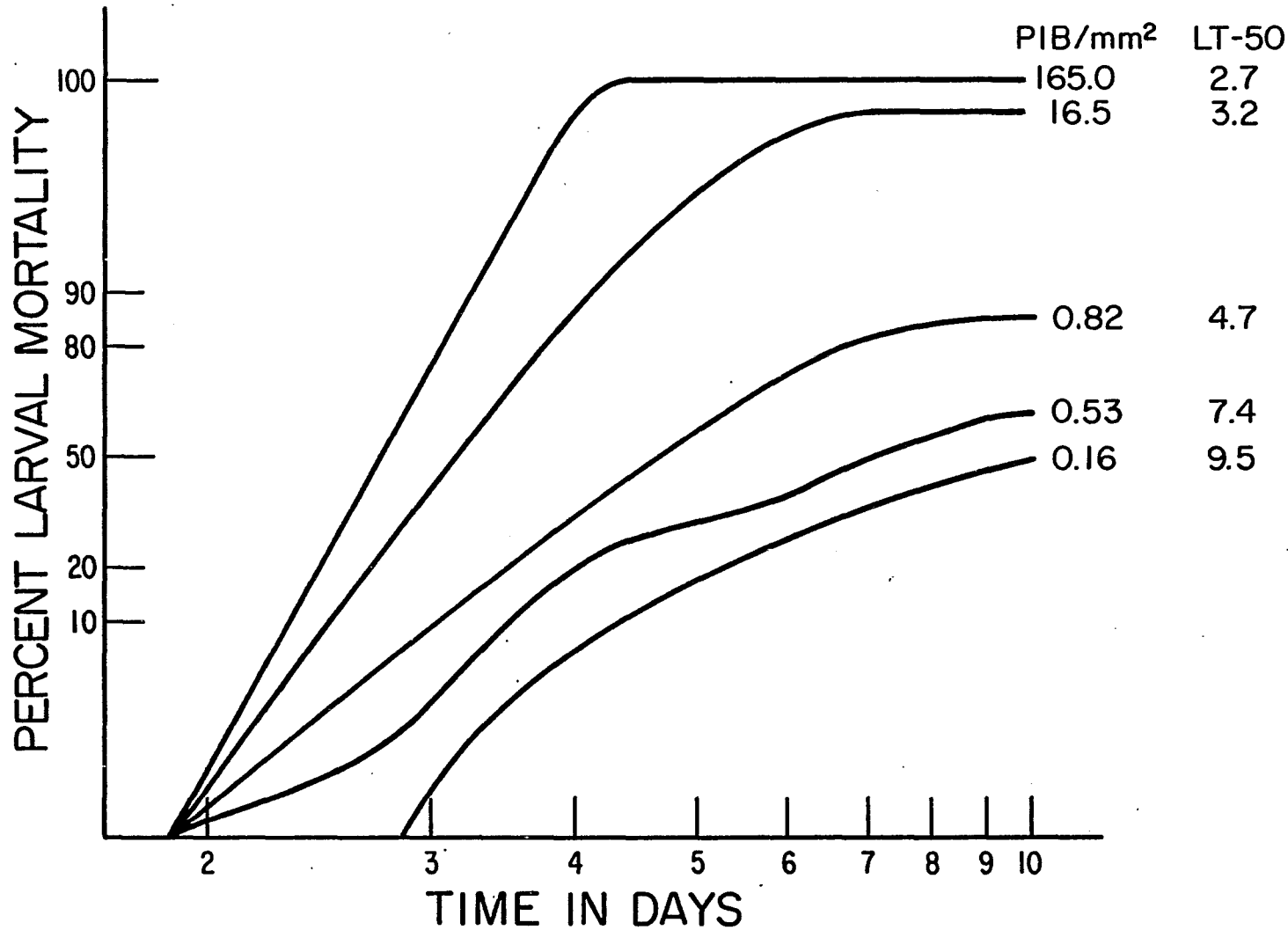

Figure 10. Time-mortality Curves for Neonate H. virescens Developing on Diet Surface-treated with Various Doses of Virus.

Virus + Feeding Stimulant Studies

Leaf Disk Bioassay

Table 3 shows that a significant increase in larval mortality resulted when virus was applied to leaf disks in combination with a cottonseed-base stimulant. This relationship was consistent at both the high and low virus dosage. The soybean flour mixture was less effective in inducing mortality and means were not significantly different from the treatment of virus in water alone. Mortality was ca. 2% when water alone was applied to leaf disks.

Young larvae were often observed feeding directly on the bait particles of the cottonseed mixture without penetrating the surface layer of the leaf disks. Frequently, no chlorophyll pigment was detected in the gut of these insects when observed under a dissecting microscope. The ability of the bait to stimulate feeding in areas of the spray deposit was further supported by the larval feeding site at time of recovery (Table 4). Whereas only ca. 31 and 37% of the larvae were recovered from the upper (treated) surface of disks receiving virus + water or water alone, ca. 60% of the larvae from virus-Coax® treated disks were found feeding on the upper disk surface. These results suggest that the incorporation of bait into virus treatments could prolong feeding by young larvae on exposed plant surfaces in the field. This would increase the probability of a larva ingesting a lethal dose of virus before moving into areas sheltered from the spray deposit, such as squares, bolls, or the undersurfaces of leaves. The ability of a bait to alter the feeding

Table 3. Percent Mortality[a] of Neonate Tobacco Budworm after 15 Hours of Feeding on Virus Alone or Virus + Feeding Stimulant-treated Leaf Disks.[b]

Treatment[c]	Virus Dosage (PIB/mm^2 of Disk)	
	1.3	13.0
Virus alone	9.6 a	43.1 b
Virus + Gustol®	15.9 a	55.7 b
Virus + Coax®	35.7 b	86.4 c

[a]Average of 4 replicates, 30-40 larvae/replicate.

[b]Means followed by the same letter do not differ at the 5% level (Newman-Keuls test).

[c]Mortality attributed to the virus in the water controls amounted to 2.2%.

Table 4. Percentage of Larvae Recovered from the Upper Surface of Leaf Disks Treated with Virus Alone or Virus + Feeding Stimulant.

Treatment	Total Number of Larvae	Percent Recovery
Water alone	245	35.3 a
Virus alone	287	31.2 a
Virus + Gustol®	264	43.9 ab
Virus + Coax®	300	59.6 b

[a]Neonate larvae allowed to feed in darkness 15 hours.

[b]Means followed by the same letter do not differ at the 5% level (Newman-Keuls tests).

habits of newly-emerged larvae was also reported by Bell and Kanavel (1977) for the pink bollworm. These authors reported that larvae feeding on bait-treated plants fed extensively on exposed surfaces of leaves and stems. Larvae on untreated plants normally feed in protected areas such as squares, bolls, or beneath the boll calyx. Besides the direct benefit of stimulating larval feeding in areas of the virus deposit, Bell and Kanavel suggested that incorporation of the bait would cause additional mortality from extended exposure to predators, parasites, and other environmental stresses. Addition of the bait could produce a similar effect with the budworm.

Greenhouse Test

A higher incidence of infection in neonates was also observed when virus was applied with baits to plants in the greenhouse (Table 5). Addition of the cottonseed-base stimulant again produced the greatest levels of mortality compared to the virus + soybean mixture or suspension of virus alone. At the 1.17×10^{11} PIB/ha rate (treatments combined) greater than 93% of all mortality occurred within 3-5 days of initial exposure. Greenhouse temperatures throughout the experiment declined from 27°C at the time of larval introduction, to 22°C at time of removal.

Duration of Exposure

Table 6 presents the mortality of larvae following different lengths of exposure to cotton terminals treated with virus alone or virus + stimulant. In these tests, mortality increased as the duration of the feeding period was lengthened, and addition of the cottonseed bait produced a significantly greater level of infection for a

Table 5. Percent Mortality[a] of Neonate Tobacco Budworm after 12 Hours of Feeding on Cotton Plants Treated with Virus or Virus + Phago-stimulant.[b]

Treatment[c]	Virus Dosage (PIB/ha)	
	2.35×10^{10}	1.17×10^{11}
Virus alone	10.7 a	39.2 b
Virus + Gustol®	18.2 ab	57.6 c
Virus + Coax®	20.9 b	63.3 c

[a]Average of 10 replicates, 14-20 larvae/replicate.

[b]Means followed by the same letter do not differ at the 5% level (Newman-Keuls test).

[c]No virus infection observed in larvae held on plants treated with water alone.

Table 6. Percent Mortality[a] of Neonate Tobacco Budworm Following Different Lengths of Exposure to Cotton Terminals Treated with Virus Alone or Virus + Feeding Stimulant.[b]

Treatment[c]	Rate (PIB/ha)	Hours of Exposure			
		3	6	18	30
Virus	2.35×10^{10}	17.8 a A	27.7 a AB	40.1 a BC	52.7 a C
Virus + Coax®		55.3 b A	56.2 b A	75.9 b B	75.6 b B
Virus	2.35×10^{11}	61.5 b A	68.9 bc AB	82.5 b BC	88.4 b C
Virus + Coax®		70.1 b A	81.2 c A	94.6 c B	99.3 c C

[a]Average of 3 replicates, 40-60 larvae/treatment/exposure period/replicate.

[b]Means followed by the same small letter (columns) and same capital letter (rows) are not significantly different at P = 0.05 (Newman-Keuls test).

[c]Control mortality less than 3% at all exposure periods.

given exposure period. This was especially apparent when larvae were allowed to feed only 3 h on terminals receiving the low virus dosage. In this case, addition of the bait resulted in a ca. 3-fold increase in mortality over the treatment of virus in water alone. Bell and Kanavel (1978) also detected ca. a 3-fold increase in mortality of neonate tobacco budworm when the NPV of the alfalfa looper, Autographa californica (Speyer), was applied in combination with a cottonseed-base adjuvant. Enhancement of activity over application of virus alone was also apparent following short feeding exposures (2 h) on greenhouse cotton plants.

The ability of the bait to enhance larval mortality following short exposures to treated leaves is especially encouraging when we consider the behavior of young larvae on cotton. Shortly after egg hatch, larvae move off of exposed leaf surfaces and into protected feeding sites such as small pinhead squares and unopened leaves. While a limited amount of feeding occurs on the lush, fully-expanded leaves in the terminal, much of this feeding is on leaf undersurfaces, where spray coverage is generally poor. As larvae continue to mature, they move down the plant attacking larger squares and bolls. If larval populations are to be effectively controlled with virus applications, ingestion of polyhedra while larvae are still within the terminal may be critical. Incorporation of a feeding stimulant may help to achieve this.

Garbanzo Bean as a Feeding Stimulant

Plant substances which act as attractants or feeding stimulants of insects (as defined by Dethier, Brown and Smith 1960) have valuable applications in host-plant resistance and insect behavior. In addition, these compounds are capable of enhancing the activity of microbial insecticides by stimulating larval feeding on pathogen-treated surfaces (Figures 11 and 12). Results of feeding trials with garbanzo bean plant extracts in which treatments were confined to the central portion of leaf disks are presented in Table 7. Differences in mean larval consumption of both the total disk area and the inner treated area were nonsignificant among treatments. There was, however, a significant increased in mortality over the virus + water alone treatment (ca. 3-fold) when virus was applied in combination with the fresh and dried bean extracts. A considerable amount of mandibular scraping was observed on the surface of disks receiving bean and cottonseed-base extracts. Feeding of this nature which did not fully penetrate the disk would not have been recorded as leaf consumption by an area meter, yet would contribute to the significant treatment differences observed in larval mortality. Both bean treatments performed as well as the commercial cottonseed-base adjuvant. Virus-water extracts of bean leaves and pods were no more effective in producing larval infection than virus applied in water alone.

The stimulatory activity of the dried bean extract was also apparent in the paired feeding comparisons (Table 8), particularly when larvae were offered a choice between dried beans and the water

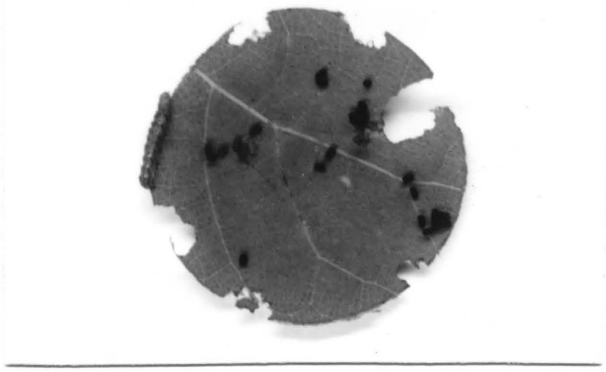

Figure 11. Larval Feeding Pattern on Leaf Disk Following Treatment of Central Portion with Virus in Water Alone.

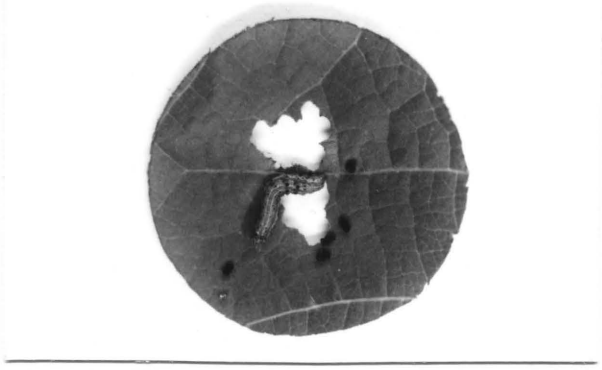

Figure 12. Larval Feeding Pattern Following Treatment of Central Portion with Virust + Feeding Stimulant.

Table 7. Average Leaf Area Consumed/Larva from Entire Cotton Disk and Central (Treated) Area Following 14 Hours of Feeding, and Resultant Mortality Due to Virus Infection.[a]

Treatment	Mean (± SE) Area of Leaf Disk Consumed (mm^2)[b]		% Larval Mortality[c]
	Total Area	Treated Area	
Water	48.4 ± 6.3	8.3 ± 1.3	1.0 a
Virus alone	36.6 ± 7.4	5.9 ± 0.8	14.7 b
Virus + garbanzo pods	31.3 ± 4.6	7.9 ± 1.4	15.2 b
Virus + garbanzo leaves	30.2 ± 7.1	9.8 ± 1.1	22.7 b
Virus + Coax®	38.3 ± 6.0	11.5 ± 3.7	45.7 c
Virus + garbanzo beans (fresh)	22.3 ± 5.2	16.5 ± 5.9	51.7 c
Virus + garbanzo beans (dried)	37.3 ± 7.3	16.2 ± 4.4	57.2 c

[a]Average of 4 replications, 25 3rd-instars/replication.

[b]Leaf disk area: total, 755.9 ± 15.2 mm^2; treated, 160.2 ± 4.3 mm^2.

[c]Means followed by the same letter are not significantly different at the 5% level (Newman-Kuels test).

Table 8. Comparative Ability of Different Plant Extracts to Stimulate Larval Feeding by Tobacco Budworm on Cotton Leaf Disks.[a]

Treatment Comparisons	Mean % of disks damaged by larval feeding for indicated no. of hours on each treatment		Mean ± SE of total disk area consumed/larva (mm^2)[b]
	3	12	
Control vs : Coax®	24.0 : 32.0	35.4 : 59.5*	22.4 ± 5.6 : 37.8 ± 7.2
Control vs : beans (dried)	6.7 : 29.3*	17.8 : 53.0*	13.5 ± 4.0 : 54.0[c] ± 8.1
Coax® vs: beans (dried)	29.3 : 40.0	69.7 : 77.8	34.6 ± 5.8 : 35.2 ± 4.9

[a]Average of 4 tests, 25 replicates/treatment/test; 1 4th-instar larva/replicate.

[b]Total disk area = 513.2 ± 12.3 mm^2.

[c]Significant difference at P = 0.05, F-test.

*Significant difference at P = 0.05, X^2-test.

control. Preference in terms of damaged disks was shown for the bean suspension within 3 h of larval release and produced ca. a 4-fold increase in total leaf area consumption per larva. Although a greater proportion of Coax®-treated disks were damaged compared to the control, differences were not significant in terms of mean consumption per larva. When extracts of bean and cottonseed meal were offered to larvae simultaneously, similar responses in feeding preference were observed.

Several materials including water extracts of corn and cottonseed oil have been reported to elicit feeding and increase the effectiveness of the nuclear polyhedrosis virus of the tobacco budworm and the corn earworm on cotton (Allen and Pate 1966, Guerra and Shaver 1969, Bell and Kanavel 1978). The commercial cottonseed-base stimulant, Coax®, contains sucrose (ca. 25%) in addition to cottonseed flour (62.3%), and crude cottonseed oil (12.3%). Sucrose is known to be a potent feeding stimulant, and was tested in combination with the virus for control of Heliothis spp. in cotton. Stacey et al. (1977) found that the combination provided significantly higher yields than application of virus alone. In view of the apparent stimulatory effect of aqueous garbanzo bean extracts on tobacco budworm feeding, a promising formulation could include oven-dried, pulverized beans in combination with invert sugars.

In addition to its possible use as a feeding stimulant, Watson (unpublished) has demonstrated the potential of the bean as an early-season trap crop for tobacco budworm. Garbanzo bean plantings are

capable of supporting large populations of Heliothis spp. in the lower Rio Grande Valley of Texas and throughout northern Mexico (H. M. Graham, personal communication). In Arizona, small acreages provide a favorable host for tobacco budworm during the spring, prior to the availability of cotton as a host (Rathman 1981). Pending further studies on adult oviposition preference toward the plant, trap-cropping with the bean may prove to be an effective means of reducing budworm populations in cotton during the early season.

Field Studies

Virus Persistence and Effect of Stimulant

The erratic performance of B. heliothis in the past (Ignoffo et al. 1965, Kinzer et al. 1976) has often been associated with a lack of persistence in the field. The principal agent responsible for this lack of persistence is sunlight in the UV spectral range of 290 to 320 nm (Bullock et al. 1970). High temperature effects and the alkaline pH of cotton dew are considered minor factors in virus degradation (Young et al. 1977), but may exert some influence throughout the arid cotton-growing regions of the Southwest. Numerous attempts have been made to enhance the persistence and efficacy of virus treatments by using microencapsulation (Ignoffo and Batzer 1971) or adjuvants (Ignoffo and Batzer 1971, Ignoffo et al. 1972). Production-processing techniques have also been modified recently, to produce more stable wettable powder formulations of B. heliothis.

Field persistence of Elcar®, determined by bioassaying treated terminals with neonate larvae, is summarized in Table 9. All treatments had excellent activity against neonates at the 0-h residue period. Only when plants were treated with the highest virus dose, however, was ca. 50% kill achieved after 4 days of field exposure. Reduction in efficacy was especially rapid when cotton was treated with virus alone at 3.1×10^{11} and 6.2×10^{12} PIB/ha. Larval mortalities at both rates were less than 40% after only 2 days of field exposure. Combination of the virus with the feeding stimulant generally resulted in greater larval mortality for a given residue period. In fact, mortalities resulting from the combination of stimulant with low doses of virus were generally comparable to those associated with higher virus rates when no stimulant was added. For example, virus applied at 3.1×10^{11} PIB/ha (1 oz/A), produced the same level of mortality as virus applied alone at 1.24×10^{12} PIB/ha (4 oz/A). This enhancement in activity was probably due to increased larval feeding in areas of the spray deposit, although the stimulant may also have limited value as a sunlight protectant. While there are no standard UV screens incorporated into the bait formulation (activated carbon, lignin sulfate, etc.), it is possible that the polyhedral inclusion bodies of the virus receive a limited amount of sunlight protection by binding to the bait particles.

When young larvae were exposed to spray residues while feeding on plants in the field (Table 10), mortalities were lower in all post-treatment samples. Furthermore, efficacy of virus treatments was

Table 9. Persistence of B. heliothis Determined by Bioassay of Treated Cotton Terminals with Neonate Tobacco Budworm.

Treatment[c]	Rate (PIB/ha)	Avg. % mortality[a,b]; days post-treatment				
		0	1	2	4	6
Virus	3.1×10^{11}	93.0 a A	55.6 a B	34.0 a C	10.5 a D	7.4 a D
Virus x Coax®		99.2 a A	91.1 bc B	56.5 bc C	24.0 ab D	9.9 a D
Virus	6.2×10^{11}	99.3 a A	84.1 b B	38.2 ab C	17.5 a D	7.3 a D
Virus x Coax®		99.1 a A	95.7 c A	64.2 cd B	40.2 bc C	11.3 a D
Virus	1.24×10^{12}	97.0 a A	90.8 bc A	59.8 cd B	48.6 c B	10.7 a C
Virus x Coax®		99.2 a A	92.2 bc A	75.4 d B	53.5 c C	18.9 a D

[a]Average of 3 replicates, 40-60 larvae/treatment/residue period/replicate.

[b]Means followed by the same small letter (columns) and same capital letter (rows) are not significantly different at P = 0.05 (Newman-Keuls test).

[c]Control mortality less than 4% at all residue periods.

Table 10. Percent Mortality[a] of Tobacco Budworm Larvae Placed on Cotton Plants at Various Days Post-treatment with Virus.[b]

Treatment[c]	Days post-treatment			
	0	1	2	4
Virus	70.6 a A	47.4 a B	27.9 a B	8.8 a C
Virus + Coax®	63.2 a A	43.0 a B	35.0 a BC	19.4 a C

[a]Average of 3 replicates, 30-40 larvae/treatment/residue period/replicate.

[b]Means followed by the same letter (columns) and same capital letter (rows) are not significantly different at P = 0.05 (Newman-Keuls test).

[c]Control mortality less than 2% at all residue periods.

generally not enhanced by addition of the feeding stimulant. The lower overall mortality in the second test can be partially explained by poorer coverage accompanying treatment of more mature plants. Greater degradation of virus would also be expected during the 48 h feeding period when larvae were maintained on plants in the field. This does not, however, account for the inability of the stimulant to enhance larval infection. It is possible that the extremes of heat and sunlight encountered in this area may have overridden the attractancy of the bait, forcing the rapid movement of young larvae off of exposed surfaces in search of protected feeding sites (i.e. pinhead squares and unfolded leaves). Fye (1971) studied the temperatures associated with various plant parts of short-staple cotton. At an air temperature of 37.8°C, he found temperatures in the upper canopy and growing terminals to be only slightly lower (ca. 1.1°C). The upper developmental temperature threshold for H. virescens larvae is ca. 35°C (Butler and Hamilton 1976). Therefore, when air temperatures are ca. 37°C and higher, significant thermal suppression of Heliothis larvae may be expected.

High light intensities in the upper canopy may also be responsible for forcing young larvae off of exposed leaf surfaces. In greenhouse studies using young plants with few fruiting structures, larvae consistently showed a preference for feeding on the undersurfaces of leaves. Fairly rapid movement of larvae off of upper leaf surfaces was noted when neonate larvae were introduced onto cotton in the field. Although all field inoculations were made ca. at dawn, it is possible

that 6- to 12-h-old neonates exhibit a natural tendency to wander before settling in an area to feed. It may be that neonates hatching from eggs in the field are initially less subject to rapid movement off of upper leaf surfaces. Shortly after eclosion, larvae in the laboratory have been observed feeding on plant surfaces immediately in the vicinity of the egg and on portions of the egg shell. If this behavior also occurs in the field, baits may have a significant impact on mortality by prolonging feeding on upper leaf surfaces.

Several plant substances have been shown to elicit feeding and enhance viral infection of Heliothis larvae in the laboratory and greenhouse (Montoya et al. 1966, Guerra and Shaver 1969). In the field, some investigators have reported greater insect mortality by the inclusion of a feeding adjuvant (McLaughlin et al. 1971, Bell and Romine 1980), while others observed no benefit from the combination (Stacey et al. 1977). In view of the results presented here, and from previously unreported work with virus-bait combinations (Watson, unpublished data), we feel that further studies are needed to properly assess the value of virus-bait combinations in Arizona cotton.

Importance of Timing
B. heliothis Applications

If the virus is to be used successfully for control of Heliothis spp. on cotton, it is generally agreed that spray applications should be directed against young larvae within the terminals (Allen et al. 1966, Falcon 1976). Shortly after egg hatch, larvae move into young, unopened leaves, pinhead squares, and other protected

areas within the terminal. As larvae continue to grow, they migrate down the plant, feeding on larger squares and eventually bolls (Kincade, Laster and Brazzel 1967). Uniform spray coverage in these areas becomes increasingly difficult, and after the first few days, little feeding occurs on the virus-covered plant leaves. Control difficulties become even greater with a material which has no contact or fuming action, and is only capable of causing death by ingestion. In light of the feeding habits of tobacco budworm on cotton, timing spray applications to coincide with the presence of eggs or young larvae in the terminal seems crucial.

Effect on Egg-stage

Tobacco budworm eggs are deposited by the female moths on new growth at the top of the plant canopy. Egg placement in this manner would suggest a good possibility for contact with the spray residue. While there is no evidence suggesting that B. heliothis directly affects the viability of eggs in the manner of a conventional ovicide (Hamm and Young 1974), it is reasonable to assume that larvae are capable of ingesting a lethal dose while chewing through the surface-contaminated egg shell (Figures 13 and 14).

Table 11 shows that larvae are capable of becoming infected by chewing out of virus-treated eggs. Furthermore, larvae hatching from eggs shortly after the spray application are more likely to ingest a lethal quantity of virus than larvae emerging from eggs laid just prior to treatment. At the highest dose, for example, mortality of

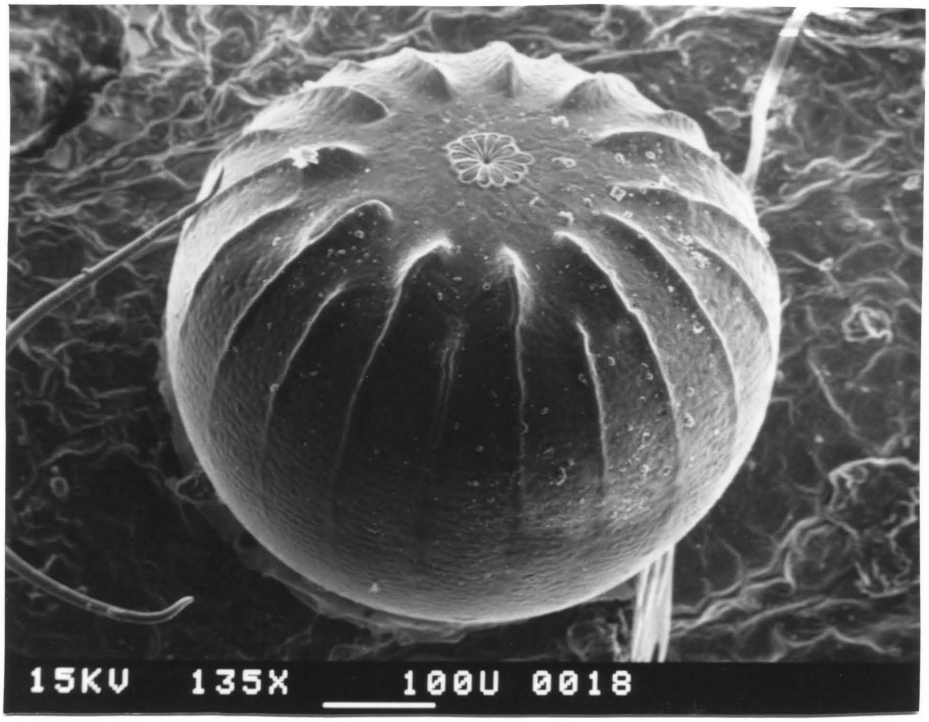

Figure 13. Scanning Electron Micrograph of Tobacco Budworm Egg (135X).

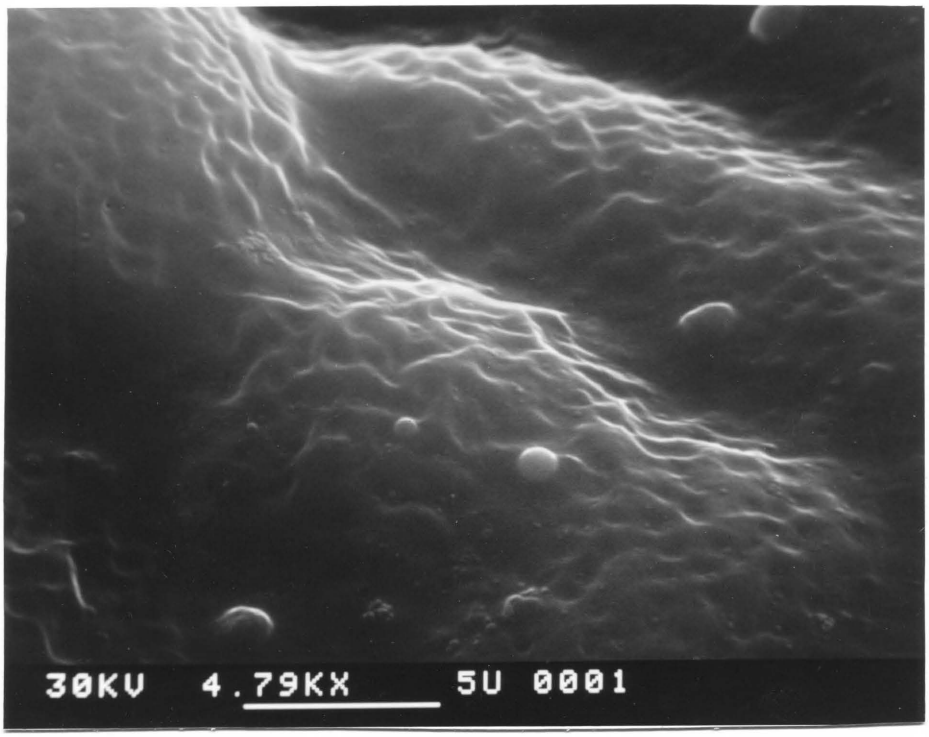

Figure 14. Polyhedral Inclusion Bodies on Surface of Treated Egg (4790X).

Table 11. Mortality of Tobacco Budworm Larvae Emerging from Virus-treated Eggs of Different Ages.[a]

Virus Rate (PIB/ha)	Avg. percent mortality[b] for egges of indicated age:		
	2-Day	1-Day	Newly-laid
Control	0.0 aA	1.6 aA	1.7 aA
3.1×10^{11}	47.7 bA	7.1 bB	5.9 bB
6.2×10^{11}	63.0 cA	20.2 cB	8.2 bC
1.24×10^{12}	79.7 dA	31.7 cB	19.4 cB

[a]Average of 3 replicates, 38-60 larvae/replicate.

[b]Means followed by the same small letter (columns) and same capital letter (rows) are not significantly different at P = 0.05 (Newman-Keuls test).

larvae declined from 79.7% when eggs were sprayed shortly before hatching (2-day sample), to 19.4% when treatment was made on newly-laid eggs. This decrease is undoubtedly a function of virus degradation, due chiefly to the effects of solar radiation. Residues on newly-laid eggs were explosed to environmental degradation ca. two days longer than those on 2-day eggs sprayed just before hatching.

The low overall mortality observed in this experiment is probably a reflection of the relatively small quantity of virus deposited on the egg shell when plants were sprayed. In previous experiments, larvae were able to ingest polyhedra while feeding over the entire surface of the treated leaf or terminal. However, the additional mortality achieved by treating H. virescens in the egg-stage (preferably just prior to hatching) encourages precisely-timed treatments based on an intensive scouting program.

Effect on Established Larval Populations

When larvae were introduced onto potted cotton plants in the greenhouse, no signficant differences in mortality occurred when virus was applied 0, 1, 2, or 4 days after the initial larval release (Table 12). Because of the tendency for developing larvae to migrate down the plant and into more protected feeding sites, a steady decline in effectiveness was expected as treatments were delayed. It is quite possible that differences were masked by the overall high mortality at each of the treatment periods. This may also have explained why

Table 12. Effect of Virus Applied Alone and in Combination with a Feeding Stimulant at Various Times after Larval Release on Greenhouse Cotton Plants.

Treatment[a,d]	Avg. percent mortality[b] associated with day of application			
	0-Day	1-Day	2-Day	4-Day
Virus	83.4	84.6	84.0	93.1
Virus + Coax®	96.2	82.2	88.5	93.6

[a]Virus applied at 9.3×10^{11} PIB/ha.

[b]Average of 10 replicates, 5 larvae/treatment/time period/replicate.

[c]All treatment comparisons nonsignificant at P = 0.05.

[d]Control mortality due to virus infection was less than 5% at all application times.

differences were nonsignificant between the virus alone and the virus + Coax® treatments.

While differences may have been more pronounced using lower rates of virus, treated plants were relatively small, supporting only 3-4 squares and a single growing terminal. On plants supporting larvae for 4 days before treatment, larvae were frequently observed feeding on leaf surfaces (ca. 26% upper, 74% lower). In field experiments, larval feeding on exposed leaves was extremely rare, except during the brief period following egg hatch. The large amount of feeding on leaves in the greenhouse experiment could have been due to (1) lower light intensities, or (2) a shortage of available fruiting structures. Greenhouse temperatures during the study ranged from 22.2° to 35.6°C. To truly ascertain the importance of timing applications of B. heliothis for control of tobacco budworm, field trials on mature cotton plants may be essential.

Table 13 shows a consistent decline in virus effectiveness as treatments were delayed following the initial release of larvae on field cotton. This was apparently due to the tendency of older larvae to avoid lethal concentrations of virus by feeding in more protected areas of the plant. For example, ca. 50% of those larvae recovered after 2 days in the field (0-day sample + 48 h of feeding) were observed feeding within the terminal bud at the top of the plant. Of the remaining larvae, ca. 44% were found on small squares in or near the terminal, and 6% were found feeding on leaves. When larvae were collected at 6 days post-inoculation (4-day sample + 48 h of

Table 13. Effect of Virus Applied Alone and in Combination with a Feeding Stimulant on Established Larval Populations in Field Cotton.

Treatment[a,d]	Avg. percent mortality[b] associated with day of application.[c]			
	0-Day	1-Day	2-Day	4-Day
Virus	87.2 aA	43.3 aB	38.1 aB	15.4 aC
Virus + Coax®	82.8 aA	60.2 aB	58.4 bB	40.0 bB

[a]Virus applied at 1.24×10^{12} PIB/ha.

[b]Average of 3 replicates, 25-40 larvae/treatment/time period/replicate.

[c]Means followed by the same small letter (columns) and same capital letter (rows) are not significantly different at P = 0.05 (Newman-Keuls test).

[d]Control mortality due to virus infection was less than 2% at all application times.

feeding), ca. 90% were recovered from larger squares and only 9% were found feeding within the terminal. Other researchers (Allen et al. 1966, House et al. 1976) have also recommended that virus treatments be directed against young larvae while still in the cotton terminal.

Throughout the entire study only ca. 2% of all larvae recovered were found feeding on exposed leaf surfaces. No differences were observed between the virus alone and virus + Coax® treatments in terms of number of larvae found feeding on exposed leaves. This conflicts with the findings of McLaughlin et al. (1971) who reported larvae in the field feeding on bait-treated leaves in areas of the spray deposit. It is possible that the microclimate occurring within a cotton canopy in Mississippi (where his study was conducted) is less extreme than that within a cotton field in Arizona.

It is true that older larvae are somewhat more resistant to infection than younger larvae. At the time of the 0-day collection (2 days after the initial release on plants), ca. 82 and 18% of larvae were in the 1st and 2nd stadia, respectively (Table 14). Of the larvae collected from the 4-day sample (6 days on plants), 67% were in the 2nd stadium and 33% were in the 3rd. The dose applied in this study, however, was capable of producing high levels of mortality in all age groups. Therefore, the decline in mortality was probably due to changes in feeding behavior rather than to maturation resistance.

Addition of Coax® did not enhance mortality over treatment with virus alone at the 0 and 1-day spray periods. Incorporation of

Table 14. Percentage of Larvae Representing Each Instar Following 2, 3, 4, and 6 Days of Feeding on Field Cotton.

Feeding Duration (days)	Larval instar		
	I	II	III
2	81.9	18.1	0.0
3	11.0	89.0	0.0
4	4.3	90.4	5.7
6	0.0	67.0	33.0

[a]Exposures include the 48 hour feeding period following virus application.

the stimulant did enhance mortality when sprays were applied 2 and 4-days post-inoculation. As larvae mature, they begin to move more actively over the plant seeking new fruiting structures. Larvae probably become more tolerant to extremes of light and temperature as they grow larger, and may be induced to take random nips from baited plant material as they move over exposed surfaces. The inability of the bait to enhance larval infection in 0 and 1-day post-inoculation sprays is consistent with the results presented in Table 10. Once again, the effect of the bait on very small larvae may have been overridden by light and/or temperature effects.

Effect of B. heliothis on Adults and Progeny

When newly-emerged adults were fed polyhedra throughout their life in a 5% sucrose solution, no significant differences were noted in the longevity or fecundity of moths compared with the control (Table 15). Differences in longevity of ♂ and ♀ moths fed virus were also nonsignificant as was egg viability compared with moths fed sugar alone. Considering the dose of virus administered (10x the recommended field rate) and the duration of exposure, it is reasonable to conclude that no visible effects result from the feeding of *B. heliothis* to tobacco budworm adults. This assumption is consistent with the results of Vail and Hall (1969) who reported no significant effect on longevity, mating or oviposition of cabbage looper adults fed NPV. Likewise, Hamm and Young (1974) reported no infection in paraffin sections of virus-fed *H. zea* adults, although polyhedra were visible in the lumen of the gut and as surface contamination near the

Table 15. Effect of Continuous Exposure to Heliothis NPV on the Longevity, Fecundity, and Fertility of Adult Tobacco Budworm.[a,b]

	Longevity (days) ♂	Longevity (days) ♀	$\bar{x}$ Eggs laid/♀	% Viable Eggs
Virus	14.0 ± 3.5	14.8 ± 1.3	769.8	77.0
Control	13.9 ± 3.6	13.4 ± 2.2	830.1	73.7

[a]Sixteen replications, 1 pair of moths/replicate.

[b]All means nonsignificant at the 5% confidence level (Students t-test).

tip of the abdomen. The absence of adverse effects due to the feeding of B. heliothis is an important consideration if PIB-fed moths are to disseminate the virus throughout a natural population.

When virus was offered to moths in feeder tubes, incidence of infection in progeny was low, regardless of dosage (Table 16). There was a decline in virus transmission on successive oviposition days following the initial exposure of moths to virus-sucrose suspensions. The low levels of infection observed in progeny of virus-fed moths were particularly disappointing considering the heavy doses of virus that were administered (5, 10, and 50x the recommended field rate). Hamm and Young (1974) were able to produce higher levels of infection in progeny of H. zea adults fed a massive dose of virus (5.9×10^8 PIB) in loop feeders. Vail and Hall (1969) also used loop feeders in their studies with the cabbage looper, but observed only ca. 2.5% infection in progeny of adults fed 1.7×10^5 to 1.7×10^7 PIB/ml. These authors concluded that the PIBs contaminate the tip of the abdomen after passing through the digestive tract and out the anus in the feces. As a result, the surface of the eggs become contaminated with PIBs and larvae become infected by chewing through the egg shell. Most larval mortality in our study arose within 3-5 days of egg hatch, suggesting that infection occurred at the time of hatch or soon thereafter.

Low levels of mortality were also observed in progeny of moths held overnight on cotton terminals sprayed with virus + sugar or virus + molasses mixtures (Table 17). These results were also

Table 16. Mean Larval Mortality of Progeny from Moths Fed Virus-Sucrose Mixtures in Feeder Tubes.[a]

Treatment (PIB/ml)	$\bar{x}$ percent mortality				
	Oviposition day				
	1	2	2	4	5
Control	0	1.2	0	0	0
5×10^7	30.0	20.0	26.2	8.5	0
1×10^8	29.0	12.4	15.2	8.9	0
5×10^8	33.7	14.7	20.2	28.3	12.4

[a]Six replications, 30-50 larvae/oviposition day/replicate.

Table 17. Mean Larval Mortality of Progeny from Moths Held Overnight on Treated Cotton Terminals in Bioassay Cups.[a]

Treatment	Rate (PIB/ml)	$\bar{x}$ percent mortality Oviposition day			
		1	2	3	4
Control		0	0	0	0
Virus + sugar	5×10^7	6.3	0.9	2.5	0.6
Virus + molasses		0.9	0	0	0
Virus + sugar	1×10^8	11.1	10.6	4.0	4.1
Virus + molasses		15.2	8.8	3.4	3.8
Virus + sugar	2×10^8	15.7	8.9	4.7	2.3
Virus + molasses		21.3	8.2	10.1	16.9

[a]Five replications, 30-50 larvae/oviposition day/replicate.

disappointing considering the powerful gustatory effect of the bait materials (Lincoln et al. 1966, Phillips and Lincoln 1968).

These studies suggest that major problems will be encountered when attempting to spread disease to progeny of moths drawn to a virus-sweet bait line in the field. Even if spray lines were widely spaced across a field, it is unlikely that the level of control would merit the cost of the required high dosages. Furthermore, the short residual activity of virus applied to newly-laid eggs in the field (Table 11) can be expected to reduce the level of transmission well below that observed in the laboratory.

CHAPTER 5

CONCLUSIONS

Throughout this study, the nuclear polyhedrosis virus, B. heliothis has proved to be a highly virulent mortality agent when directed against early-instar tobacco budworm larvae. Dosage-mortality studies using a diet surface inoculation technique have shown the LC_{50} to be less than 2 PIB/mm^2 for continuously exposed 1- to 5-day-old larvae. A greater quantity of inoculum was generally required to produce the same level of mortality as larvae matured. The onset of mortality was also delayed in older larvae, and this may be an important consideration in crop protection programs where prolonged feeding by pests cannot be tolerated. The length of this incubation period can be shortened by increasing the quantity of inoculum ingested. While cost factors currently prohibit the application of very high doses of virus, efforts should be made to maximize persistence, and encourage larval feeding on spray residues by incorporation of a feeding stimulant and selective placement of the pathogen in areas of greatest larval feeding.

In laboratory and greenhouse studies, mortality of neonates was repeatedly enhanced when virus was applied to cotton in combination with commercial feeding stimulants. The cottonseed-base adjuvant, Coax®, was more effective than either virus alone or virus mixed

with soybean flour (Gustol®). The stimulatory activity of these materials was supported by observations of larval feeding in areas of the spray deposit as well as directly on the bait particles. Under laboratory conditions, the cottonseed bait is apparently able to alter the natural preference of neonates for feeding on leaf undersurfaces in favor of more exposed, upper surfaces. The value of the bait in enhancing infection was particularly apparent when larvae were held for short durations on virus-treated terminals. In the field, where spray coverage is not uniform and, therefore, exposure to the pathogen is not continuous, application of feeding stimulants with microbial insecticides could mean the difference between inadequate and acceptable levels of crop protection.

Water extracts of fresh and dried garbanzo bean were shown to be highly attractive to 3rd and 4th-instar tobacco budworm larvae, suggesting their potential for use as a feeding stimulant. Both bean treatments performed as well as the commercial cottonseed adjuvant. Virus-water extracts of bean leaves or pods were no more effective in producing larval infection than virus applied in water alone. An oven-dried, powdered formulation of the bean, possibly combined with 10-20% invert sugars is recommended for further study.

Investigations to determine persistence of a commercial virus preparation (Elcar®), showed that a rate of 1.24×10^{12} PIB/ha (4 oz/A) is required to produce ca. 50% kill after 4 days of field exposure. Incorporation of the cottonseed feeding stimulant into

virus sprays generally resulted in greater larval mortality for a given residue period. Enhancement of activity was probably due to increased larval feeding in areas of the spray deposit. The stimulant may also have limited value as a sunlight protectant.

When young larvae were exposed to spray residues while feeding on plants in the field, the bait was surprisingly ineffective in enhancing virus infection. It may be that the extremes of light and heat encountered at the top of the plant canopy can override the attractancy of the bait, thereby forcing larvae off of exposed, virus-covered leaf surfaces. Further studies are necessary before the stimulant can be recommended as a spray additive for use with microbial insecticides in this area.

There is a tendency for tobacco budworm larvae to migrate down the plant and into more protected feeding sites as they mature. If the virus is to be used successfully, treatments must be directed against eggs and young larvae while in the cotton terminal. Larvae are capable of ingesting a lethal quantity of the pathogen while chewing out of the treated egg, and applications should coincide as closely as possible with egg hatch in order to maximize virus transmission. Following emergence of larvae from eggs, there is a consistent decline in virus effectiveness as treatments are delayed. This is largely a result of changes in location of larval feeding, although maturation resistance may also be a factor. Once, again, addition of Coax® did not enhance mortality of very young larvae (0 and 1 day old) over

treatment with virus alone. Significant increases were observed, however, when virus-bait combinations were directed against older larvae. It is possible that larger larvae are less vulnerable to extremes of light and temperature and may be induced to feed on bait-covered foliage while moving between fruiting structures.

When the virus was fed to adult tobacco budworm in a sucrose solution, no apparent differences were noted in longevity or fecundity of moths compared with the control. Viability of eggs was similarly unaffected. Transovum transmission of virus infection (occurring as larvae chew through a surface-contaminated egg shell), was inefficient despite the feeding of heavy doses of virus to moths in sugar or molasses baits. Results of these studies suggest that major difficulties will be encountered during attempts to spread infection throughout a population by drawing moths to a virus-sweet bait source in the field.

It is highly unlikely that B. heliothis will replace conventional insecticides for control of tobacco budworm on cotton. To date, attempts to use the virus as a substitute insecticide have been generally unsuccessful. A more realistic role for the pathogen currently, is as a supplement to naturally occurring biological control agents which are temporarily at low levels. This condition may be due to a natural, environmentally-related lag in predator or parasite populations, or to some disruptive action such as application of a synthetic insecticide. The unique nature of the virus makes it

particularly well-suited for incorporation into an ecologically oriented pest control program. By acting as an early season suppressant of Heliothis populations, it may serve to reduce the need for further insecticide applications later in the season.

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