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EFFICACY OF BACILLUS THURINGIENSIS BERLINER AND ITS COMPATIBILITY WITH THE PREDATOR, GEOCORIS PUNCTIPES (SAY) FOR CONTROL OF HELIOTHIS VIRESCENS (F.), ON COTTON IN ARIZONA

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EFFICACY OF *BACILLUS THURINGIENSIS* BERLINER AND ITS COMPATIBILITY WITH THE PREDATOR, *GEOCORIS PUNCTIPES* (SAY) FOR CONTROL OF *HELIOTHIS VIRESCENS* (F.), ON COTTON IN ARIZONA

by

Abdul-Sattar Arif Ali

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THE UNIVERSITY OF ARIZONA
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As members of the Final Examination Committee, we certify that we have read

the dissertation prepared by Abdul-Sattar Arif Ali

entitled Efficacy of *Bacillus thuringiensis* Berliner and its compatibility with the predator, *Geocoris punctipes* (Say), for control of *Heliothis virescens* (F.), on cotton in Arizona

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for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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Dissertation Director

April 22, 1981
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ABSTRACT

Efficacy of Bacillus thuringiensis Berliner against the first three larval instars of Heliothis virescens (F.) was studied under greenhouse and field conditions. The primary objective of these studies was to determine the susceptibility of the tobacco budworm to Dipel, a commercial preparation of Bacillus thuringiensis.

Both greenhouse and field studies showed that first-instar larvae were more susceptible to all dosage levels of Dipel tested (0.125, 0.25, 0.5, and 1.0 lb/A). The higher rates of 0.5 and 1.0 lb/A gave better control and remained effective for longer periods against the first-, second- and third-larval instars than did the lower rates of 0.125 and 0.25 lb/A. The efficacy of all Dipel rates decreased significantly with each subsequent post-application-date bioassay, and only the higher dosages of 0.5 and 1.0 lb/A remained effective at the three-day residue period in the field studies.

The addition of a feeding stimulant, Coax, at 0.25 lb/A, to all Dipel treatments resulted in a significant increase in larval mortality of all instars tested. Dipel plus Coax exhibited longer residual activity than did Dipel alone, indicating that Coax may protect the spores and toxin from degradation by the direct sunlight.

When second-instar tobacco budworm larvae were allowed to feed on Dipel-treated terminals for 6, 18 or 30 hours and then transferred to untreated diet, they showed a great capacity to recover from the effect of the toxin. However, as the Dipel rate or

exposure time was increased, the ability of larvae to recover was decreased.

Dipel did have an effect on the biological activity of treatment-surviving larvae. Larval and pupal development was delayed, pupal weight was decreased, and female fecundity was reduced. In all cases the changes were proportional with the Dipel rate and with the length of exposure. No apparent effect was shown relative to adult longevity or egg viability for those surviving Dipel treatments as larvae.

When Dipel was applied directly on tobacco budworm eggs, it had no effect on egg hatch but did significantly affect survival of newly-emerged larvae, indicating the larvae consumed a lethal dose of the toxin during eclosion.

The compatibility of Dipel in combination with Geocoris punctipes (Say) against the first-instar larvae of Heliothis virescens was also studied under greenhouse and field conditions. Results of these studies indicated the possibility of utilizing these two biological agents to suppress tobacco budworm populations in the field. A minimum density of one Geocoris nymph per 4-row feet is required, plus an effective field rate of Dipel.

When adult tobacco budworms were fed on Dipel at 32000 IU/mil, their longevity and fecundity were significanlty reduced. An adult feeding stimulant is needed to lure the adults to a Dipel source.

INTRODUCTION

The tobacco budworm, Heliothis virescens (F.), is a relatively recent pest of cotton in Arizona, with the first major outbreak occuring in 1972 (Watson 1974). Sporadic infestations were reported from 1973 to 1976 and usually occurred late in the season. In the summer of 1976, infestations of this pest were reported in Peoria, Buckeye, and Phoenix (Tollefson 1979). During the 1977 and 1978 growing season, damaging infestations of the pest spread throughout Maricopa, Pinal and Yuma Counties (Tollefson 1979). High population buildup by mid-season usually resulted in insecticide applications beginning in mid-July to early-August and continuing through September.

The ability of H. virescens to develop resistance to synthetic organic insecticides is well documented (Harris et al. 1972). Recent investigations indicated this pest developed a high level of resistance to methyl parathion (Harp and Turner 1976, Davis et al. 1977) and is showing tolerance to the synthetic pyrethroids (Crowder et al. 1979).

Insect control, as well as environmental protection, has been and continues to be a prime concern to applicators, producers and users of insecticides in the United States. Pest management is one widely advocated technique that can help provide efficient productivity while increasing environmental safety. One of the potential pest management tools is the use of microbial insecticides. These insecticides are generally nontoxic to both plants and vertebrates and are quite specific to certain insect species (Jacques 1973).

Natural enemies are important as mortality factors, but are inadequate to prevent crop damage without insecticide treatments when pest populations reach the economic injury level. The use of selective insecticides, based on need rather than on regular schedules, helps to maximize the value of these natural enemies in a pest management program.

Species of the genus *Geocoris* have been reported as abundant insect predators in many cotton growing areas of the United States. *Geocoris punctipes* (Say) is the most common species found in southern Arizona (Wene et al. 1965). These predators are known to feed on several of the common arthropod pests of cotton. Their effectiveness as natural agents has been frequently reported in the results of large-scale field tests (Whitcomb and Bell 1964). Lawrence (1974) studied the predator-prey relationship of *G. punctipes* and *H. virescens* and determined that this predator is most effective as an egg predator of tobacco budworm but is also very effective against first-instar larvae.

Past efforts to quantify the combined action of *Bacillus thuringiensis* and *Geocoris punctipes* against *H. virescens* populations in Arizona cotton fields have been limited. The potential of the combined benefit of these biological agents to suppress *H. virescens* and other Lepidopterous species is needed to evaluate the importance of this type of integration.

This study is concerned with the significance of using *B. thuringiensis* and *G. punctipes* to enhance biological control of the tobacco budworm in the field. The primary objectives of this study were: 1) to determine tobacco budworm susceptibility to *B. thuringiensis*, and 2) the usefulness of a commercial preparation of *B. thuringiensis* var. *kurstaki* (Dipel ®) plus *G. punctipes* to suppress *H. virescens* population.

LITERATURE REVIEW

Heliothis Species

The genus *Heliothis* contains a group of insect species of extreme economic importance to many of the cultivated and non-cultivated crops of the world. Species in this genus exhibit a wide host range, high fecundity and great genetic variability. In the United States, two principal species of this genus, the cotton bollworm, *Heliothis zea* (Boddie) and the tobacco budworm, *Heliothis virescens* (F.), are serious pests of cotton and many other important crops. Both species occur in most of North, Central, and South America, and they appear to be restricted to the New World (Kogan et al. 1978). Populations of these two species have been present in Arizona and California for many years with the first major budworm outbreak reported on cotton in Arizona in 1972 (Watson 1974). Prior to this outbreak, the budworm had not been documented as a serious agricultural pest, occurring primarily on flowers in yards and occasionally damaging commercial ornamental crops (Sharma et al. 1977). These investigators reported the presence of tobacco budworm in Texas, Louisiana, and Mississippi for many years, causing extreme damage to cotton production in those states.

Both the tobacco budworm and the bollworm feed on leaves and fruits of the host plant. Generally, however, the fruiting structure is preferred. One or both species can cause economic loss to soybean, cotton, tobacco, field and sweet corn, tomato, sorghum, peanut, and many species of minor crops. In addition to the direct damage done by their feeding, the larvae are reported to be responsible for the mechanical transmission of virus, fungal and bacterial rots, causing additional damage and crop losses, especially under moist conditions (Kogan et al. 1978).

A comprehensive survey of the literature concerning the description of stages, host range, geographical and seasonal distribution, the life-cycle, and the importance of natural enemies of H. virescens, was made by Lawrence (1974), Tollefson (1979), and Potter (1979). In his studies on the biology and ecology of tobacco budworm, Tollefson reported that the phenology of the cotton plant influenced larval diet and micro-climatic conditions. He added that a more mature plant would provide a more balanced diet resulting in shorter developmental time and higher survival.

Natural Enemies of Heliothis Species

Several predator and parasite species normally destroy a relatively large number of budworm eggs and larvae, but repeated applications of broad-spectrum insecticides to suppress pink bollworm populations severely reduces the level of predators and parasites and causes an elevation to major pest status of the tobacco budworm. Lincoln et al. (1967) gave an extensive list of predators and the corresponding stage of Heliothis spp. attacked. They reported that all stages of the pest were preyed upon by a complex of beneficial insects and spiders. Field studies showed that Chrysopa carnea (Stephen), Geocoris spp. and Orius tristicolor (White), were the most effective predators, especially against eggs and first-instar larvae (Lingren et al. 1968, Van den Bosch et al. 1969, Tejada 1971, Lingren and Wolfenbarger 1976). Tollefson (1979) made a more comprehensive review of predators and parasites associated with various Heliothis stages.

Pathogens, representing a variety of taxonomic categories have also been reported for Heliothis spp. (Kogan et al. 1978). The viruses are represented by a cytoplasmic polyhedrosis, a granulosis, and nuclear polyhedrosis. A fungus, Nomuraea rileyi (Farlaw) Sampson, was reported to attack last-instar larvae in soybean and cotton. Two species of microsporidea, Vairimopha necatrix (Cramer) and Nosema heliothisdis Lutz and Splendore, were also associated with Heliothis infections. One species of nematode, Heterorhabditis (chromonema), heliothisdis Khan, Brooks, and Hirshmann, has been found parasitizing pupae, and the feasibility of its use as a biological control agent is being investigated (Kogan et al. 1978).

Geocoris Species

In Arizona, the genus Geocoris is represented by the following species: G. punctipes (Say), G. pallens, Stal, G. atricolor Montandon, and G. carinatus Mcatee. G. punctipes, however, is the most commonly found (Wene et al. 1965), and can be differentiated from the other species by its slightly larger size and lighter coloration. Torre-Bueno (1946) described G. punctipes as having a fine longitudinal sulcus extending from the sulcus of the tylus on the vertex. The head is smooth, polished and not granulose. Lawrence

(1974) made a comprehensive review of *Geocoris* spp. concerning the description, geographical and seasonal distribution, life history, importance as a predator and feeding habits.

All studies on the predator-prey relationships of *Geocoris* spp. and *Heliothis* spp. agree that *Geocoris punctipes* is most effective as a predator against the egg stages and the first larval-instar (Lawrence and Watson 1979). Information concerning host suitability based on prey activity was reported by Dunbar and Bacon (1972). These investigators used eight different diets to rear *G. punctipes* nymphs. They found that the diets producing highest growth rates were those composed of insect eggs or dead larvae. Prey that were active or possessed a defensive behavior were apparently less suitable as food sources.

In addition to its role as a predator, *G. punctipes* has been cited as an insect-pathogen vector. The nymphs of this species were capable of transmitting the nuclear polyhedrosis virus of the cabbage looper, *Trichoplusia ni* (Hubner). The virus was transmitted to larvae during non-fatal feeding attempts (Staten 1970).

Natural Enemies of *Geocoris* Species

Geocoris spp. have natural enemies which serve as limiting factors on *Geocoris* populations in the field. Nymphal *G. punctipes* and *G. uliginosus* are preyed upon by all species of *Nabis*, nymphal assassin bugs, and large nymphs of *Lygus hesperus* Knight (Whitcomb and Bell 1964, Champlain and Sholdt 1967). Dunbar (1971) reported parasitization of *Geocoris* spp. by the tachanid, *Hyaloma aldrichii*

Townsend. Parasitism of *Geocoris* eggs by the scelionid, *Telenomus* sp., was reported by Van den Bosch and Hagen (1966), and Claney and Pierce (1966). Percent parasitism of eggs collected from alfalfa in Yuma, Arizona and Southern California ranged from 27 to 68%.

Bacillus thuringiensis

Description

The crystalliferous spore forming bacterium, *Bacillus thuringiensis* Berliner, has shown considerable promise as a microbial control agent of agricultural and forest insect pests. The vegetative cell of this bacterium produces a proteinaceous crystal of endotoxin, pathogenic to many lepidopterous larvae. The proteinaceous crystal or parasporal body is the principal insecticidal agent in commercial preparations of *B. thuringiensis*. It is a stomach poison and must be ingested to be effective.

According to Dulmage (1979), the bacterium, *B. thuringiensis* is a rod-shaped, spore-forming, gram-positive microbe, 1.0 to 1.2 um wide and 3 to 5 um long. Most strains are motile in the early stages of growth, moving by the use of peritrichous flagella. The crystal or the parasporal body appears within the cell at the time of sporulation. This crystal is the most distinguishing characteristic of *B. thuringiensis*.

Discovery

Dulmage (1979) outlined the historical aspect that led to the discovery, identification and insecticidal uses of *B. thuringiensis*.

He noted that this bacterium was first discovered by Ishiwata in Japan in 1901 but Ishiwata was unable to identify the microbe. It was rediscovered by Berliner in 1911 in diseased Mediterranean flourmoth, Anagasta kuehniella (Zeller) and named B. thuringiensis after the province Thuringia in Germany where it was found. In 1927, Mattes rediscovered the Berliner strain of B. thuringiensis and re-established interest in the bacterium as a tool in the control of insects. The parasporal body in sporulated cells of B. thuringiensis was observed by Berliner in 1915 (Dulmage 1979). The insecticidal activity of the parasporal crystal against Lepidopterous insects was confirmed in the mid-1950's and the term delta-endotoxin was assigned to the parasporal crystal by Heimpel in 1967 (Dulmage 1979).

Properties of B. thuringiensis

The different toxins produced by strains of B. thuringiensis were classified by Heimpel (1967). The B-exotoxin (= exotocin) was first described by McConnell and Richards (1959). This toxin is heat-stable and characterized by its ability to prevent pupae derived from treated housefly larvae from developing into normal adults. Kim and Huang (1970) described a method of isolation and characterization of B-exotoxin. This toxin is not directly associated with spores or the crystal toxin (Heimpel and Angus 1960, Kim and Huang 1970). The second toxin is the delta-endotoxin or the crystal toxin which is associated with the sporulation of the bacterium cell. This toxin is widely used in the commercial control of insects. Dulmage (1970) reported the isolation and formulation of the delta-endotoxin of a new isolate of B. thuringiensis var alesti (= vari kurstaki). He found that the insecticidal activity of this variant was much more toxic than previous formulations. Sharpe et al (1979) outlined a more recent method of separation of spores and parasporal crystals of B. thuringiensis by floatation. A reliable bioassay method for formulation and standardization of B. thuringiensis was described by Van Der Geest and Wassink (1972).

Ignoffo et al. (1974) studied the stability of B. thuringiensis and Baculovirus heliothis on soybean foliage. They found that the half-life of spores and endocrystals of B. thuringiensis was less than 24 hours, and that 65% of insecticidal activity and spore viability was lost during the first day. However, some insecticidal activity was detected at seven days post-application.

Several isolates of B. thuringiensis var. kurstaki have been described which display differing toxicities to H. virescens (Dulmage et al. 1971). Accumulated evidence suggested that B. thuringiensis kurstaki is not a homogeneous group with respect to either crystal serotype or toxicity spectrum (Krywienczyk et al. 1978).

Insectidical Importance of B. thuringiensis

Several investigators have reported on the use of B. thuringiensis formulations against lepidopterous insects attacking field crops, vegetables, forest trees and shrubs. Some of these reports include the use of B. thuringiensis formulations against the gypsy moths, Lymantria dispar (L.) (Lewis et al. 1974). The orangestriped oakworm, Anisota senatoria (Smith) (Kaya 1974), the navel orange worms, Paramgelois transitella (Walker) (Kellen et al. 1977), the Cabbage Looper, Trichoplusia ni (Hubner) (Schuster and Clark, 1977; Vail et al. 1980), the Cornborer, Ostrinia nubilalis (Hubner) (Lynch et al. 1980), the pear fruit tree leaf-roller Archips argyrospilus (Walker) (Sorensen and Falcon 1980), the forest tent caterpillar, Malacosoma disstria Hubner (Harper and Abrahamson (1979), the indian meal moth Plodia interpunctella (Hubner) (McGaughey 1979), the almond moth Ephestia cautella (Walker) (McGaughey and Dicke 1980), and the California oakworm, Phrygonidia California Packard (Milstead et al. 1980). B. thuringiensis has also been used against the tobacco budworm and other Heliothis spp. for many years (Mistric and Smith, 1974, Johnson 1978, Pfrimmer 1979, Durant 1979).

Research on control of the tobacco budworm on tobacco with B. thuringiensis sprays was first initiated by Rabb et al. (1957). They found that this agent was infective against the budworm. The initial report of satisfactory control of H. virescens on tobacco with B. thuringiensis sprays was made by Creighton et al. (1961). Spray applications of B. thuringiensis have been recommended for budworm, hornworm and cabbage looper control on tobacco in most tobacco producing areas of the U.S.A. since 1968 (Mistric and Smith 1973). These investigators reported that the use of some formulations of B. thuringiensis spray gave maximum crop protection. They also reported that lower dosages were frequently as effective as higher dosages. Johnson (1974) evaluated the effectiveness of certain formulations of new delta-endotoxin of B. thuringiensis kurstaki in controlling the tobacco budworm on flue-cured tobacco in South Carolina and reported that when applied as sprays, Dipel^R, Biotrol^R, XK^R, Thuricide^{R}Hp and Thuricide HPC^R, were usually as effective as standard chemical insecticides, although the rate varied depending on the material used. Johnson (1974) also noted that the residual toxicity of spray formulations decreased significantly after four days exposure to field conditions.

The use of microbial insecticides has tremendous advantages in the tobacco insect control program since it is safe for man to handle and would reduce some of the other problems of using toxic chemicals. Commercial preparations of B. thuringiensis registered with the U.S.D.A. are exempt from residue tolerance requirements .

B. thuringiensis has been formulated in many forms such as spray, wp, dust, baits, and granules. The formulation used depends on the type of insect pest to be controlled as good coverage is still essential to maximize effectiveness. Falcon and Sorensen (1976) described a new method of application using microdroplet applicator techniques to enhance coverage on field crops.

Lynch et al. (1980) used different formulations of B. thuringiensis kurstaki to control Ostrinia nubilalis (Hubner) and found granular formulations to be more effective than sprays against this pest. They also found the granular and foam formulations superior to the spray in persistance and efficacy.

Biological Activities of B. thuringiensis

Kim et al. (1972) studied the effect of B-exotoxin of B. thuringiensis on invitro synthesis of macromolecules in an insect cell line. They found the growth of an established line of H. Zea cells significantly retarded after treatment with B-exotoxin of B. thuringiensis. Reduction in growth was attributed to inhibition of RNA synthesis, and 50% of inhibition of RNA synthesis was attained 4-6 hours after inoculation.

Dulmage and Martinez (1973) reared H. virescens on diets containing low concentrations of the spore/delta-endotoxin complex of B. thuringiensis var. kurstaki. They found as the concentration of the complex was increased, the developmental time increased, pupal weights of the surviving larvae decreased, and the number of larvae able to complete development through the adult stage was reduced. In all cases, the changes were directly proportional to the concentration of the complex in the diet. Fertility and fecundity were reduced in adult tobacco budworms emerging from larvae reared in the presence of the toxin.

Bell (1978) studied the development and mortality of budworm fed on resistant and susceptible soybean cultivators treated with the fungus Nomuraea rileyi (Farlow, Sampson) and the bacterium B. thuringiensis. On susceptible leaves, the fungus resulted in 100% mortality after nine days and the bacterium 62% after 13 days. On resistant leaves, highest mortalities were 100% after 10 days and 98% after 13 days with the fungus and bacterium, respectively. Development time from larvae to pupae was extended when either pathogen was fed with susceptible foliage. With the bacteria, pupal weight was lower, and none of the larvae reached pupation when they were fed on a resistant foliage (Bell, 1978).

The B-exotoxin of B. thuringiensis was reported to have some adverse effects on the biological activities of the insect. Ignoffo, and Gregory (1972) demonstrated the prevention of mouthpart development after feeding B-exotoxin to Heliothis Zea, H. virescens, Trichoplusia ni, spodoptera exigua, estigmene acrea (Drury) and Pectinophora gossypiella (Saunders). They also found the development of neonatal H. Zea and mature T. ni larvae to be affected by exposure to diet surface treated with B-exotoxin, mortality in many cases resulting from the failure to molt or discard exuvia. Longevity and fecundity was reduced in moths emerged from mature larvae fed on treated diet.

Dulmage et al. (1978) studied the relationship between length of exposure to the toxin and survival of budworm larvae. They reported that budworm larvae fed on diets containing various levels of the B. thuringiensis spore/delta-endotoxin complex, and then transferred to untreated diet, showed unexpected capacity to recover from the effect of the toxin. However, as the length of exposure increased, the capacity decreased. Observation on larvae held to emergence indicated that recovery from the toxin was complete. X-ray studies using Ba^{2+} incorporated into the diet showed that, although the toxin paralyzed the mid-gut of the treated animals, many animals recovered after the toxin was removed, with food once again passing through the gut (Dulmage et al. 1978).

The histopathological effect of B. thuringiensis against insects was well documented by Kinsinger and McGaughey (1979). These investigators demonstrated that the midgut epithelium in larvae of the Indian meal moth, Plodia interpunctella (Hubner), and the almond moth, Ephestia Cautella (Walker), was damaged within one hour after they ingested spores and parasporal crystals of B. thuringiensis Kurstaki. Midgut epithelial cells progressively degenerated until the midgut was totally disrupted and the larvae died. Larval death followed extensive septicemia, and thus apparently was caused by a loss of integrity of the gut wall (Kinsinger and McGauphey 1979).

Factors Influencing B. thuringiensis Effectiveness

B. thuringiensis acts against an insect either by the toxin crystal, the spore or by both. The activity of these components, however, is reduced by several factors. Ignoffo & Garcia (1978) reported that the half life of unpasteurized and pasteurized dried films of B. thuringiensis exposed to a laboratory UV source was 30 and 40 minutes, respectively. Films of B. thuringiensis containing peroxidase and exposed to UV were significantly more stable than those not containing peroxidose. These findings supported the assumption that sunlight inactivation of entomopathogens is probably caused by peroxide or the peroxide radical produced by UV irradiation of amino acids. Other factors influencing the activity of B. thuringiensis include temperature and pH (Lacey et al. 1978; Neisess 1980).

Compatability of B. thuringiensis With Other Control Methods

The microbial insecticide has shown promise in combination with other biotic or chemical compounds against many lepidopterous pests (Lam and Webster 1972, Daoust and Roome 1974). Kennedy and

Oatman (1976) studied the effectiveness and selectivity of Dipel^R, a commercial preparation of B. thuringiensis, plus pirimicarb^R for the control of lepidopterous larvae on broccoli in California. They found that weekly applications of Dipel^R at 1.01 lb./A plus pirimicarb ^{R} at 0.25 lb./A during head formation were as effective as methomyl at 0.45 lb./A in preventing insect infestation of the heads. These investigators also found that the effectiveness of Dipel^R + Pirimicarb^R and methomyl treatments on parasitizm of T. ni and Plutella xylostella at 14 days post-treatment were minimal. At six and seven days post-treatment, Dipel + Pirimicarb interferred less with parasitism than did methomyl.

The use of chemical insecticides in combination with insect pathogens such as B. thuringiensis has received considerable attention in pest control programs (Angus and Luthy 1971, Benz 1971, Dougherty et al 1971, Ker-Sangchen et al. 1974, and Sutter et al. 1971). This integration of biological and chemical control methods is one way of minimizing the environmental hazards associated with chemical pesticides while still receiving the benefit of their use.

Ker-Sung chen et al. (1974) studied the effects of certain organophosphate and carbamate insecticides on the survival of commercial spore preparations of B. thuringiensis when applied to inert surfaces on insect larvae in combination treatment. On an inert surface, they found carbaryl and stirophos decreased viability of spores from Biotrol XK^R preparation, but propoxur ^{R} enhanced spore survival. Trichlorfon, phomet, methomyl and carbofuran had no effect on the preparation. Ker-Sung Chen et al. (1974) also reported that carbaryl, phomet and methomyl had synergistic activity when combined with Biotrol XK^{R} for use against the tobacco budworm. Strophos had an antagonistic effect and Trichlorfon and propoxur had no significant effect. Creighton and McFadden (1974) reported that a spray mixture of Dipel ^{R} and chlordimeform hydroxide provided effective protection against the cabbage looper, T. ni, and imported cabbage worm, Pieris rapae (L.), at a rate as low as 1/16 lb/A of each ingredient. These investigators noted that Dipel ^{R} and chlordimeform hydroxide had a synergistic effect.

Pieters et al. (1978) evaluated the mixture of Elcar ^{R}, chlordimeform and Dipel ^{R} + chlordimeform for control of Heliothis spp. on cotton. They noted that Elcar ^{R} and Dipel ^{R} chlordimeform treatments compared favorably with conventional chemical insecticides for control of Heliothis spp. Fuxa (1979) studied the interaction of the microsporidium, Vairimorpha necatrix, with a bacterium, virus, and fungus in H. Zea. He found the effect of the microsporidium combined with B. thuringiensis was at least additive with indications of synergism. Hamilton and Atia (1977) reported that chlordimeform and fentin hydroxide synergized B. thuringiensis against third-instar larvae of the diamond back moth, Thyracella collaris.

Dunbar and Johnson (1975) studied the effect of B. thuringiensis on the survival of a tobacco budworm parasitoid and predator in the laboratory. They noted that ingestion of B. thuringiensis by Cardiochiles nigriceps viereck reduced post-treatment longevity but treating topically did not decrease post-treatment longevity.

Post-treatment longevity of the stilt bug, Jalysus spinosus (Sag), was not decreased by exposure to tobacco leaves treated with B. thuringiensis. Wilkenson et al. (1975) reported that mortality of parasitoids and predators exposed to B. thuringiensis and NPV preparations was 4% while it was 27% for the same dosages of chemical insecticides and 31% for pyrethrin.

Several investigators have applied commercial formulations that were modified by including attractants, feeding stimulants, thickeners or other additives and have reported increased insect mortality due to the inclusion of additives (Smith et al. 1980, and Bell and Romine, 1980). These studies suggested that an increase in biological efficiency, i.e. increased mortality at a given dose, could be due to factors such as increased consumption by insects, increased activity of the insecticide in the insect gut or reduced particle losses. Bell and Romine (1980) evaluated a feeding adjuvant, NPV and B. thuringiensis (HD-1) for controlling tobacco budworm on cotton. They found that cotton treated with HD-1 + NPV + adjuvant resulted in a reduction of square and boll damage, and larvae present by 38-50% when compared to an untreated check. Bell and Romine (1980) also found that the yield of seed cotton was 32% greater in cotton treated with B. thuringiensis + adjuvant compared to cotton treated with the bacterium alone.

Bell and Kanavel (1978) described the feeding stimulant used with B. thuringiensis as a wp mixture of cotton seed flour (ca. 62.3%), sucrose (ca. 25.0%), crude cotton seed oil (12.3%), and Tween 80 (ca. .33%). This adjuvent was prepared and provided by Traders Oil

Mill Co., Fort Worth, Tx. and is now commercially available as Coax R.

Watson et al. (1979) reported that addition of Coax R (1-2 lb./A) resulted in significantly greater mortality in both early-morning and late-evening applications. The higher rate of Dipel (0.5 lb./A) with either 1.0 or 2.0 lb./A of Coax R was significantly better against first-instar tobacco budworm than the lower rate of Dipel R (0.25) at either Coax R rate. The one pound per acre rate of Coax R was generally as effective as was the two pound rate (Watson et al. 1979).

MATERIALS AND METHODS

Source of Insects and *B. thuringiensis* Preparation

Geocoris adults and nymphs were collected from alfalfa fields near Tucson and Yuma, Arizona in the summer of 1978. Identification of the *Geocoris* spp. was based on the characteristics described by Torre Bueno (1946).

Budworm eggs and larvae used in the various field and greenhouse studies were obtained from a culture that had been maintained in the laboratory for less than one year. This stock culture was developed from larvae collections from cotton fields throughout Arizona.

The source of *B. thuringiensis* evaluated was Dipel Wp. This preparation was manufactured by Abbott Laboratories, North Chicago, IL., and contained 16,000 IU of potency. Fig. (1) shows the spore and the parasporal body which are the main components of Dipel ^{R}.

Rearing of *Heliothis* spp.

The basic rearing procedures used for *H. virescens* were adapted from those described by Patana (1969), with minor modifications as described by Tollefson (1979). Adults were held in 4.4 liter wide-mouthed glass jars equipped with an inverted glass vial filled with a 5% sugar solution. Oviposition and resting sites were provided in the form of paper-towel strips. Egg sheets were surface sterilized by washing with a 5% solution of Clorox and sodium thiosulfate and then rinsed with water. After drying, egg sheets were

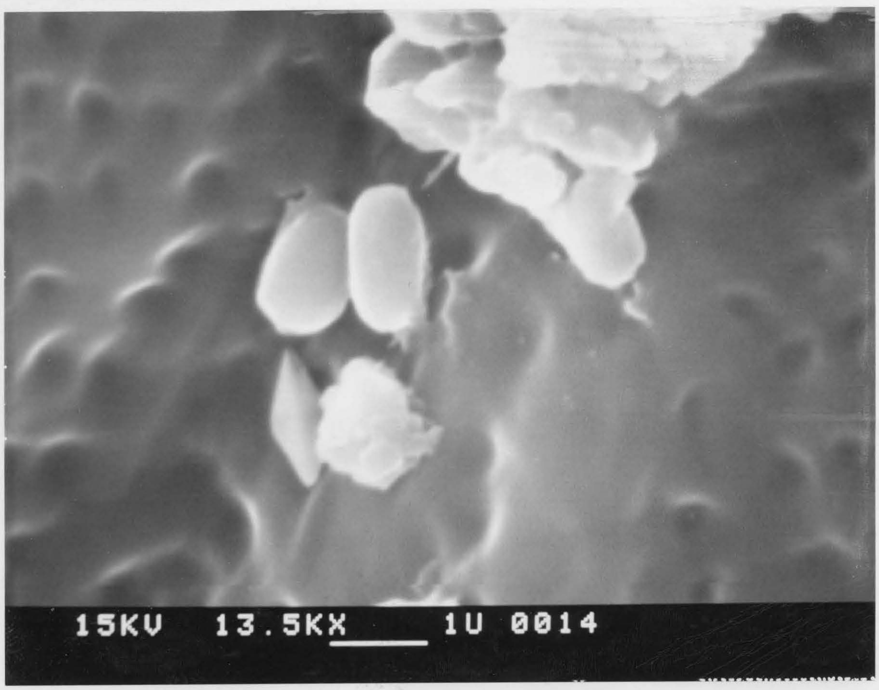

Fig. 1. The diamond-shaped parasporal body and the spores of B. thuringiensis, on H. virescens egg shell.

placed in 572 mil. cardboard cups until hatching occurred. Newly-emerged larvae were transferred from egg sheets to rearing cups at a rate of ca. 100 larvae per container. The larval-rearing containers consisted of 572 mil. cups ca. one-half full of a modified lima bean diet (Patana 1969). The diet in the cup was diced to provide added surface area for minimizing larval cannibalism. Cups were then sealed with clear plastic lids glued to the tops. Five slits were made in the sides for aeration with a thin scalpel blade. Most of the larvae reaching the second- or third-instar were used in the experimental tests, while some larvae were left in the rearing cups through pupation. All stages except the first-larval instar were held in an air-conditioned room (26 ± 2°C) under naturally-occuring day lengths. The first-larval instar was held in an environator at (26 ± 2°C) in complete darkness until they moulted to the second instar, then moved out of the environator. Pupae were transferred to 237 mil. cups capped with screen lids and left until adult emergence occurred.

Rearing of Geocoris Punctipes

A stock culture of *G. punctipes* was maintained in a converted refrigerator which served as a rearing cabinet. Temperature and relative humidity were 26 ± 2°C and 30 ± 20%, respectively. Fluorescent lights were used to provide 14 hour light/day. Rearing procedures for the adults were adopted from those used by Lawrence (1974). Adults were caged in transparent plastic storage boxes (34.6 x 27.0 x 8.7 cm.). Five 1.6 cm. diam. holes were bored into each of the longest sides of each box. Trays to hold the food and oviposition substrate were constructed from 7 cm sections of rigid plastic tubing (1.6 cm diam.) which had been cut in half longitudinally. Each concave half was attached at one end to a cork stopper, and the opposite end was inserted through the cage wall to form an interiorly projecting tray. Five of these trays were used to hold the food and the remaining five were used to hold the oviposition substrate. Food consisted of fifth-instar H. virescens larvae that had been killed by placing them in hot tap water for about 20 seconds. Two larvae were placed in each tray and were replaced by fresh ones every two days. Distilled water served as the moisture source for the predator. A glass vial served as a reservoir, stoppered with a cotton dental wick and mounted in an inverted position in the roof of the adult rearing cage.

The oviposition site in the rearing trays consisted of loosely packed cotton lint. The cotton was replaced at weekly intervals, and the egg-laden cotton from each cage was held in smaller boxes which served as rearing cages for the emerging nymphs. When nymphs reached the fourth-instar, the cotton lint was removed leaving the nymphs to complete development.

Nymphal rearing cages consisted of transparent plastic boxes (17 x 12.5 x 6.4 cm), with no holes on the sides. Food was provided in the bottom of the cage. The number of fifth-instar, killed Heliothis larvae offered to the nymphs, was varied with the age of the predator. Older nymphs required more food but no more than six larvae per time. The egg-laden cotton remained in the cage to provide a site upon which exuvia were shed during the molting process.

Most of the fourth-instar nymphs were used for experimental testing, leaving the remaining nymphs to develop to adults. Newly-emerged adults were removed from nymphal rearing containers with an aspirator and introduced into adult cages.

Greenhouse Studies

Dipel vs. *H. virescens* Larvae.

Greenhouse studies were conducted to investigate the effect of Dipel [R] and its residues on the mortality of first-, second- and third-instar tobacco budworm larvae. Weight of the pupae, longevity of the adults and fecundity of the females were recorded for ten individuals surviving through the larval instars to the adult stages. At the initiation of the test, potted cotton plants in the bloom or early boll stage, were taken outside the greenhouse and sprayed with selected Dipel [R] dosages (0.125, 0.25, 0.5 and 1.0 lb./A) using a back-pack sprayer. Soon after application, the pots were returned to the greenhouse and arranged in a row of 25 plants onto which 100 larvae, of the desired instars (first-, second- or third-), were introduced. For each treatment, there was a comparison untreated plant containing 100 larvae from the same group as those on the treated plant. This will be referred to as a check or control and served as a larval culture control.

To study the persistence of Dipel [R] on the plant, budworm larvae were introduced on treated plants at 0, 2, 4, and 6 days post-application. The Dipel [R] dosages used were 0, 0.125, 0.250, 0.500 and 1.0 lb./A., and each test was replicated five times.

Larvae were left on the plants for four days after which they were recovered from the plants and placed individually in a 1 oz. plastic cup containing synthetic diet. Accumulated mortality data were taken for seven days, including the four days on the plants. Ten pupae from the survivors were weighed, and longevity of ten males and ten females was recorded for each treatment dose. Fecundity was determined for ten egg-laying females surviving the test.

To determine the longevity and fecundity, two males and one female were held in individual cages. Adult rearing cages consisted of 947 ml wax paper cups covered with a piece of fine cheese cloth (ca. 11.4 cm diameter). The cheese cloth lid ensured proper ventilation and provided females with a favorable oviposition site. Moths were provided with a 5% sugar solution dispensed from an inverted glass vial attached to the rearing jar. Cages were inspected daily for moth mortality and egg counts. Moths were held at room temperature in the open lab at ca. 26 ± 2°C.

Field Studies

Dipel vs. *H. virescens* Larvae

Field experiments were conducted at the Campbell Ave. Farm in the summer of 1979. Plots of 100 row-feet of cotton were sprayed with the experimental dosages of Dipel[R] described previously. Each row was considered as a block and a buffer zone consisting of three untreated rows was maintained between treated blocks. Tests were conducted in one-half of the field after the cotton reached an optimum growth stage, i.e., with blooms and bolls. Plants in the

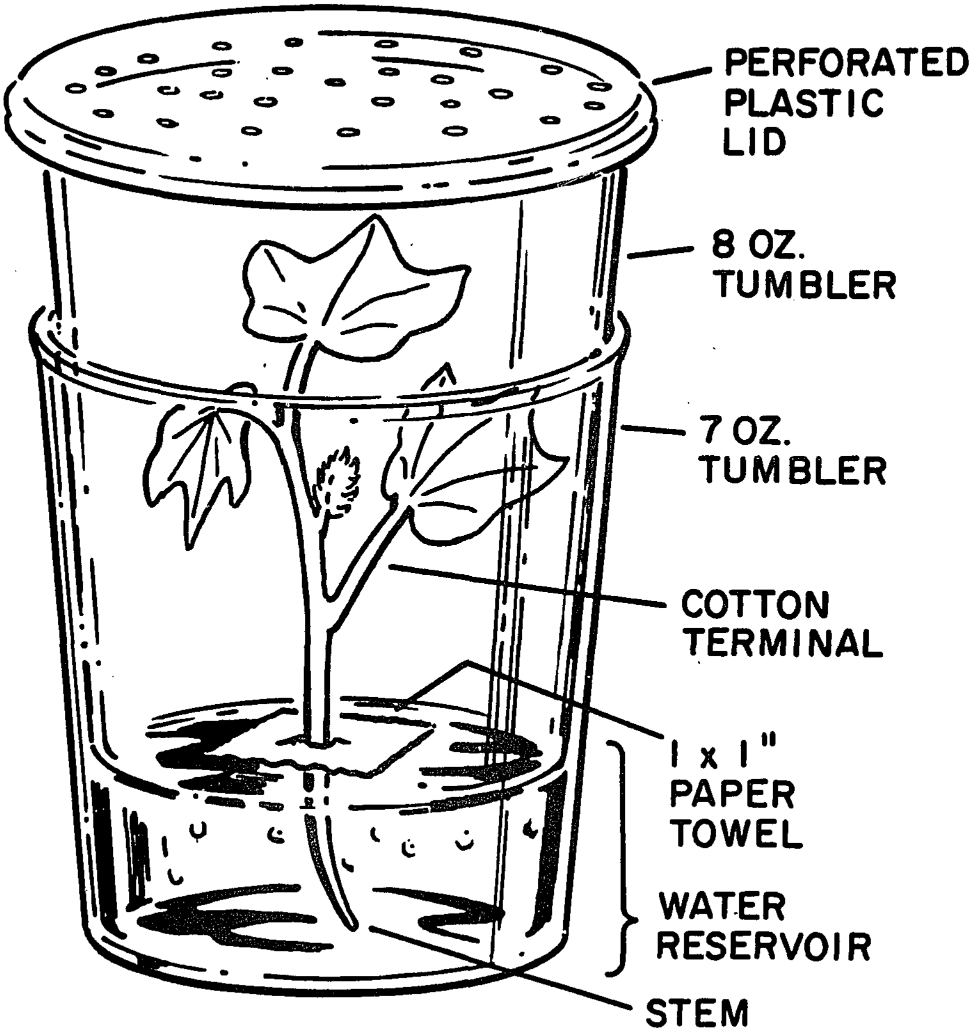

Fig. 2. Micro-cages used for bioassay tests.

other half of the field were cut back so that a similar stage of plants would be available for subsequent tests.

A back-pack sprayer was calibrated to apply the required spray material (30 gal./acre). Soon after application, random samples of cotton terminals were taken from each treatment, returned to the laboratory, and placed individually in bioassay containers in which budworm larvae were then introduced at a rate of three larvae per cage. Larvae were checked at 24-hour intervals for three days for mortality. The surviving larvae were then transferred into a media cup for further observations. Accumulated mortality was recorded for six days, three days on the terminal and three days on the media. Surviving larvae were reared on modified media described earlier for further developmental observations.

To determine the effect of the residue period on each dosage, cotton terminals were brought into the laboratory, and the same larval testing procedure repeated for four subsequent days. The tests were replicated twice. Residue period in the tests were 0, 1, 2, 3, and 4 days. These dosages and their subsequent residue periods were tested against first-, second- and third-larval instars of budworm.

Micro cages used in these tests (Fig. 2) consisted of two tumblers, 7 oz. and 8 oz. in size, with the 8 oz. tumbler inserted in the 7 oz. cup as far as possible to form a seal on the sides and a water chamber in the bottom into which the stem of the terminal was inserted. A perforated plastic lid was used to cover the 8 oz. cup containing the terminal, providing ventilation and minimizing excessive humidity inside the cage.

Dipel + Coax vs. H. *virescens* Larvae

The 1980 studies utilized the same techniques as in 1979 with the exception of a comparison between a combination of the feeding stimulant, Coax ^{R}, and Dipel ^{R}, and Dipel ^{R} alone. The primary objective of the test was to see the effectiveness of Coax ^{R} as an adjuvent with Dipel ^{R} when used at a lower than recommended field rate. Coax ^{R} was used at a rate of 0.25 lb./acre, lower than the recommended field rate of 1-2 lb./acre. The 100-row-feet plots were sprayed with each of ten combinations of Dipel ^{R} and Coax ^{R}. Treatments were made on cotton in the field but efficacy was determined by removing terminals and returning them to the laboratory for bioassay with tobacco budworm larvae in micro-cages as described earlier. Mortality counts were made at 24-hour intervals following placement of larvae in the cages. However, results are presented for mortality at 144 hours. The same procedure was followed in the untreated check. Sample size for each treatment consisted of 51 larvae. However, results were based only on the sample size accounted for at the end of the six days observation period. Missing larvae were not included in the final sample size. All tests on dosages and residue periods were replicated three times.

Larvae surviving the treatments were reared on media for further mortality assessment and for observations on larval and pupal development. The number of days from initiaton of the tests to pupation and to adult emergence was recorded for surviving individuals. Weight of pupae, longevity of males and females and females' fecundity were determined as described in the greenhouse study.

To determine the effect of Dipel on fertility of eggs obtained from moths that developed from Dipel-fed larvae, 100 eggs were taken from the moths during the first three days of oviposition. Eggs were incubated in an environator until eclosion and number of larvae were recorded. This test was replicated three times. Twenty-five newly-hatched larvae from each treatment were transferred into media cups to observe the subsequent effect of previous Dipel treatment on F_1 first-instar larvae. Mortality readings were taken for six subsequent days. Four groups of 25 larvae were used for each Dipel dose and Dipel residue.

Larval Exposure Time vs. Recovery.

Dulmage et al. (1978) reported that tobacco larvae recovered from HD-1 toxin when removed from toxin-containing medium and placed in non-toxin-containing medium in the laboratory. These investigators suggested the existance of recovery under field conditions.

Based on this information, it was desired to determine the kind of interaction that exists between *B. thuringiensis* and *H. virescens* under Arizona conditions. Field plots were sprayed with two levels of Dipel (0.5 and 1.0 lb./A) and a water control. Plant terminals were immediately brought into the laboratory and placed in micro-cages with newly-emerged second-instar larvae (three days old). After 6, 18, and 30 hours of feeding, respectively, 50 larvae were transferred to media cups. The number of individuals surviving to third-instar, to pupae, and to adults, respectively, were recorded. There were three replications of each treatment. The number of days from beginning of feeding to pupation and then to adult emergence was also recorded. Ten pupae were weighed and observations were made on the longevity of ten males and ten females and on the fecundity of ten females. Egg fertility and viability of F_1 first-instar larvae were determined as described earlier in the long-term study.

Dipel vs. *H. virescens* Egg

This study was designed to determine the efficacy of *B. thuringiensis* when sprayed on budworm eggs in the field. Paper towel sheets, on which the eggs were laid, were taped on the plant and then sprayed by one of the following Dipel dosages: 0.0, 0.125, 0.250, 0.50 and 1.0 lb./A. Eggs used in these tests were newly-laid, one-day-old, and two-days-old. After spraying, the egg sheets were left until dry, then returned to the laboratory to be incubated at 25 ± 1°C in 237 ml cardboard cups covered with plastic lids. A hundred eggs of each treatment were observed to determine the percent egg hatch at each Dipel level tested. Also, 30 newly-hatched larvae at each dose tested were transferred individually into 1.0 oz. plastic cups containing modified media and survival recorded for six subsequent days. The purpose of this test was to determine if newly-hatched larvae could obtain lethal quantities of the toxin from the egg sheet and the length of time Dipel remained effective on the egg sheet. These tests were replicated six times.

Dipel Plus Geocoris punctipes vs. 1st-instar H. virescens Larvae

Greenhouse Studies

The objective of this study was to determine the combined action of fourth-instar G. punctipes and various dosages of Dipel. For this experiment, potted cotton was grown in the greenhouse and testing began when the plants reached the bloom or early-boll stage. Cotton plants were arranged in a fifty-foot long row outside the greenhouse and sprayed with the desired rate of Dipel using a back-pack sprayer. Cotton plants were then returned to the greenhouse and arranged inside experimental cages. These cages were constructed of metal or wood frames, 71.5 x 57.5 x 70 cm., covered with lucite screen (32 x 32 mesh).

The number of plants introduced into each cage depended on the density of Geocoris to plant population desired, i.e., individual sums of one, two, three or four plants were used for determining the effectiveness of G. punctipes nymphs at the different densities. Ten rows per test were maintained using five Heliothis larvae per row totaling fifty larvae per test. Each test was replicated three times. Geocoris nymphs were introduced at a rate of one nymph per row.

To determine the effectiveness of G. punctipes plus B. thuringiensis residue, fifty larvae were introduced as described earlier, on Dipel^{R}-treated cotton at two, four and six days post-application and a predator nymph was then introduced.

First-instar Heliothis larvae were transferred to plants by means of a number-one camel's hair brush. Larvae were randomly distributed over the terminals and maintained with the predator for four days, after which a comprehensive search was made to recover the surviving larvae. Mortality due to the combined activity of Dipel and/or *Geocoris* predation was evaluated by subtracting the number that survived from the total number of larvae initially placed on the plants (50 larvae). Control treatments were conducted in the same manner excluding the Dipel treatment and the predator. Larval mortality was also observed for Dipel alone, *Geocoris* alone, and for the control.

All experimental tests were conducted in the greenhouse at temperatures of 25.1 ± 1.0°C and humidity of 45 ± 20% as recorded by a hygrothermograph. Fluorescent lights provided extended photoperiods in seasons when days were short.

Field Studies

Field studies were conducted at The University of Arizona Campbell Ave. Farm, Tucson, Arizona. The study area (ca. one acre) was planted with Delta Pine - 61 cotton on drill rows with 40 inch spacing. Field cages were arranged north to south along the marginal rows of either side of the field, leaving two outer rows as a buffer zone. Cotton plants inside the cages were thinned to one plant per row-foot.

Due to the presence of many arthropods in the field, it was impractical in an open field to conduct an experimental test designed to evaluate the predator and the biological insecticide. Each cage was considered a plot.

Field cages were constructed of 3.6 x 1.8 x 1.8 m metal frame fitted with saran screen (16 mesh) cover. The door of the cage was oriented to the north and was equipped with a zipper for opening and closing. The bottom of the cage was sealed with soil. A D-Vac ^{R} machine was used to rid the plants of any associated arthropods before and after each treatment. However, some small ants and spiders were still inside the cages but in very low densities.

The caged plants were sprayed with various dosages of Dipel (0.0, 0.125, 0.250, 0.500 and 1.0 lb./A) using the back-pack sprayer described earlier. Fifty newly-emerged, first-instar H. virescens larvae were transferred with a number-one camel's hair brush onto the plants shortly after treatment.

The larvae were distributed randomly on the terminals of the plants. At the same time, G. punctipes nymphs were introduced at the rates of one per four row-foot and one per two row-foot. This set of treatments was repeated at two days post-application. Readings were taken four days after the larvae and nymphs were introduced into the cages. A treatment with Dipel alone, Geocoris alone and neither was also conducted at the same time in each test. The number of budworm larvae recovered after four days from the treated cages was compared with that recovered from the untreated cage.

The sprayer used in all tests was powered by CO_2. The CO_2 cylinder was equipped with gauges and a valve to provide a pressure of 40 lb./in.^{2} inside the sprayer. Constant pressure was maintained to ensure uniform application. The boom of the sprayer was equipped with three Tee-Jet, 6x, nozzles, one directed over the row and the others directed towards the sides of the row to ensure complete row coverage. Nozzle output and rate of travel were coordinated to deliver the desired 30 gal./A spray solution.

Statistical Analysis of Data

The mean and the standard deviation of the mean were calculated for all greenhouse and field studies. Analysis of variance (ANOVA) was made to determine group means. When dealing with mortality or survival data, the ANOVA was made on the arcsin transformation of the means. Computer programing was designed to do the ANOVA. The least significant difference (L.S.D., Steel Torrie 1960) was then computed, separating means that were significantly different at the 0.05 level of probability. Due to the variability in the numbers involved in each mean of the larval and pupal development, it was suggested by Dr.R. Kuehl (Center of Quantitative Studies, University of Arizona) to use the Average L.S.D. to separate those means.

RESULTS AND DISCUSSION

Greenhouse Studies

Dipel vs. *H. virescens* Larvae

Efficacy of any insecticide is dependent upon a number of factors. Among these are: application rate, degredation rate, and age distribution of the target pest. This study was initiated to determine the effects of *Bacillus thuringiensis* on the first three larval instars of the tobacco budworm. Four rates and four residual periods were investigated. Results of these studies are presented in tables 1, 2 and 3.

In general, the first two larval instars of tobacco budworm were quite similar in susceptibility to all dosage levels of *B. thuringiensis*, although at the longer residue periods, the first-instar was slightly more susceptible. The third-instar was least susceptible of all. With each increase in dosage, higher mortality was obtained in all instars. However, little difference was observed between the 0.5 and 1.0 lb./A rates (Appendix A).

The most effective residue period was that where larvae were placed on foliage immediately after it was sprayed (0-day residue). Effectiveness declined with each successive residue period (2-, 4- and 6-days). However, the two higher dosages rates (0.5 and 1.0 lb./A) had greater residual activity and resulted in higher mortality for the longer residue periods.

Table 1. Mortality of first-instar tobacco budworm fed on Dipel-treated greenhouse cotton at various intervals after application.

Dipel Rate (lb/A)	Mean percent mortality for indicated residue period 1/			
	0-day	2-days	4-days	6-days
0	13.8 ± 2.8 2/	14.2 ± 1.8	14.4 ± 1.8	13.6 ± 0.5
0.125	81.4 ± 3.0	64.2 ± 9.4	47.2 ± 13.8	37.0 ± 9.7
0.250	84.2 ± 3.2	69.8 ± 4.8	49.6 ± 2.7	33.8 ± 5.1
0.500	90.6 ± 1.3	78.0 ± 6.2	57.0 ± 11.2	44.2 ± 9.3
1.000	95.2 ± 2.0	87.8 ± 2.7	67.0 ± 5.6	45.6 ± 8.5

1/ accumulated mortality for 7 days from inoculation

2/ $\bar{x} \pm$ standard deviation of the mean

Table 2. Mortality of second-instar tobacco budworm fed on Dipel-treated greenhouse cotton at various intervals after application.

Dipel Rate	Mean percent mortality for indicated residue period 1/			
(lb/A)	0-day	2-day	4-day	6-day
0	16.6 ± 1.8 2/	17.8 ± 1.5	17.2 ± 1.6	17.6 ± 1.1
0.125	80.4 ± 3.2	61.4 ± 14.3	44.2 ± 17.6	27.8 ± 11.5
0.250	85.5 ± 4.2	72.6 ± 6.2	49.2 ± 6.8	27.8 ± 4.6
0.500	91.4 ± 1.7	80.2 ± 7.0	59.8 ± 7.2	40.2 ± 12.7
1.000	92.2 ± 1.5	81.4 ± 5.3	60.2 ± 1.3	40.8 ± 8.6

1/ accumulated mortality for 7 days from inoculation

2/ $\bar{x}$ ± standard deviation of the mean

Table 3. Mortality of third-instar tobacco budworm fed on Dipel-treated greenhouse cotton at various intervals after application.

Dipel Rate	Mean percent mortality for indicated residue period [1/]			
(lb/A)	0-day	2-day	4-day	6-day
0	19.6 ± 2.9[2/]	21.0 ± 2.5	23.4 ± 2.2	21.4 ± 6.0
0.125	68.2 ± 2.2	55.8 ± 4.0	37.8 ± 4.1	25.0 ± 2.0
0.250	83.4 ± 1.5	67.4 ± 1.8	49.0 ± 4.7	32.0 ± 7.1
0.500	86.2 ± 1.8	72.0 ± 3.2	51.2 ± 4.3	35.4 ± 6.3
1.000	91.2 ± 1.6	74.4 ± 3.6	54.4 ± 5.0	36.8 ± 4.0

1/ accumulated mortality for 7 days from inoculation

2/ $\bar{x}$ ± standard deviation of the mean

Dipel-Fed Larvae vs. Pupal Weight

Larvae surviving the B. thuringiensis (= Dipel) treatments were held for evaluating the effects on subsequent stages of development. Tables 4, 5 and 6 present the results of pupae obtained from surviving first-, second- and third-instars, respectively, of Dipel-fed larvae. Effects were measured by pupal weight.

Table 4 shows that with first instar-fed larvae the only effect on subsequent pupae resulted from the two higher dosages and only at the 0-day post-application residue period. These pupae were significantly smaller (0.05 level of probability). A similar comparison of pupae from second instar, Dipel-fed larvae is shown in Table 5. At the 0-day residue period, pupal weights from larvae surviving the 0.25, 0.50 and 1.0 lb./A rates of Dipel were significantly less than those in the 0.125 lb./A and untreated check treatments. Only the two higher rates showed significantly smaller pupae at the 2-day residue period. At the 4- and 6-day residue periods there was a trend for reduced pupal weight in the successively higher dosage levels although they were not significantly different.

Table 6 shows the results of pupal weights from surviving third-instar Dipel-fed larvae. In this group less difference in pupae weights was noted between treated and untreated larvae although the trend toward smaller pupae with each successive increase in dosage still occurred.

Table 4. Tobacco budworm pupal weights resulting from exposure of first-instar larvae to various rates and residue periods of Dipel-treated greenhouse cotton.

Dipel Rate	Pupal weight (mg) for indicated residue period			
(lb/A)	0-day	2-day	4-day	6-day
0	323 ± 34[1/] [2/]	323 ± 19	317 ± 28	323 ± 34
0.125	307 ± 34	315 ± 33	320 ± 30	321 ± 26
0.250	302 ± 20	313 ± 31	315 ± 29	319 ± 32
0.500	285 ± 22	309 ± 25	313 ± 27	321 ± 22
1.000	282 ± 24	310 ± 24	309 ± 20	319 ± 27

1/ L.S.D. = 22

2/ $\bar{x}$ ± standard deviation of the mean

Table 5. Tobacco budworm pupal weights resulting from exposure of second-instar larvae to various rates and residue periods of Dipel-treated greenhouse cotton.

Dipel Rate	Pupal weight (mg) for indicated residue period			
(lb/A)	0-day	2-day	4-day	6-day
0	315 ± 15[1/ 2/]	322 ± 23	321 ± 19	332 ± 25
0.125	321 ± 18	327 ± 30	325 ± 31	326 ± 32
0.250	287 ± 32	304 ± 34	317 ± 28	312 ± 25
0.500	280 ± 23	292 ± 23	305 ± 16	310 ± 19
1.000	275 ± 16	294 ± 20	314 ± 21	311 ± 10

1/ L.S.D. = 22

2/ $\bar{x} \pm$ standard deviation of the mean

Table 6. Tobacco budworm pupal weights resulting from exposure of third-instar larvae to various rates and residue periods of Dipel-treated greenhouse cotton.

Dipel Rate (lb/A)	Pupal weight (mg) for indicated residue period			
	0-day	2-day	4-day	6-day
0	323 ± 19 [1/ 2/]	324 ± 24	327 ± 29	327 ± 24
0.125	309 ± 25	317 ± 18	319 ± 25	318 ± 25
0.250	302 ± 23	315 ± 17	324 ± 19	326 ± 18
0.500	297 ± 19	301 ± 26	316 ± 27	321 ± 28
1.000	285 ± 20	295 ± 23	316 ± 26	317 ± 24

1/ L.S.D. = 22

2/ $\bar{x} \pm$ standard deviation of the mean

Dipel-Fed Larvae vs. Adult Longevity

Both males and females originating from larvae surviving Dipel treatments were observed for adult longevity. Results of these observations are presented in Tables 7 through 12. No significant difference in adult longevity was noted among any of the treatments nor between Dipel treatments and the untreated check. There was a trend toward reduced longevity, however, from the Dipel treatment at the 0- and 2-day post-application residue periods where first- and second-instar larvae were used in the tests.

Dipel-Fed Larvae vs. Female Fecundity

Larvae surviving the various Dipel treatments were held for adult emergence to ascertain treatment effects on fecundity. Tables 13, 14 and 15 present the results on fecundity from females originating from Dipel-fed first-, second- and third-instar larvae, respectively.

With the exception of the lowest Dipel-treatment rate (0.125 lb./A), significantly fewer eggs were laid by females originating from first-instar larvae exposed to the higher rates at the 0-day residue period (Table 13); at the two-days residue period fewer eggs were laid by females from all Dipel treatments although not significantly different from the check. At the extended residue periods of 4- and 6-days no differences among treatments and the check were noted.

Table 14 shows fecundity of females from the second-instar larvae test group. Only the two higher dosage treatments (0.5 and

and 1.0 lb./A resulted in significantly fewer eggs per female and that, only at the 0-day post-application residue period. Females from the longer residue periods produced fewer eggs but they were not significantly different from the untreated check (Table 14).

The number of eggs laid by females that had developed from Dipel-exposed third-instar larvae, showed a significant reduction at the 0-day post-application residue period (Table 15). No treatment effects were observed among the other residue periods (Table 15).

These results indicate that the effects of *B. thuringiensis* on adult tobacco budworm which originated from Dipel-exposed larvae are functions of dosage level and residue period to which the pathogen is exposed to environmental conditions, i.e., the higher the dosage and the shorter the residue period, the greater the effect. There is also an indication that the younger the larvae when exposed to Dipel, the greater the effect on the adult.

Field Studies

Dipel vs. *H. Virescens* Larvae

Field studies similar to the greenhouse studies involved four rates of Dipel and the first three larval instars of the tobacco budworm. Field persistance of the pathogen was determined using the bioassay method for residue periods of 0-, 1-, 2-, 3-, and 4-days post application.

Results of these studies are presented in Tables 16, 17 and 18 for the first-, second- and third-instars, respectively, of the tobacco budworm. Mortality of first-instar larvae increased

Table 7. Longevity of tobacco budworm males which developed from first-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate (lb/A)	Longevity (days) for indicated residue period			
	0-day	2-day	4-day	6-day
0	18.0 ± 4.8 1/	18.3 ± 4.1	16.7 ± 5.4	17.8 ± 5.8
0.125	16.1 ± 6.0	16.9 ± 4.0	16.1 ± 5.7	16.4 ± 5.0
0.250	16.6 ± 6.0	16.7 ± 4.2	18.0 ± 3.9	17.7 ± 3.7
0.500	16.7 ± 3.9	14.8 ± 4.9	17.5 ± 5.1	17.5 ± 5.2
1.000	15.3 ± 5.0	17.1 ± 3.4	18.8 ± 3.9	17.5 ± 4.3

1/ $\bar{x}$ ± standard deviation of the mean

Table 8. Longevity of tobacco budworm females which developed from first-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate	Longevity (days) for indicated residue period			
(lb/A)	0-day	2-day	4-day	6-day
0	18.9 ± 2.3 [1/]	18.3 ± 5.0	17.1 ± 6.1	17.6 ± 4.3
0.125	17.5 ± 5.5	17.4 ± 4.4	16.3 ± 5.1	16.0 ± 5.0
0.250	17.5 ± 4.7	16.7 ± 4.6	16.3 ± 5.5	15.8 ± 5.9
0.500	16.2 ± 5.8	15.6 ± 4.9	18.3 ± 4.7	16.0 ± 5.3
1.000	16.5 ± 3.3	17.4 ± 4.1	16.9 ± 4.7	18.1 ± 4.3

1/ $\bar{x}$ ± standard deviation of the mean

Table 9. Longevity of tobacco budworm males which developed from second-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate (lb/A)	Longevity (days) at indicated residue period			
	0-day	2-day	4-day	6-day
0	18.5 ± 5.3 1/	16.4 ± 4.0	16.3 ± 5.5	16.6 ± 5.7
0.125	17.1 ± 4.7	17.5 ± 6.0	17.2 ± 4.6	17.9 ± 5.5
0.250	16.4 ± 4.2	16.7 ± 4.6	17.3 ± 4.4	18.3 ± 4.3
0.500	16.1 ± 3.6	14.6 ± 5.2	18.0 ± 4.4	17.9 ± 4.3
1.000	16.9 ± 5.2	17.5 ± 4.7	17.3 ± 4.5	19.7 ± 3.7

1/ $\bar{x}$ ± standard deviation of the mean

Table 10. Longevity of tobacco budworm females which developed from second-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate (lb/A)	Longevity (days) for indicated residue period			
	0-day	2-day	4-day	6-day
0	17.7 ± 4.4[1/]	17.8 ± 3.6	16.3 ± 4.8	17.4 ± 4.3
0.125	15.4 ± 4.3	17.2 ± 4.6	17.4 ± 5.1	17.2 ± 4.8
0.250	15.6 ± 4.8	15.7 ± 4.4	17.2 ± 5.7	16.2 ± 4.6
0.500	16.6 ± 3.1	14.4 ± 3.8	16.2 ± 7.0	17.3 ± 5.1
1.000	16.0 ± 3.8	15.7 ± 5.5	16.7 ± 5.6	18.7 ± 4.4

1/ $\bar{x}$ ± standard deviation of the mean

Table 11. Longevity of tobacco budworm males which developed from third-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate (lb/A)	Longevity (days) for indicated residue period			
	0-day	2-day	4-day	6-day
0	18.5 ± 3.4[1/]	18.6 ± 5.8	17.3 ± 5.4	16.7 ± 6.2
0.125	19.1 ± 4.1	16.4 ± 4.8	16.8 ± 5.5	17.8 ± 5.6
0.250	18.6 ± 4.5	19.2 ± 4.5	17.1 ± 4.6	18.8 ± 4.5
0.500	16.7 ± 4.7	18.9 ± 3.9	17.6 ± 5.3	18.3 ± 3.8
1.000	18.6 ± 4.6	18.2 ± 4.9	17.2 ± 4.4	16.7 ± 3.5

1/ $\bar{x}$ ± standard deviation of the mean

Table 12. Longevity of tobacco budworm females which developed from third-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate	Longevity (days) for indicated residue period			
(lb/A)	0-day	2-day	4-day	6-day
0	18.5 ± 4.1 [1/]	17.5 ± 4.3	17.2 ± 4.1	17.8 ± 4.3
0.125	18.7 ± 3.6	19.0 ± 4.4	17.7 ± 5.2	15.6 ± 5.3
0.250	19.0 ± 3.2	19.2 ± 3.9	15.5 ± 5.2	17.3 ± 5.3
0.500	16.7 ± 5.1	18.7 ± 4.3	17.3 ± 5.8	18.2 ± 3.6
1.000	18.6 ± 4.9	18.3 ± 4.4	17.6 ± 5.7	18.6 ± 3.1

1/ $\bar{x}$ ± standard deviation of the mean

Table 13. Fecundity of tobacco budworm females which developed from first-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate (lb/A)	Fecundity (# egg/female) for indicated residue period			
	0-day	2-day	4-day	6-day
0	983 ± 283 1/ 2/	1071 ± 377	916 ± 241	1041 ± 304
0.125	722 ± 194	866 ± 176	906 ± 200	914 ± 282
0.250	588 ± 156	926 ± 223	1026 ± 259	953 ± 173
0.500	690 ± 175	753 ± 139	965 ± 171	931 ± 321
1.000	667 ± 183	783 ± 156	916 ± 233	932 ± 248

1/ L.S.D. = 243

2/ $\bar{x}$ ± standard deviation of the mean

Table 14. Fecundity of tobacco budworm females which developed from second-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate	Fecundity (# egg/female) for indicated residue period			
(lb/A)	0-day	2-day	4-day	6-day
0	957 ± 271[1/ 2/]	982 ± 298	1090 ± 346	1119 ± 313
0.125	726 ± 138	912 ± 277	1110 ± 263	1049 ± 347
0.250	764 ± 259	958 ± 263	968 ± 251	1034 ± 283
0.500	698 ± 195	867 ± 232	882 ± 248	967 ± 252
1.000	687 ± 186	970 ± 243	890 ± 191	982 ± 232

1/ L.S.D. = 243

2/ $\bar{x}$ ± standard deviation of the mean

Table 15. Fecundity of tobacco budworm females which developed from third-instar larvae that fed on Dipel-treated greenhouse cotton.

Dipel Rate (lb/A)	Fecundity (# egg/female) for indicated residue period			
	0-day	2-day	4-day	6-day
0	1038 ± 445[1/ 2/]	1169 ± 397	1211 ± 425	1223 ± 379
0.125	1000 ± 540	1099 ± 397	1123 ± 230	1171 ± 402
0.250	716 ± 212	902 ± 259	1057 ± 320	1017 ± 344
0.500	915 ± 218	1053 ± 223	1007 ± 303	1124 ± 348
1.000	687 ± 186	970 ± 243	890 ± 191	982 ± 232

1/ L.S.D. = 243

2/ $\bar{x}$ ± standard deviation of the mean

significantly as dosage level was increased at the 0-, 1-, and 2-day post-application residue periods (Table 16, Appendix B_1). After that, effectiveness was significantly reduced, indicating inactivation of the pathogen. At the 2-day residue period, mortality ranged from 59 to 72%, respectively, for the four dosage rates; at three days it ranged from ca. 28 to 37%. Results were quite similar with the second-instar larvae, indicating comparable susceptibility between the two instars (Table 17, Appendix B_2).

Effectiveness of Dipel against third-instar larvae is shown in Table 18. Again, significantly higher mortality was obtained with increased dosages. With the third-instar, however, only the two higher dosages (0.5 and 1.0 lb./A) were comparable to those for the first- and second-instar larvae. The lower dosages of 0.125 and 0.25 lb./A were considerably less effective against third-instar larvae than against either of the earlier instars.

Factors purportedly influencing persistence of Dipel in the field include UV light, pH and enzymatic activity on the leaf surface. In field-persistence studies of _B_. _thuringiensis_, Leong et al. (1980), reported that endospore viability and pathogenecity were affected by solar radiation, leaf temperature and vapor pressure deficit. These factors would likely be common in the degredation of this pathogen in Arizona.

Dipel plus Coax vs. _H_. _Virescens_ Larvae

Studies were conducted to determine the efficacy of Coax, a cottonseed-based stimulant, in increasing the effectiveness of Dipel

Table 16. Mean mortality of first-instar tobacco budworm fed on Dipel-treated field cotton.

Dipel Rate	Percent mortality for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	13.3 ± 1.5[1/] [2/]	12.4 ± 0.2	14.6 ± 3.4	14.3 ± 5.7	12.4 ± 0.2
0.125	72.5 ± 1.5	56.7 ± 3.5	59.3 ± 5.7	27.6 ± 1.5	20.6 ± 2.5
0.250	81.7 ± 5.7	66.0 ± 4.9	65.7 ± 0.5	29.6 ± 1.4	24.8 ± 0.4
0.500	86.8 ± 4.3	76.3 ± 10.5	67.8 ± 3.5	33.6 ± 4.2	25.8 ± 1.8
1.000	91.4 ± 2.2	80.4 ± 10.5	72.0 ± 3.6	36.8 ± 8.7	26.9 ± 3.3

1/ based on cumulative mortality at 6 days post-inoculation

2/ $\bar{x}$ ± standard deviation of the mean

Table 17. Mean mortality of second-instar tobacco budworm fed on Dipel-treated field cotton.

Dipel Rate (lb/A)	Percent mortality for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	11.5 ± 4.5[1/] [2/]	9.3 ± 1.6	12.3 ± 2.9	8.4 ± 2.9	10.2 ± 0.0
0.125	79.2 ± 0.0	60.7 ± 12.3	50.0 ± 18.8	29.6 ± 27.1	18.4 ± 5.7
0.250	83.3 ± 0.0	79.4 ± 3.2	69.4 ± 11.6	39.6 ± 32.4	27.6 ± 16.0
0.500	88.6 ± 1.5	84.6 ± 1.2	79.6 ± 0.0	39.6 ± 32.4	29.6 ± 18.8
1.000	90.7 ± 1.5	85.6 ± 0.2	81.7 ± 2.9	49.0 ± 25.0	32.7 ± 17.3

1/ Based on cumulative mortality at 6 days post-inoculation

2/ $\bar{x}$ ± standard deviation of the mean

Table 18. Mean mortality of third-instar tobacco budworm fed on Dipel-treated field cotton.

Dipel Rate (lb/A)	Percent mortality for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	9.2 ± 1.4 1/ 2/	7.2 ± 1.5	9.1 ± 1.6	8.0 ± 2.8	5.2 ± 1.3
0.125	56.1 ± 1.4	46.0 ± 1.4	30.3 ± 1.5	22.0 ± 0.4	10.3 ± 2.8
0.250	69.9 ± 2.1	61.3 ± 2.9	43.4 ± 3.7	23.0 ± 1.4	18.6 ± 0.3
0.500	84.7 ± 1.4	80.6 ± 1.4	69.7 ± 2.4	36.0 ± 5.7	19.6 ± 1.7
1.000	89.8 ± 2.8	85.8 ± 2.9	76.8 ± 1.8	40.0 ± 2.8	22.8 ± 0.4

1/ Based on cumulative mortality at 6 days post-inoculation

2/ $\bar{x}$ ± standard deviation of the mean

against the first three larval instars of the tobacco budworm. Another purpose was to determine the most satisfactory combination of Dipel and Coax when the stimulant was used at 0.25 lb./A, lower than the recommended field rate. Tables 19, 20 and 21 present the results for first-, second- and third-instars, respectively. Higher dosages were significantly more effective against all instars tested (Appendix C). In general, the addition of Coax resulted in significantly higher mortality at all dosage levels and against all instars tested. However, the first-larval instar was most susceptible to either treatment. Efficacy of either treatment dropped with each successive instar.

Relative to stability in the field, the higher dosages of 0.5 and 1.0 lb./A of Dipel with Coax at 0.25 lb./A, showed greater persistence than the lower dosages of 0.125 and 0.25 lb./A with Coax at the 0.25 lb./A rate, (Appendix C). Previous reports showed that the addition of Coax at 1.0 lb./A was as effective as 2.0 lb./A against the first three instars of tobacco budworm larvae (Watson et al. 1979). Results of the present studies indicated that Coax can be used at rates even lower, i.e. 0.25 lb./A, than those recommended and still give satisfactory results. Coax may enhance effectiveness in either of two ways. It may stimulate greater feeding by the larvae or it may serve as a protectant, increasing stability of the pathogen. If the lower rates prove satisfactory, as indicated in the present tests, the addition of this material to an application will be much more economical than that presently used.

Table 19. Relative efficacy of various combinations of Dipel and a feeding stimulant, Coax, against first-instar tobacco budworm.

	Rate	Percent mortality[1] for indicated residue period				
Treatment	(lb/A)	0-day	1-day	2-days	3-days	4-days
Dipel	0	9.2 ± 3.1[2]	11.1 ± 4.9	12.3 ± 5.4	10.4 ± 3.6	5.5 ± 1.2
Dipel + Coax	0 + 0.250	17.4 ± 3.2	12.2 ± 5.4	12.3 ± 2.1	12.6 ± 3.5	9.1 ± 3.4
Dipel	0.125	85.1 ± 7.6	53.1 ± 8.0	43.1 ± 8.5	32.0 ± 6.7	29.1 ± 1.8
Dipel + Coax	0.125 + 0.250	91.0 ± 3.2	81.6 ± 0.0	70.5 ± 3.1	44.2 ± 17.3	24.4 ± 7.6
Dipel	0.250	87.2 ± 7.4	71.0 ± 9.3	55.4 ± 4.8	41.0 ± 6.7	33.1 ± 2.4
Dipel + Coax	0.250 + 0.250	95.1 ± 1.2	91.1 ± 1.2	83.5 ± 3.8	65.8 ± 3.2	52.8 ± 8.9
Dipel	0.500	90.8 ± 1.2	79.3 ± 9.7	68.5 ± 1.6	52.8 ± 6.1	37.3 ± 5.9
Dipel + Coax	0.500 + 0.250	97.9 ± 2.1	95.3 ± 1.2	87.0 ± 1.1	73.5 ± 4.1	62.5 ± 3.8
Dipel	1.000	92.9 ± 2.5	81.3 ± 7.6	73.9 ± 6.6	59.7 ± 3.2	38.7 ± 7.6
Dipel + Coax	1.000 + 0.250	98.7 ± 1.2	96.7 ± 1.2	92.5 ± 1.2	79.8 ± 2.3	69.5 ± 5.4

1/ Based on cumulative mortality at 6-days post-inoculation.

2/ $\bar{x}$ ± standard deviation of the mean

Table 20. Relative efficacy of various combinations of Dipel and a feeding stimulant, Coax, against second-instar tobacco budworm.

	Rate	Percent mortality[1/] for indicated residue period				
Treatment	(lb/A)	0-day	1-day	2-days	3-days	4-days
Dipel	0	9.8 ± 5.3[2/]	6.8 ± 4.6	6.2 ± 1.9	10.3 ± 6.0	6.2 ± 3.5
Dipel + Coax	0 + 0.250	20.8 ± 4.2	15.3 ± 2.4	16.4 ± 4.0	11.8 ± 4.4	9.6 ± 2.3
Dipel	0.125	77.6 ± 5.4	58.2 ± 12.3	34.9 ± 10.7	28.3 ± 5.0	11.0 ± 6.5
Dipel + Coax	0.125 + 0.250	79.2 ± 8.4	68.1 ± 2.4	60.9 ± 3.8	41.3 ± 18.0	22.6 ± 8.2
Dipel	0.250	84.0 ± 3.2	65.1 ± 16.3	51.9 ± 14.4	41.4 ± 5.2	16.4 ± 4.0
Dipel + Coax	0.250 + 0.250	88.2 ± 4.8	81.9 ± 4.3	69.9 ± 9.5	57.9 ± 12.8	35.6 ± 2.9
Dipel	0.500	86.7 ± 4.3	76.6 ± 9.8	63.0 ± 6.3	55.9 ± 9.2	23.3 ± 5.0
Dipel + Coax	0.500 + 0.250	92.4 ± 3.2	88.9 ± 2.4	80.1 ± 4.1	68.9 ± 12.8	52.1 ± 8.0
Dipel	1.000	90.9 ± 4.4	82.8 ± 7.4	65.8 ± 7.4	60.7 ± 6.7	29.4 ± 7.5
Dipel + Coax	1.000 + 0.250	97.2 ± 2.4	93.1 ± 2.4	86.3 ± 2.4	76.4 ± 10.6	59.6 ± 5.9

1/ Based on cumulative mortality at 6 days post-inoculation

2/ $\bar{x}$ ± standard deviation of the mean

Table 21. Relative efficacy of various combinations of Dipel and a feeding stimulant, Coax, against third-instar tobacco budworm.

		Percent mortality[1/] for indicated residue period				
Treatment	Rate	0-day	1-day	2-days	3-days	4-days
Dipel	0	5.4 ± 4.1[2/]	4.8 ± 4.3	4.0 ± 2.0	2.7 ± 1.2	4.0 ± 2.0
Dipel + Coax	0 + 0.250	9.7 ± 8.2	8.8 ± 3.0	6.8 ± 3.0	4.1 ± 5.4	2.7 ± 1.2
Dipel	0.125	53.5 ± 10.7	42.2 ± 10.3	21.6 ± 8.3	9.5 ± 3.1	7.3 ± 2.3
Dipel + Coax	0.125 + 0.250	68.6 ± 20.9	50.4 ± 13.8	40.5 ± 8.4	26.6 ± 13.2	8.1 ± 2.0
Dipel	0.250	60.9 ± 13.9	58.5 ± 4.3	37.8 ± 10.8	17.0 ± 5.1	10.7 ± 5.0
Dipel + Coax	0.250 + 0.250	79.7 ± 12.3	65.5 ± 16.1	55.2 ± 18.3	34.8 ± 22.6	14.1 ± 7.3
Dipel	0.500	69.0 ± 8.0	64.0 ± 5.1	52.7 ± 3.3	24.5 ± 3.6	12.7 ± 3.1
Dipel + Coax	0.500 + 0.250	83.3 ± 9.7	73.0 ± 14.9	61.9 ± 11.8	41.0 ± 17.9	29.4 ± 10.8
Dipel	1.000	80.4 ± 4.3	72.1 ± 3.1	57.4 ± 2.5	34.0 ± 3.1	18.0 ± 2.0
Dipel + Coax	1.000 + 0.250	88.1 ± 8.6	85.8 ± 5.1	66.7 ± 13.0	47.1 ± 15.6	33.6 ± 8.3

1/ Based on cumulative mortality at 6-days post inoculation

2/ $\bar{x}$ ± standard deviation of the mean

These results indicate that Dipel plus Coax can be used to reduce the build-up of tobacco budworm population. A four-day interval between treatments is suggested if population pressure persists. The Dipel rate of 0.25 lb./A plus Coax at 0.25 lb./A can be used for quick knock-out of an increasing larval population. However, for longer-lasting periods, higher dosages of Dipel are recommended (i.e., 0.5 or 1.0 lb./A).

First- and second-instar larvae were observed to spend more time on tender leaves, especially on those treated with Coax, than on buds or squares. Third-instar larvae fed on whatever was available.

Dipel vs. Larval Development

The developmental times for larvae that survived Dipel treatments are presented in Tables 22, 23 and 24. Developmental time increased significantly as the Dipel rate was increased. This correlation was evident with first-, second-, and third-larval instars. The influence of Dipel on larval development continued up to two days post-application; residues greater than two days caused no retardation in larval development. For sample periods beyond two days, the effect of Dipel on surviving larvae declined, apparently because of deactivation in the field. Highly significant differences can be seen among dosage levels at the zero-day sample period (Tables 22, 23, 24); in contrast, no significant difference is shown among dosage levels at the three-day sample period. Higher dosage levels influenced larval development for longer periods than did the lower dosages. For example, at the zero-day sample period, average developmental times for first-instar larvae were 16.3, 18.9 and 20.2 days at the 0, 0.125, and 1.0 lb./A rates of Dipel, respectively. At the three-day sample period for the same Dipel rates, average larval developmental times were 16.7, 17.0 and 17.9 days, respectively. Similar trends were shown with second- and third-instar larvae. Dulmage and Martinez (1973) reported an increase of larval developmental time when they fed continuously on a diet containing low concentrations of Dipel. In the present study higher dosages of Dipel were used and delayed larval development was observed even when they were exposed to Dipel-treated cotton for only 72 hours.

Dipel-Fed Larvae vs. Weight of Pupae

As in the greenhouse studies, larvae surviving the Dipel treatments were held for evaluating the effects on subsequent stages of development. Tables 25, 26 and 27, respectively, present the results of pupae obtained from surviving first-, second- and third-instar Dipel-fed larvae.

Table 25 shows that with surviving first-instar Dipel-fed larvae all dosages had a significant effect on subsequent pupal weight at the 0-day post-application residue period; these were significantly smaller. A trend of reduced pupal weights was also evident at the first-day post-application residue period. However, only the higher dosages (0.5, 1.00 lb./A) had a significant effect on the pupae.

Table 22. Developmental time of first-instar tobacco budworm that fed on cotton treated with various rates of Dipel and at various post-application residue periods.

Dipel Rate	Days from inoculation to pupation for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	16.6 ± 1.5 1/ 2/	16.1 ± 1.5	16.9 ± 1.4	16.7 ± 1.2	16.2 ± 1.1
0.125	18.9 ± 2.2	18.1 ± 1.7	17.3 ± 1.4	17.0 ± 1.3	17.1 ± 1.2
0.250	19.0 ± 2.0	18.4 ± 2.2	17.8 ± 1.9	17.4 ± 1.6	16.9 ± 1.4
0.500	20.0 ± 0.9	19.0 ± 1.9	18.3 ± 1.4	17.6 ± 1.9	16.2 ± 0.8
1.000	20.2 ± 1.6	19.3 ± 2.2	18.0 ± 1.3	17.9 ± 2.0	17.1 ± 1.5

1/ Ave. L.S.D. = 0.7

2/ $\bar{x}$ ± standard deviation of the mean

Table 23. Developmental time of second-instar tobacco budworm that fed on cotton treated with various rates of Dipel and at various post-application residue periods.

Dipel Rate (lb/A)	Days from inoculation to pupation for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	16.4 ± 1.2[1/] [2/]	17.4 ± 1.5	17.2 ± 0.9	17.4 ± 1.3	16.6 ± 1.4
0.125	18.3 ± 1.9	18.5 ± 1.8	17.5 ± 0.9	17.6 ± 1.3	17.0 ± 1.6
0.250	19.8 ± 2.0	18.8 ± 1.6	17.7 ± 0.9	17.1 ± 1.2	17.4 ± 2.0
0.500	20.4 ± 1.6	18.6 ± 1.9	18.1 ± 1.8	18.2 ± 1.7	17.2 ± 1.7
1.000	21.4 ± 2.2	18.8 ± 2.0	18.3 ± 1.5	18.3 ± 1.2	17.3 ± 1.6

1/ Ave. L.S.D. = 0.7

2/ $\bar{x}$ ± standard deviation of the mean

Table 24. Developmental time of third-instar tobacco budworm that fed on cotton treated with various rates of Dipel and at various post-application residue periods.

Dipel Rate (lb/A)	Days from inoculation to pupation for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	15.1 ± 1.2 1/ 2/	16.7 ± 0.7	15.3 ± 1.1	15.2 ± 1.1	15.3 ± 1.2
0.125	17.0 ± 1.9	16.3 ± 0.8	16.3 ± 1.1	15.4 ± 1.2	15.4 ± 1.2
0.250	17.0 ± 1.9	18.3 ± 1.4	16.6 ± 2.3	15.7 ± 1.1	15.5 ± 1.6
0.500	18.6 ± 2.0	18.1 ± 1.5	17.1 ± 1.3	16.2 ± 1.4	15.7 ± 1.2
1.000	18.8 ± 1.5	18.9 ± 1.4	17.0 ± 1.5	16.0 ± 1.4	16.0 ± 1.4

1/ Ave. L.S.D. = 0.7

2/ $\bar{x}$ ± standard deviation of the mean

Similar comparisons of pupae from second-instar, Dipel-fed larvae are shown in Table 26. At the zero-day residue period, pupal weights from larvae surviving Dipel treatment were not significantly different from the untreated control; however, a trend of reduced pupal weight was evident with the increasing Dipel dosages. All Dipel rates resulted in significantly smaller pupae at the 1-day residue period, as compared to those in the untreated check. Only the two higher rates showed significantly smaller pupae at the 2-day residue period. At the 3- and 4-day residue periods there was no correlation between Dipel rate and pupal weight.

Table 27 presents the results of pupal weights from surviving third-instar Dipel-fed larvae. In this group less difference in pupal weight was noted between treated and untreated larvae. Only the highest rate of 1.0 lb./A showed significantly smaller pupae and only at the zero-day residue period.

These data show that Dipel-fed larvae do have an influence on the size of the subsequent pupae even with short exposure periods but higher dosages are required to give this response. Previous studies reported reductions in pupal weight only when larvae were exposed continuously to sublethal doses of Dipel (Dulmage and Martinez, 1973; Dulmage et al. 1978).

Dipel-Fed Larvae vs. Pupal Development

Similar to larval developmental rates, pupal development was also retarded with increasing concentrations of Dipel. Only higher dosages showed significant differences from the control at the

Table 25. Weight of pupae that developed from first-instar tobacco budworm fed on various rates of Dipel and at various post-application residue periods.

Dipel Rate (lb/A)	Weight of pupae (mg) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	312 ± 29 1/ 2/	271 ± 36	302 ± 24	333 ± 25	326 ± 35
0.125	284 ± 32	248 ± 27	306 ± 15	344 ± 26	330 ± 23
0.250	284 ± 29	259 ± 46	311 ± 31	340 ± 30	346 ± 29
0.500	250 ± 25	222 ± 32	320 ± 20	310 ± 36	301 ± 26
1.000	254 ± 38	228 ± 32	301 ± 39	321 ± 20	306 ± 19

1/ L.S.D. = 25

2/ $\bar{x}$ ± standard deviation of the mean

Table 26. Weight of pupae that developed from second-instar tobacco budworm which fed on various rates of Dipel at various post-application residue periods.

Dipel Rate (lb/A)	Weight of pupae (mg) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	274 ± 27[1/ 2/]	281 ± 31	301 ± 26	309 ± 11	311 ± 29
0.125	271 ± 18	253 ± 44	281 ± 24	308 ± 10	320 ± 23
0.250	266 ± 26	254 ± 38	285 ± 24	308 ± 15	310 ± 22
0.500	267 ± 30	237 ± 42	274 ± 34	290 ± 22	307 ± 14
1.000	253 ± 30	220 ± 32	268 ± 35	294 ± 26	306 ± 27

1/ L.S.D. = 25

2/ $\bar{x}$ ± standard deviation of the mean.

Table 27. Weight of pupae that developed from third-instar tobacco budworm which fed on various rates of Dipel at various post-application residue periods.

Dipel Rates (lb/A)	Weight of pupae (mg) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	312 ± 22[1/ 2/]	292 ± 26	288 ± 21	320 ± 32	316 ± 30
0.125	289 ± 28	287 ± 31	291 ± 23	317 ± 25	317 ± 25
0.250	289 ± 32	286 ± 20	293 ± 24	311 ± 26	317 ± 25
0.500	288 ± 25	286 ± 20	289 ± 28	314 ± 33	306 ± 33
1.000	286 ± 26	286 ± 22	285 ± 24	307 ± 29	310 ± 27

1/ L.S.D. = 25

2/ $\bar{x}$ ± standard deviation of the mean

zero-day sample period. Results of these studies are shown in Tables 28, 29 and 30. Retardation was higher for those pupae which originated from Dipel-exposed third-instar larvae than those which originated from first- or second-instar larvae. Dulmage and Martinez (1973) reported longer developmental time for pupae originating from larvae that had been exposed continuously to sublethal doses of Dipel. No correlation can be found between Dipel concentration and pupal development at 2-, 3-, or 4-days post-treatment sample periods. Under Arizona conditions, it appears that this biological insecticide loses most of its effectiveness after two days in the field.

Dipel-Fed Larvae vs. Adult Longevity

Adults which developed from surviving Dipel-exposed larvae were evaluated for carry-over effects. Normal longevity was exhibited regardless of Dipel dosages or larval instar tested. However, there was an indication of slightly reduced adult longevity, though not significant, of those originating from first-instar Dipel-exposed larvae at the zero-day residue period. Results are presented in Tables 31 through 36. Dulmage and Martinez (1973) reported reduced longevity of tobacco budworm adults when they developed from larvae that were exposed continuously to sublethal doses of Dipel. The present study indicates that even with higher dosages of Dipel, the tobacco budworm can recover from the toxin if the exposure is 72 hours or less, with moths exhibiting normal life expectancy.

Table 28. Duration of tobacco budworm pupae which developed from surviving first-instar larvae that fed on Dipel-treated cotton.

Dipel Rate (lb/A)	Pupal duration (days) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	11.3 ± 1.8 1/ 2/	11.1 ± 1.5	11.2 ± 1.5	11.2 ± 1.4	11.2 ± 2.3
0.125	12.3 ± 1.6	12.5 ± 1.8	11.8 ± 1.2	12.0 ± 1.2	11.6 ± 1.5
0.250	12.4 ± 1.9	12.7 ± 1.8	12.3 ± 1.5	11.4 ± 1.2	11.9 ± 1.6
0.500	13.2 ± 0.8	13.0 ± 1.5	12.8 ± 1.5	12.4 ± 1.3	12.1 ± 1.7
1.000	13.3 ± 1.4	13.7 ± 1.5	13.1 ± 1.2	13.3 ± 1.5	11.9 ± 1.7

1/ Ave. L.S.D. = 0.6

2/ $\bar{x}$ ± standard deviation of the mean

Table 29. Duration of tobacco budworm pupae which developed from surviving second-instar larvae that fed on Dipel-treated cotton.

Dipel Rate	Pupal duration (days) for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	11.9 ± 1.5[1/ 2/]	11.2 ± 1.0	11.7 ± 1.0	11.7 ± 1.0	11.8 ± 1.2
0.125	11.8 ± 1.4	12.5 ± 1.3	12.2 ± 1.1	12.4 ± 1.3	12.2 ± 1.2
0.250	12.2 ± 0.9	12.7 ± 1.4	12.9 ± 1.3	12.4 ± 1.3	12.0 ± 1.4
0.500	13.3 ± 1.9	12.5 ± 1.1	12.8 ± 1.3	12.1 ± 1.3	12.0 ± 1.5
1.000	13.0 ± 1.0	12.2 ± 1.2	12.9 ± 1.3	12.7 ± 1.4	12.1 ± 1.3

1/ Ave. L.S.D. = 0.6

2/ $\bar{x}$ ± standard deviation of the mean

Table 30. Duration of tobacco budworm pupae which developed from surviving third-instar larvae that fed on Dipel-treated cotton.

Dipel Rate (lb/A)	Pupal duration (days) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	13.0 ± 1.0[1/] [2/]	13.5 ± 1.9	13.5 ± 1.2	13.0 ± 1.2	12.7 ± 1.3
0.125	14.0 ± 1.3	13.4 ± 2.0	13.9 ± 2.5	13.1 ± 1.1	12.8 ± 1.4
0.250	13.8 ± 1.5	14.6 ± 1.8	13.3 ± 1.0	13.2 ± 1.3	12.7 ± 1.5
0.500	15.4 ± 2.3	14.2 ± 2.1	13.4 ± 0.9	13.0 ± 1.4	12.8 ± 1.6
1.000	15.2 ± 1.5	14.1 ± 1.8	13.4 ± 1.1	13.2 ± 1.3	12.8 ± 1.4

1/ Ave. L.S.D. = 0.6

2/ $\bar{x}$ ± standard deviation of the mean

Table 31. Longevity of male tobacco budworm that emerged from survivors of Dipel-fed, first-instar larvae.

Dipel Rate (lb/A)	Longevity (days) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	18.0 ± 4.8[1/] [2/]	18.3 ± 4.2	18.1 ± 5.0	17.1 ± 6.1	17.5 ± 5.1
0.125	16.2 ± 6.4	17.7 ± 3.3	19.9 ± 3.4	17.0 ± 5.9	15.8 ± 5.4
0.250	15.8 ± 4.8	16.7 ± 4.4	17.8 ± 6.0	14.6 ± 5.4	17.7 ± 4.0
0.500	15.3 ± 4.4	14.5 ± 4.5	17.8 ± 5.5	18.0 ± 5.3	16.0 ± 5.2
1.000	16.0 ± 5.7	17.4 ± 4.1	16.6 ± 7.1	18.1 ± 4.0	17.3 ± 4.4

1/ L.S.D. = 4.3

2/ $\bar{x}$ ± standard deviation of the mean

Table 32. Longevity of female tobacco budworm that emerged from survivors of Dipel-fed first-instar larvae.

Dipel Rate (lb/A)	Longevity (days) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	18.9 ± 2.3 1/ 2/	17.9 ± 4.8	18.4 ± 5.9	16.8 ± 5.4	17.5 ± 6.0
0.125	17.1 ± 5.8	17.3 ± 4.3	18.7 ± 4.0	16.8 ± 6.0	16.0 ± 5.6
0.250	14.6 ± 6.4	16.3 ± 5.0	18.6 ± 4.1	17.5 ± 4.8	15.8 ± 5.7
0.500	16.4 ± 3.6	15.2 ± 5.2	19.8 ± 4.1	16.4 ± 6.6	17.5 ± 5.1
1.000	16.6 ± 4.2	17.1 ± 3.4	17.0 ± 4.6	18.8 ± 3.9	18.2 ± 4.4

1/ L.S.D. = 4.3

2/ $\bar{x}$ ± standard deviation of the mean

Table 33. Longevity of male tobacco budworm emerged from survivors of Dipel-fed, second-instar larvae.

Dipel Rate	Longevity (days) for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	16.8 ± 4.9 [1/ 2/]	17.5 ± 6.0	15.7 ± 6.4	16.1 ± 4.7	17.1 ± 4.7
0.125	14.7 ± 4.5	17.8 ± 3.6	16.6 ± 3.4	17.4 ± 5.1	17.2 ± 5.4
0.250	16.2 ± 5.0	15.3 ± 4.2	15.9 ± 6.2	17.1 ± 5.8	15.8 ± 5.1
0.500	16.5 ± 3.2	13.9 ± 3.8	17.2 ± 5.0	16.2 ± 6.3	17.2 ± 5.6
1.000	15.6 ± 3.8	15.7 ± 5.7	15.4 ± 5.1	16.5 ± 5.8	18.9 ± 4.0

1/ L.S.D = 4.3

2/ $\bar{x}$ ± standrad deviation of the mean

Table 34. Longevity of female tobacco budworm emerged from survivors of Dipel-fed, second-instar larvae.

Dipel Rate (lb/A)	Longevity (days) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	18.8 ± 6.2[1/ 2/]	17.0 ± 4.6	16.6 ± 4.6	16.0 ± 5.9	16.2 ± 5.7
0.125	17.0 ± 5.1	17.0 ± 4.1	14.3 ± 5.1	17.2 ± 4.6	17.9 ± 5.3
0.250	15.4 ± 4.9	15.9 ± 4.8	15.8 ± 5.7	17.9 ± 5.7	18.2 ± 4.9
0.500	16.0 ± 3.4	13.7 ± 5.9	16.8 ± 5.2	17.6 ± 4.5	17.8 ± 4.0
1.000	16.8 ± 4.9	17.5 ± 4.4	15.8 ± 4.1	17.3 ± 4.3	19.9 ± 2.8

1/ L.S.D. = 4.3

2/ $\bar{x}$ ± standard deviation of the mean

Table 35. Longevity of male tobacco budworm that emerged from suvivors of Dipel-fed, third-instar larvae.

Dipel Rate (lb/A)	Longevity (days) for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	18.5 ± 3.8 1/ 2/	15.6 ± 4.8	17.5 ± 4.3	17.1 ± 4.2	17.8 ± 4.3
0.125	19.1 ± 3.4	17.3 ± 4.3	19.0 ± 4.4	17.6 ± 5.2	15.2 ± 5.9
0.250	19.0 ± 3.4	17.9 ± 3.9	19.2 ± 3.9	15.1 ± 5.6	18.3 ± 4.7
0.500	17.7 ± 4.5	17.8 ± 4.2	18.7 ± 3.8	17.3 ± 5.4	18.2 ± 3.9
1.000	18.6 ± 4.9	19.5 ± 4.0	18.3 ± 4.1	17.6 ± 6.0	18.6 ± 3.1

1/ L.S.D = 4.3

2/ $\bar{x}$ ± standard deviation of the mean

Table 36. Longevity of female tobacco budworm that emerged from survivors of Dipel-fed, third-instar larvae.

Dipel Rate	Longevity (days) for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	18.5 ± 2.8 [1/ 2/]	17.2 ± 3.4	18.6 ± 5.8	17.2 ± 5.4	16.4 ± 6.3
0.125	19.2 ± 3.3	17.2 ± 5.4	16.3 ± 4.9	16.7 ± 5.4	17.8 ± 5.5
0.250	18.6 ± 4.4	16.6 ± 5.5	19.2 ± 4.4	17.2 ± 4.2	18.8 ± 4.5
0.500	18.0 ± 4.6	16.7 ± 4.1	19.0 ± 3.5	17.6 ± 5.3	18.3 ± 3.8
1.000	18.4 ± 4.6	18.9 ± 4.9	18.2 ± 4.7	17.2 ± 4.4	16.5 ± 3.7

1/ L.S.D. = 4.3

2/ $\bar{x} \pm$ standard deviation of the mean

Dipel-Fed Larvae vs. Female Fecundity

Females emerging from Dipel-treated larvae, regardless of instar treated, showed a trend of reduced fecundity for the zero-day sample period. Only the highest rate (1.0 lb./A) showed a significant difference from the control treatment. Tables 37, 38 and 39, respectively, present the average number of eggs per female for moths developing from Dipel-exposed first-, second- and third-instar tobacco budworm larvae. No correlation was found between fecundity and Dipel dose at any residue period except for those at the zero-day sample period. Ignoffo and Gregory (1972) and Dulmage and Martinez (1973) reported reduced fecundity of tobacco budworm females that developed from larvae that had been exposed continuously to Dipel-treated diet. In the present study, larvae were left on Dipel-treated terminals for 72 hours and then moved on to Dipel-free diet. Only the higher dosages influenced fecundity of tobacco budworm moths and then, only at the zero-day residue period. Dulmage and Martinez (1973) noted that the reduced fecundity was not related to the toxin but resulted indirectly from the general debilitation produced by the toxin.

Dipel-Fed Larvae vs. Egg Fertility and Viability

Eggs from moths originating from Dipel-exposed larvae showed no effects of the earlier Dipel treatments. Normal egg hatch was obtained for all treatments (Table 40) and ensuing larvae reared on artificial diet exhibited normal development (Table 41). There was no significant difference among treatments and the control.

Table 37. Fecundity of female tobacco budworm emerging from survivors of Dipel-fed, first-instar larvae.

Dipel Rate (lb/A)	Mean no. eggs/female for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	898 ± 269[1/ 2/]	810 ± 353	896 ± 175	896 ± 209	1016 ± 369
0.125	666 ± 208	813 ± 171	815 ± 185	859 ± 177	931 ± 283
0.250	672 ± 264	742 ± 160	730 ± 200	791 ± 183	970 ± 366
0.500	684 ± 172	683 ± 211	780 ± 181	817 ± 182	915 ± 246
1.000	604 ± 221	687 ± 172	758 ± 152	854 ± 167	967 ± 211

1/ L.S.D. = 236

2/ $\bar{x}$ ± standard deviation of the mean

Table 38. Fecundity of female tobacco budworm emerging from survivors of Dipel-fed, second-instar larvae.

Dipel Rate	Mean no. eggs/female for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	717 ± 147 1/ 2/	899 ± 233	936 ± 316	1201 ± 349	1068 ± 300
0.125	673 ± 195	818 ± 205	920 ± 253	1011 ± 353	957 ± 335
0.250	692 ± 164	684 ± 185	853 ± 197	948 ± 233	983 ± 205
0.500	654 ± 164	715 ± 205	841 ± 211	939 ± 259	909 ± 282
1.000	621 ± 178	717 ± 169	950 ± 283	887 ± 203	1000 ± 235

1/ L.S.D. = 236

2/ $\bar{x}$ ± standard deviation of the mean

Table 39. Fecundity of female tobacco budworm emerging from survivors of Dipel-fed, third-instar larvae.

Dipel Rate	Mean no. egg/female for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	1233 ± 417[1/ 2/]	1147 ± 381	1063 ± 388	1075 ± 335	1161 ± 407
0.125	1217 ± 477	1032 ± 259	1022 ± 372	1032 ± 255	1123 ± 341
0.250	864 ± 195	968 ± 286	1013 ± 324	1020 ± 315	1048 ± 317
0.500	999 ± 295	1019 ± 341	1041 ± 316	991 ± 293	1185 ± 400
1.000	928 ± 293	1019 ± 339	1029 ± 269	1078 ± 304	968 ± 234

1/ L.S.D. = 236

2/ $\bar{x}$ ± standard deviation of the mean

Table 40. Percent egg hatch of tobacco budworm surviving Dipel-fed first-, second-, and third-instar larvae.

Treated Instar	Dipel Rate (lb/A)	Percent hatch for indicated residue period				
		0-day	1-day	2-days	3-days	4-days
1st instar	0	88	88	91	90	93
	0.125	90	86	90	89	88
	0.250	83	90	92	89	91
	0.500	90	86	94	91	87
	1.000	90	86	94	91	87
2nd instar	0	90	95	89	93	90
	0.125	92	92	92	93	90
	0.250	93	93	94	91	87
	0.500	95	93	89	95	89
	1.000	92	93	89	89	88
3rd instar	0	92	93	93	93	88
	0.125	93	95	91	94	89
	0.250	91	89	92	92	89
	0.500	93	93	89	95	90
	1.000	90	92	89	91	87

Table 41. Survival of F_1 larvae from tobacco budworm moths, originating from Dipel-fed larvae.

Treated instar	Dipel Rate (lb/A)	Mean larval survival for indicated residue period				
		0-day	1-day	2-day	3-day	4-day
1st instar	0	24.3 ± 1.0	23.8 ± 1.3	24.3 ± 1.0	23.2 ± 1.3	23.5 ± 1.9
	0.125	23.3 ± 1.3	23.3 ± 1.0	23.3 ± 0.5	23.5 ± 1.3	23.5 ± 1.3
	0.250	24.0 ± 0.8	23.8 ± 1.0	24.0 ± 0.8	22.3 ± 2.2	23.5 ± 0.6
	0.500	22.8 ± 1.7	23.3 ± 1.7	23.0 ± 0.8	23.5 ± 1.0	23.5 ± 1.0
	1.000	22.8 ± 1.7	22.5 ± 0.6	23.0 ± 0.6	23.3 ± 1.0	23.0 ± 0.8
2nd instar	0	23.5 ± 1.3	23.5 ± 1.0	23.8 ± 1.5	23.8 ± 0.5	23.5 ± 1.9
	0.125	21.0 ± 1.4	24.8 ± 0.5	24.0 ± 0.8	23.5 ± 1.3	22.5 ± 1.7
	0.250	22.5 ± 1.7	23.5 ± 0.6	23.8 ± 1.0	23.3 ± 1.3	23.0 ± 1.4
	0.500	22.3 ± 1.7	24.5 ± 1.0	24.8 ± 0.5	23.3 ± 1.7	23.0 ± 0.8
	1.000	22.5 ± 2.1	23.8 ± 1.7	23.5 ± 1.3	23.3 ± 1.3	23.0 ± 1.6

(continued)

Table 41 continued.

3rd instar	0	23.5 ± 1.3	22.3 ± 1.7	23.5 ± 1.3	23.0 ± 1.6	24.0 ± 0.8
	0.125	22.0 ± 1.8	24.3 ± 0.5	22.0 ± 1.8	24.3 ± 1.0	23.0 ± 0.8
	0.250	22.5 ± 1.7	23.0 ± 2.2	24.3 ± 1.0	23.8 ± 0.5	23.5 ± 0.6
	0.500	21.3 ± 1.5	24.0 ± 0.8	23.5 ± 0.6	23.3 ± 1.7	23.8 ± 1.0
	1.000	24.3 ± 1.0	22.5 ± 2.1	23.5 ± 1.9	23.8 ± 1.3	22.3 ± 1.8

1/ Survival based on 25 larvae after 6 days of introduction.

2/ $\bar{x}$ ± standard deviation of the mean

Dipel vs. Tobacco Budworm Egg

Dipel had no apparent effect on egg-hatch when the toxin was applied directly to the egg. Normal eclosion resulted with all dosages tested (Table 42). However, Dipel did have a significant effect on survival of larvae emerging from treated eggs (Table 43). Survival was inversely correlated with dosage. Apparently, larvae obtained the toxin from the egg shell during the hatching process since no other source of Dipel was available on which to feed. Highly significant differences were evident among all treatments (Appendix E). Relative to residual effects, Dipel-treated eggs significantly reduced larval survival through 3-days post-application, the time required for newly-laid eggs to hatch. The rate of 1.0 lb./A resulted in 100% mortality when egg-hatch occurred 24 hours after application; only 8.9% of the larvae survived from eggs hatching 72 hours post-application. At the lowest dosage, 0.125 lb./A, survival was 11.7% and 33.9%, respectively, at the 24- and 72-hour hatch periods (Table 43).

This discovery suggests the usefulness of Dipel as an indirect ovicide, as well as a larvicide. However, in this case Dipel does not affect the embryo inside the egg but only subsequently as the larva emerges from the egg. This would suggest that the maximum effects of Dipel treatment against the tobacco budworm could be obtained when field sampling indicated maximum egg-laying and newly-hatched larvae. Rates of 0.25 lb./A or higher would give better results because of their greater residual effectiveness. Repeated applications would probably be required at 4-day intervals, if

Table 42. Influence of various rates of Dipel on the egg hatch of the tobacco budworm.

Dipel Rate (lb/A)	Percent Egg Hatch
0	87 ± 9 1/ 2/
0.125	78 ± 7
0.250	81 ± 8
0.500	81 ± 6
1.000	86 ± 9

1/ Based on sample size of 100

2/ $\bar{x}$ ± standard deviation of the mean

Table 43. Percent survival of 1st-instar tobacco budworm larvae emerging from Dipel-treated egg.

Dipel Rate (lb/A)	Percent survival for Indicated Residue Period		
	1-day	2-days	3-days
0	90.6 ± 2.5 1/ 2/	88.9 ± 5.4	87.2 ± 6.5
0.125	11.7 ± 3.5	24.4 ± 2.7	33.9 ± 4.9
0.250	7.2 ± 3.9	15.0 ± 2.8	18.9 ± 1.7
0.500	5.0 ± 3.5	12.2 ± 2.7	12.8 ± 3.3
1.000	0.0 ± 0.0	5.0 ± 1.8	8.9 ± 2.7

1/ Mortality based on that occuring at 6 days after eclosion.

2/ $\bar{x}$ ± standard deviation of the mean

population pressure persisted. If beneficial insects are present and it becomes apparent that they are incapable of controlling the infestation, Dipel application can be made as a supplementary control measure without disrupting natural-enemy effectiveness.

Larval Exposure Time vs. Recovery

Based on reports that tobacco budworm larvae can recover after moving from Dipel-treated to untreated foliage (Dulmage et al. 1978), studies were conducted to confirm or negate such reports. In the present studies, only second-instar larvae were used as a test insect. The rates of Dipel tested were 0.5 and 1.0 lb./A. Table 44 presents the results of these studies.

When larvae were left to feed on treated cotton for 30 hours, the number that recovered was significantly less than that of larvae allowed to feed for only six hours. Significant differences were shown in the capacity of larvae to recover and survive, and in their subsequent development to adults, based upon dosage level and feeding period. The ability to recover was reduced as dosage or time of exposure was increased.

These data confirm previous reports that tobacco budworm larvae have the ability to recover from the effect of the toxin once they are removed from treated feeding sites. This situation is likely to happen in the field as larvae may move about on treated plants and gain access to untreated areas.

When larvae are allowed to feed on treated foliage the toxin is removed with the food and passed through the gut. Exuviae-like

Table 44. Survival of second-instar tobacco budworm larvae exposed to Dipel-treated cotton for various periods of time.

Dipel Rate (lb/A)	Feeding Period (hrs)	Mean no. surviving to indicated stage		
		to third instar	to pupation	to adult emergence
0	6	46.0 ± 1.7 [1/ 2/]	43.3 ± 1.5	41.7 ± 0.6
	18	47.0 ± 0.0	44.3 ± 0.6	42.3 ± 1.2
	30	46.0 ± 0.7	43.3 ± 2.1	42.3 ± 2.5
0.500	6	41.3 ± 2.3	38.0 ± 4.0	36.7 ± 3.5
	18	31.0 ± 4.6	29.0 ± 6.1	28.7 ± 5.8
	30	25.3 ± 4.2	22.0 ± 3.6	21.0 ± 3.5
1.000	6	38.7 ± 6.8	35.3 ± 5.0	34.0 ± 5.6
	18	28.0 ± 2.0	24.0 ± 4.6	23.0 ± 3.6
	30	22.7 ± 6.0	19.7 ± 6.1	19.3 ± 6.0

1/ Data based on sample size of 50

2/ $\bar{x}$ ± standard deviation of the mean

material was observed in the first feces passed when the larvae started feeding on the untreated diet. Most mortality occurred during the period between the second- and third-instars and once larvae had molted to the third-instar, mortality was reduced significantly.

Developmental Time of Surviving Larvae. Table 45 presents the developmental time of larvae that recovered from various periods of Dipel feeding. Where larvae had fed for six hours on the terminal, there was no significant difference in developmental time between either dose and the untreated control; however, highly significant differences were shown in both treatments where the larvae had fed on treated terminals for 18 or 30 hours.

Pupal Development. Average pupal time from survivors of various larval Dipel-feeding times is presented in Table 46. Again, it is evident that with an increase in Dipel dosage or with an increase of exposure time the pupal developmental period was increased. Dulmage et al. (1978) reported normal pupal development for those emerging from Dipel-treated larvae even with the 72-hour exposure period. However, higher Dipel dosages were used in this study than those used by Dulmage.

Weight of Pupae. A trend of decreasing pupal weights was evident with increasing larval exposure time to Dipel (Table 47). However, no significant differences were found. Larval exposure to Dipel probably was not long enough to influence pupal weight.

Longevity of the Adults. Tables 48 and 49 show longevity of tobacco budworm males and females, respectively. Adults emerging

Table 45. Effect of various Dipel-feeding periods of second-instar tobacco budworm on developmental time of surviving larvae.

Dipel Rate (lb/A)	Larval developmental time (days) for indicated feeding period: 6 hrs.	18 hrs.	30 hrs.
0	15.6 ± 2.1 1/ 2/	16.1 ± 1.9	16.5 ± 2.1
0.50	15.9 ± 2.3	16.7 ± 2.1	17.0 ± 2.3
1.00	15.9 ± 2.2	17.2 ± 2.4	17.2 ± 2.2

1/ Ave. L.S.D. = 0.4

2/ $\bar{x}$ ± standard deviation of the mean

Table 46. Pupal duration of tobacco budworm surviving various Dipel-feeding periods of second-instar larvae.

Dipel Rate (lb/A)	Pupal duration (days) for indicated larval feeding period 6 hrs.	18 hrs.	30 hrs.
0	11.5 ± 1.1 [1/ 2/]	11.5 ± 1.3	11.7 ± 1.3
0.50	11.8 ± 1.4	12.1 ± 1.2	12.3 ± 1.6
1.00	12.2 ± 1.5	12.4 ± 1.2	12.7 ± 1.8

1/ Ave. L.S.D. = 0.3

2/ $\bar{x}$ ± standard deviation of the mean

Table 47. Pupal weight of tobacco budworm which developed from second-instar larvae exposed to various Dipel-feeding periods.

Dipel Rate (lb/A)	Pupal weight (mg) for indicated feeding period		
	6 hrs.	18 hrs.	30 hrs.
0	322 ± 27 [1/] [2/]	312 ± 27	305 ± 24
0.50	314 ± 24	305 ± 36	285 ± 27
1.00	295 ± 26	296 ± 27	284 ± 38

1/ L.S.D. = 26

2/ $\bar{x}$ ± standard deviation of the mean

Table 48. Longevity of tobacco budworm males which developed from second-instar larvae exposed to various Dipel-feeding periods.

Dipel Rate (lb/A)	Longevity (days) for indicated larval feeding period		
	6 hrs.	18 hrs.	30 hrs.
0	16.8 + 5.3 1/	16.2 + 4.4	16.9 + 5.6
0.50	16.2 + 6.9	15.3 + 4.3	17.3 + 4.7
1.00	16.4 + 3.7	16.8 + 4.2	15.5 + 6.2

1/ $\bar{x} \pm$ standard deviation of the mean

Table 49. Longevity of tobacco budworm females which developed from second-instar larvae exposed to various Dipel-feeding periods.

Dipel Rate (lb/A)	Longevity (days) for indicated larval feeding period		
	6 hrs.	18 hrs.	30 hrs.
0	17.9 ± 5.4[1/]	17.6 ± 5.0	17.6 ± 4.6
0.50	16.6 ± 5.1	15.8 ± 4.8	15.4 ± 5.4
1.00	15.3 ± 3.6	17.5 ± 4.7	17.6 ± 3.9

1/ $\bar{x} \pm$ standard deviation of the mean

Table 50. Fecundity of tobacco budworm females which developed from second-instar larvae exposed to various Dipel-feeding periods.

Dipel Rate	Mean no. eggs/female for indicated larval feeding period		
(lb/A)	6 hrs.	18 hrs.	30 hrs.
0	1278 ± 378[1/ 2/]	1101 ± 404	1107 ± 396
0.50	1128 ± 616	789 ± 336	1110 ± 332
1.00	1174 ± 478	948 ± 341	982 ± 422

1/ L.S.D. = 374

2/ $\bar{x}$ ± standard deviation of the mean

Table 51. Percent egg-hatch from tobacco budworm females which developed from second-instar larvae exposed to various Dipel-feeding periods.

Dipel Rate (lb/A)	Percent egg hatch for indicated larval feeding period		
	6 hrs	18 hrs	30 hrs
0	93	91	93
0.50	94	91	95
1.00	93	91	95

Table 52. Survival of F_1, first-instar, tobacco budworm larvae following exposure of parental generation during the second-larval instar to various feeding periods.

Dipel Rate	Mean survival for indicated feeding period		
(lb/A)	6 hrs.	18 hrs.	30 hrs.
0	22.5 ± 1.3 1/ 2/	23.8 ± 1.0	23.7 ± 0.6
0.50	22.5 ± 0.6	24.3 ± 1.0	23.8 ± 1.3
1.00	22.5 ± 1.3	24.0 ± 0.8	23.5 ± 1.3

1/ survival of sample of 25 larvae that occurred after 6 days of inoculation.

2/ $\bar{x}$ ± standard deviation of the mean

from either larval treatment exhibited normal life spans and no significant difference was obtained between treatments and the control.

Fecundity and Egg Viability. No correlation was exhibited between Dipel treatments and the control relative to fecundity of females surviving from Dipel-exposed, second-instar larvae (Table 50). All females exhibited normal oviposition. Also, there was no carryover effect on egg fertility (Table 51) or on the first-instar larvae that hatched from those eggs (Table 52). Dipel did have some longterm effects on development of tobacco budworm but none related to fecundity and egg viability.

Dipel Plus *G. Punctipes* vs. First-Instar *H. Virescens* Larvae

Greenhouse Studies

These studies were designed to determine the effect of various combinations of Dipel and fourth-instar *G. punctipes* against first-instar tobacco budworm larvae. Table 53 presents the results of these studies.

The addition of *G. punctipes* resulted in significantly higher mortality with each rate of Dipel tested (Appendix F1). With each increase in density of *Geocoris* there was a decrease in the number of tobacco budworm larvae recovered at the end of the four-day test period; the trend was similar with each residue period. The rate of 0.125 lb./A with the *Geocoris* nymphs resulted in 10.7, 8.7, 5.3 and 4.0%, respectively, of surviving larvae, for the four densities of the predator at the zero-day post-application. For the same residue periods the rate of 1.0 lb./A with Geocoris nymphs resulted in 5.3, 3.3, 2.0 and 0%, respectively, of surviving larvae for the above predator densities. At the 6-day post-application period, numbers of larvae recovered were 55.3, 50.7, 23.3 and 7.3, and 50.0, 50.0, 20.7 and 6.0%, respectively, for the above Dipel-predator combination. These data indicate that Geocoris is adding a significant mortality factor in combination with either dose of Dipel against first-instar tobacco budworm larvae. One predator per four plants (= ca. 3287/acre) was still giving satisfactory results when combined with either Dipel dosage. If this density of predators is present in the field and is incapable of controlling tobacco budworm infestations, any Dipel dosage tested would be adequate. However, higher dosages would give better control because they persist longer in the field.

Field Studies

Field studies were conducted inside larger field cages (3.6 x 1.8 x 1.8 m.). As with the greenhouse studies, various combinations of the pathogen and predator were evaluated against first-instar tobacco budworm larvae. Results of these studies are shown in Table 54.

Geocoris nymphs at a density of one per two row-feet were significantly more effective than one per four row-feet at all Dipel levels tested (Appendix F_2). All Dipel treatments with either density of Geocoris gave significantly better control than did Dipel alone.

Table 53. Combined effects of fourth-instar *Geocoris punctipes* and Dipel on percent survival of first-instar tobacco budworm larvae on greenhouse cotton.

Dipel Rate (lb/A)	Residue Periods (days)	*Geocoris* Levels[1/]				
		I	II	III	IV	V
0	0	71.3 ± 2.3[2/3/]	56.0 ± 2.0	51.3 ± 1.2	22.0 ± 2.0	6.7 ± 1.2
	2	72.0 ± 0.0	58.0 ± 2.0	53.3 ± 1.2	22.7 ± 1.2	6.7 ± 1.2
	4	69.3 ± 1.2	58.0 ± 2.0	52.0 ± 0.0	21.3 ± 1.2	6.0 ± 2.0
	6	69.3 ± 1.2	55.3 ± 1.2	50.7 ± 1.2	23.3 ± 2.3	6.0 ± 2.0
0.125	0	12.7 ± 1.2	10.7 ± 1.2	8.7 ± 2.3	5.3 ± 1.2	4.0 ± 2.0
	2	38.7 ± 5.8	34.7 ± 3.1	18.7 ± 4.2	12.7 ± 1.2	2.7 ± 1.2
	4	58.7 ± 2.3	50.0 ± 6.0	45.3 ± 7.6	20.7 ± 2.3	7.3 ± 1.2
	6	66.7 ± 5.8	55.3 ± 4.2	50.7 ± 5.0	23.3 ± 2.3	7.3 ± 1.2

(continued)

Table 53 Continued

0.250	0	8.7 ± 1.2	5.3 ± 1.2	6.0 ± 2.0	2.0 ± 2.0	2.7 ± 3.1
	2	25.3 ± 1.2	17.3 ± 1.2	16.7 ± 1.2	7.3 ± 1.2	4.7 ± 1.2
	4	42.7 ± 1.2	43.3 ± 4.6	41.3 ± 1.2	18.0 ± 4.0	6.7 ± 1.2
	6	60.0 ± 2.0	54.0 ± 2.0	53.3 ± 3.1	22.7 ± 3.1	8.0 ± 0.0
0.500	0	8.7 ± 1.2	5.3 ± 1.2	5.3 ± 1.2	2.0 ± 2.0	2.0 ± 0.0
	2	23.3 ± 1.2	16.0 ± 2.0	14.0 ± 2.0	7.3 ± 2.3	4.0 ± 0.0
	4	45.3 ± 1.2	34.7 ± 1.2	34.0 ± 4.0	15.3 ± 1.2	7.3 ± 1.2
	6	62.0 ± 10.4	52.7 ± 1.2	52.7 ± 1.2	21.3 ± 3.1	6.7 ± 1.2
1.000	0	7.3 ± 1.2	5.3 ± 1.2	3.3 ± 1.2	2.0 ± 2.0	0.0 ± 0.0
	2	19.3 ± 5.0	15.3 ± 2.3	12.7 ± 3.1	6.7 ± 1.2	4.0 ± 2.0
	4	39.3 ± 8.1	28.7 ± 8.1	30.7 ± 4.2	14.7 ± 2.3	5.3 ± 1.2
	6	60.7 ± 9.5	50.0 ± 2.0	50.0 ± 5.3	20.7 ± 2.3	6.0 ± 0.0

1/ G coris levels: I = 0, II = one per 4 pots/row, III = one per 3 pots/row, IV = one per 2 pots/row, and V = one per one pot/row.

2/ $\bar{x}$ ± standard deviation of the mean

3/ Data based on sample size of 50 larvae

This was true when predator and prey were introduced to Dipel treatments at 2-days post-application. However, the magnitude of effectiveness of either combination was reduced at the 2-day post-application period when compared to that at the zero-day residue period.

Results obtained from these studies indicate the possibility of good integrated biological control, using a satisfactory density of *G. punctipes* (i.e. one predator per four row-feet) plus an effective field rate of Dipel. The need for subsequent applications will depend totally on the effectiveness of the previous combination of control measures. Environmental factors would have a significant influence on the stability of the biological insecticide in the field.

Only the fourth-instar *G. punctipes* was evaluated in these studies. However, under field conditions, all stages are found and act together with other natural enemies against *H. virescens* population. Fifth-instar *G. punctipes* is more effective as a predator than the fourth-instar (Lawrence and Watson 1979). The adult stage is better yet due to its agility on the same plant or between plants in the field.

Since Dipel has no observed direct or indirect effect on *G. punctipes*, the possibilities of using these biological control agents against tobacco budworm is encouraging. By minimizing the application of toxic chemicals and/or by making periodic release of *Geocoris* from laboratory established cultures, the predator population can be maintained at an effective level.

Table 54. Combined effects of fourth-instar Geocoris punctipes and Dipel on survival of first-instar tobacco budworm larvae on field cotton.

Dipel Rate (lb/A)	Geocoris Level 1/	Percent survival for indicated residue period	
		0-day	2-days
0	1	49.3 ± 3.1 2/	51.3 ± 4.6
	2	30.7 ± 9.5	36.7 ± 5.0
	3	25.3 ± 9.9	26.0 ± 8.0
0.125	1	13.3 ± 4.2	41.3 ± 7.0
	2	10.0 ± 5.3	30.7 ± 4.6
	3	4.7 ± 3.1	20.7 ± 4.6
0.250	1	8.7 ± 2.3	30.7 ± 4.2
	2	5.3 ± 1.2	22.0 ± 3.5
	3	3.3 ± 3.1	15.3 ± 3.1
0.500	1	6.7 ± 2.3	24.0 ± 5.3
	2	3.3 ± 3.1	17.3 ± 7.6
	3	2.0 ± 2.0	12.0 ± 5.3
1.000	1	5.3 ± 1.2	19.3 ± 4.2
	2	0.7 ± 1.2	14.0 ± 2.0
	3	0.0 ± 0.0	6.7 ± 3.1

1/ Geocoris levels: 1 = 0, 2 = 1 per 4 ft., 3 = 1 per 2 ft.

2/ $\bar{x} \pm$ standard deviation of the mean

General Observations

Moth Transmission of the Pathogen

Since moths move actively within the cotton canopy and over leaf surfaces during feeding and oviposition, they are likely to come in contact with spray residue. It was desirable to determine if moths are capable of picking up and transferring the pathogen to their eggs during oviposition. At least two modes of transmission are probably involved: (1) external pick-up of Dipel residues by moths, and (2) ingestion of the pathogen during feeding, followed by transovarian transmission as an external contaminant of the egg shell.

Tobacco budworm adults were sprayed with or dipped in a Dipel solution of 64000 IU/ml. Treated moths were caged in adult rearing containers described elsewhere in the text. Egg sheets collected during the first 24 hours post-treatment were incubated in an environator at 30 ± 1°C. Fifty neonate larvae from each treatment were transferred to 1 oz. plastic cups (two per cup) containing a modified diet and held for mortality observations. Results of these tests were compared with a control treatment that had no exposure to Dipel. Mortality readings were taken for six consecutive days after transferring larvae to the diet.

Data obtained from these studies showed no indication of pathogen transmission (Table 55). No significant difference was found between the control and either of the treatments.

Table 55. Survival of first-instar tobacco budworm larvae using various methods of Dipel transmission.

Dipel Treatment	Rate (lb/A)	Percent Mortality [1/]
Spray	0	17 ± 3[2/]
	1.000	17 ± 6
Dip	0	16 ± 6
	1.000	18 ± 6
Feeding	0	14 ± 5
	0.500	15 ± 5
Surface Contamination	0	12 ± 2a
	0.500	31 ± 7b

1/ Mean of three replications; 50 larvae per replication

2/ $\bar{x}$ ± standard deviation of the mean

In another test, moths were fed sugar solution containing 64000 IU/ml of Dipel. Eggs obtained from the females during the first 24 hour period were incubated as described previously, and 50 newly-hatched larvae per treatment were evaluated for Dipel effects as described above.

Results of these tests are shown in Table 55. These data also indicated that adults were not transmitting the pathogen to their eggs.

Surface Contamination and Pathogen Transmission

Cheese-cloth sheets were sprayed with Dipel, at the rate of 0.5 lb./A, using a hand-gun sprayer. Contaminated sheets then served as covers for containers that served as oviposition sites for three-day old females. The next day sheets containing eggs were removed and incubated in an environator at 30 ± 1°C. Fifty larvae emerging from those eggs were transferred into media cups for mortality observations. Readings were taken for six subsequent days.

Results indicated that newly-laid eggs could obtain the pathogen from the contaminated surface (Table 55). Emerging larvae apparently obtained the pathogen by consuming part of the contaminated egg shell.

Dipel vs. Tobacco Budworm Moths

This study was designed to investigate the effects of Dipel on longevity and fecundity of H. virescens moths. The literature indicated that previous work overlooked the direct influence of Dipel on the adult stage of the tobacco budworm.

Dipel was mixed with 5% sugar solution to give 32000 IU/ml. This mixture served as a continuously-available food source for the moths. In another treatment Dipel was deactivated and fed to moths at the same rate as the active Dipel (2 mg/ml). Deactivation was accomplished by the dry-goods autoclave method for two hours at 104°C. Moths fed only 5% sugar solution served as a control treatment.

Data from these tests are shown in Table 56. The results indicated that Dipel does have an adverse effect on the adult stage. Longevity and fecundity were significantly reduced when adults were fed Dipel compared to those fed deactivated Dipel or only 5% sugar solution. Egg-laying occurred only with 3- and 4-day-old females and that ceased on the second or third day after introduction. In most cases, females died within one to two days after they ceased oviposition. One- and two-day-old males and females did not mate and no eggs were observed. No significant difference was found between the inactive Dipel and control treatments. These data point to the possibility of utilizing Dipel against the adult stage in addition to the egg and larvae stages. Dipel could be used in combination with a feeding stimulant and/or a pheromone to suppress populations by reducing adult longevity and/or fecundity.

Dipel-Fed Moths vs. Recovery

The purpose of this test was to determine if tobacco budworm moths could recover after short feeding periods on a Dipel solution. Cotton terminals were sprayed with either Dipel in water, Dipel in

Table 56. Influence of Dipel on longevity and fecundity of tobacco budworm moths

Age of moths	Treatment	# moths	Longevity (days)1/	# eggs/female
newly-emerged	Dipel	70	4.1 + 1.3a 3/ 4/	2/
	inactive Dipel	35	13.1 + 3.4b	
	control	20	14.4 + 3.5b	
two-day old	Dipel	16	3.8 + 1.2a	2/
	inactive Dipel	15	14.2 + 3.2b	
	control	15	14.1 + 3.7b	
three-day old	Dipel	20	4.2 + 1.0a	258 + 66 5/
	inactive Dipel	20	13.8 + 4.0b	835 + 179b
	control	20	14.2 + 3.5b	825 + 294b
four-day old	Dipel	40	4.1 + 0.9a	419 + 91a 6/
	inactive Dipel	30	12.5 + 3.8b	846 + 366b
	control	30	13.8 + 4.0b	934 + 343b

1/ days after beginning of feeding

2/ newly-emerged and two-day old moth did not lay eggs

3/ $\bar{x} \pm$ standard deviation of mean

4/ means in the same column followed by the same letter are not significantly different at 0.05 level of probability

5/ mean of 12 females

6/ mean of 10 females

sugar solution or with 5% sugar solution alone. Dipel was used at the rate of 4 mg/ml (64000 IU/ml). Treated terminals were placed individually in microcages with a pair of moths introduced into each. The moths were left in the microcages for 24 hours, and then transferred to moth-rearing cages with a 5% sugar-solution provided as food.

Data presented in Tables 57 and 58 indicate that Dipel + sugar solution was more effective against the moths than was the Dipel in water. Most moths died within one to three days after the initial feeding; however, some moths were able to recover from the effect of Dipel. Recovery was higher when adults were fed only Dipel (Table 57). Apparently, sugar solution was needed to stimulate feeding. If the moth failed to obtain a lethal quantity of the toxin, it resumed normal activities two to three days after exposure to the sugar solution alone. Mating and egg-laying were normal for the surviving adults, however, fecundity was reduced compared to the control treatment (Table 58). These results confirm the previous observation on the need for a stimulant to enhance adult feeding. A more comprehensive study is needed, however, to conclusively demonstrate the utility of this method of pest population suppression.

Dipel vs. *G. Punctipes*

Geocoris adults and nymphs were provided with Dipel solution, Dipel plus sugar solution or sugar solution alone. In all treatments, the predators were provided with freshly-killed fifth-instar *H. virescens* larvae. During the ten-day observation period, there was no

Table 57. Influence of Dipel on mortality of tobacco budworm moths.

Age of Treated Moth	Treatment[1]	Initial No.	No. Dying[2] in $\leq$ 4 days	% Mortality	No. Dying in $>$ 4 days	% Mortality
Newly-emerged	Control	10	--	0 a[3]	10	100a
	Dipel	18	5	28ab	13	72ab
	Dipel + 5% sugar solution	20	10	50b	10	50b
Three-Days Old	Control	23	5	22ab	18	78ab
	Dipel	25	10	40ab	15	60ab
	Dipel + 5% sugar solution	28	20	71b	8	29b

1/ moths were exposed to Dipel for only 24 hours.

2/ Days after the initiation of the treatment.

3/ Numbers on the same column that are not followed by a common letter are significantly different at 0.05 level of probability.

Tabe 58. Influence of Dipel on longevity and fecundity of tobacco budworm moths.

Age of moths	Treatment 1/	Initial Number	No. Dying in ≤ 4 Days	$\bar{x}$ Longevity (days)	No. Dying in > 4 Days	$\bar{x}$ Longevity (days)	Fecundity of Surviving Females (# eggs/ female) # female	$\bar{\bar{x}}$ ± S.D.
newly-emerged	Control	10	---	---	10	12.7 + 5.1a 2/3/	5	683 + 196a
	Dipel	18	5	3.8 + 0.5a	13	10.6 + 3.7ab	5	481 + 132ab
	Dipel + 5% sugar solution	20	10	3.0 + 1.1b	10	9.2 + 3.3b	5	341 + 156b
three-days old	Control	23	5	3.7 + 0.5ab	18	12.6 + 4.0 a	5	715 + 153ac
	Dipel	25	10	3.1 + 0.8ab	15	10.2 + 3.1ab	5	441 + 202ab
	Dipel + 5% sugar solution	28	20	2.7 + 1.0b	8	7.8 + 2.5b	5	427 + 221b

1/ moths were exposed to Dipel for only 24 hours.

2/ $\bar{x}$ ± standard deviation of the mean.

3/ numbers in the same column that are not followed by a common letter are significantly different at the 0.05 level of probability.

apparent effect on either stage. Nymphs molted normally to the next instar, adults mated, and females laid fertile eggs. Dipel was successfully used to adversely affect the tobacco budworm and at the same time, it showed no deleterious effect on the predator, *Geocoris punctipes*.

CONCLUSIONS

Greenhouse and field studies showed that first-larval instar of the tobacco budworm was more susceptible to all dosage levels of Dipel tested. The higher dosages of the Dipel (0.5 and 1.0 lb./A) gave better control against the second- and third-instars and remained effective for longer periods than did the lower dosages of 0.125 and 0.250 lb./A.

When field-treated terminals were returned to the laboratory for bioassay against the first three larval instars of tobacco budworm, a considerable reduction in efficacy was evident with each subsequent post-application date bioassay. However, higher dosages persisted longer than did the lower dosages.

The addition of the feeding stimulant, Coax, at 0.25 lb./A, to all Dipel treatments resulted in significant increases in larval mortality of all instars tested, but the first was the most susceptible. Coax enhanced Dipel activity by either increasing larval feeding on the treated plant parts or by protecting the pathogen and its associated toxin from natural environmental elements such as ultraviolet light.

Data concerning the ability of larvae to recover from exposure to Dipel indicated that recovery does occur, and that this should be taken into consideration when dealing with field applications against natural populations of the tobacco budworm. Since larvae wander about the plant, their continuous exposure to the toxin is by no means certain.

The effects of Dipel on certain characteristics of the larvae and pupae were directly proportionate to the dosage level used in the exposure period. Larval and pupal developmental times were increased with exposure to Dipel-treated food and larval survival and pupal weights were decreased. Fecundity was reduced but no apparent effect was shown relative to longevity or egg viability of adults surviving larval treatment.

Dipel had significant effects on newly-hatched larvae when it was applied directly on the egg. All dosages tested were effective up to three days post-application. Once egg laying reaches the maximum and first-instar larvae start to emerge, Dipel treatments should be applied.

Data concerning the efficacy of Geocoris plus various dosages of Dipel against the first-larval instar of the tobacco budworm, indicate that these two biological agents can be utilized in the field to suppress an increasing population of H. virescens. A minimum of about 3287 Geocoris per acre is needed in the field to keep a tobacco budworm population in check. However, other natural enemies are usually present, thereby reducing the number of Geocoris needed for suppression of tobacco budworm populations. If the tobacco budworm population exceeds the economic threshold, any dosage of Dipel can be applied for a quick knock-out; however, for longer-lasting effects, higher dosages of 0.5 to 1.0 lb./A are required.

Finally, these data have provided additional information on the use of biological insecticides for management of tobacco budworm populations. Biological insecticides are particularly important environmentally and are compatible with other natural control agents.

APPENDIX A

ARCSIN TRANSFORMATION OF PERCENT MORTALITY FOR LISTED TOBACCO BUDWORM LARVAL INSTAR FED ON DIPEL-TREATED GREENHOUSE COTTON.

Table A1. First-instar.

Dipel Rate	Mortality for indicated residue period			
(lb/A)	0-day	2-day	4-day	6-day
0	0.38[1/]	0.39	0.39	0.38
0.125	1.13	0.93	0.76	0.65
0.250	1.16	0.99	0.78	0.62
0.500	1.26	1.09	0.86	0.73
1.000	1.35	1.22	0.96	0.74

1/ L.S.D. = 0.08

Table A2. Second-instar.

Dipel Rate (lb/A)	Mortality for indicated residue period 0-day	2-day	4-day	6-day
0	0.42[1/]	0.44	0.43	0.43
0.125	1.11	1.70	0.72	0.55
0.250	1.19	1.02	0.78	0.55
0.500	1.27	1.12	0.88	0.68
1.000	1.29	1.13	0.89	0.69

1/ L.S.D. = 0.08

Table A3. Third-instar.

Dipal Rate (lb/A)	Mortality for indicated residue period 0-day	2-day	4-day	6-day
0	0.46[1/]	0.48	0.50	0.48
0.125	0.97	0.84	0.66	0.52
0.250	1.15	0.96	0.78	0.60
0.500	1.19	1.01	0.80	0.64
1.000	1.27	1.04	0.83	0.65

1/ L.S.D. = 0.08

APPENDIX B

ARCSIN TRANSFORMATION OF PERCENT MORTALITY FOR LISTED TOBACCO BUDWORM LARVAL INSTAR FED ON DIPEL-TREATED FIELD COTTON.

Table B1. First-instar.

Dipel Rate (lb/A)	Arcsin of mortality for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	0.37[1/]	0.36	0.39	0.38	0.36
0.125	1.02	0.85	0.87	0.55	0.47
0.250	1.13	0.94	0.94	0.58	0.52
0.500	1.20	1.07	0.97	0.62	0.53
1.000	1.27	1.11	1.01	0.65	0.54

1/ L.S.D. = 0.19

Table B2. Second-instar.

Dipel Rate (lb/A)	Arcsin of mortality for indicated residue period				
	0-day	1-day	2-day	3-day	4-day
0	0.34 1/	0.31	0.36	0.29	0.33
0.125	1.10	0.90	0.79	0.55	0.44
0.250	1.15	1.10	0.99	0.67	0.54
0.500	1.22	1.19	1.10	0.67	0.56
1.000	1.26	1.18	1.13	0.77	0.60

1/ L.S.D. = 0.19

Table B3. Third-instar.

Dipel Rate	Arcsin of mortality for indicated residue period				
(lb/A)	0-day	1-day	2-day	3-day	4-day
0	0.31 1/	0.27	0.31	0.28	0.23
0.125	0.85	0.74	0.58	0.49	0.32
0.250	0.99	0.90	0.72	0.50	0.45
0.500	1.17	1.11	0.99	0.64	0.46
1.000	1.24	1.18	1.07	0.68	0.49

1/ L.S.D. = 0.19

APPENDIX C

ARCSIN TRANSFORMATION OF PERCENT MORTALITY FOR LISTED TOBACCO BUDWORM LARVAL-INSTAR FED ON VARIOUS COMBINATIONS OF DIPEL AND COAX

Table C_1 First-instar

	Rate	Mortality for indicated residue period				
Treatment	(lb/A)	0-day	1-day	2-day	3-day	4-day
Dipel	0	0.30[1/]	0.33	0.35	0.32	0.23
Dipel + Coax	0 + 0.250	0.42	0.35	0.35	0.36	0.30
Dipel	0.125	1.18	0.81	0.71	0.60	0.57
Dipel + Coax	0.125 + 0.250	1.26	1.13	1.00	0.73	0.51
Dipel	0.250	1.21	1.00	0.84	0.69	0.61
Dipel + Coax	0.250 + 0.250	1.34	1.26	1.15	0.94	0.81
Dipel	0.500	1.26	1.10	0.98	0.81	0.65
Dipel + Coax	0.500 + 0.250	1.40	1.35	1.20	1.03	0.91
Dipel	1.000	1.30	1.12	1.03	0.88	0.67
Dipel + Coax	1.000 + 0.250	1.42	1.39	1.30	1.10	0.98

L/ L.S.D. = 0.14

Table C2 Second-instar

	Rate	Mortality for indicated residue period				
Treatment	(lb/A)	0-day	1-day	2-days	3-days	4-days
Dipel	0	0.30	0.30	0.30	0.30	0.30
Dipel + Coax	0 + 0.250	0.50	0.40	0.40	0.30	0.30
Dipel	0.125	1.08	0.87	0.63	0.56	0.33
Dipel + Coax	0.125 + 0.250	1.10	0.97	0.89	0.69	0.49
Dipel	0.250	1.16	0.94	0.80	0.70	0.42
Dipel + Coax	0.250 + 0.250	1.23	1.13	0.99	0.87	0.64
Dipel	0.500	1.20	1.07	0.92	0.84	0.50
Dipel + Coax	0.500 + 0.250	1.30	1.23	1.11	0.98	0.80
Dipel	1.000	1.27	1.14	0.94	0.89	0.57
Dipel + Coax	1.000 + 0.250	1.36	1.30	1.19	1.07	0.88

1/ L.S.D. = 0.14

Table C_3 Third-instar

	Rate	Mortality at indicated residue period				
Treatment	(lb/A)	0-day	1-day	2-days	3-days	4-days
Dipel	0	0.22[1/]	0.18	0.19	0.16	0.20
Dipel + Coax	0 + 0.250	0.29	0.30	0.26	0.16	0.16
Dipel	0.125	0.82	0.71	0.48	0.31	0.27
Dipel + Coax	0.125 + 0.250	0.99	0.79	0.68	0.53	0.28
Dipel	0.250	0.90	0.87	0.66	0.42	0.33
Dipel + Coax	0.250 + 0.250	1.12	0.95	0.84	0.62	0.38
Dipel	0.500	0.98	0.92	0.81	0.52	0.36
Dipel + Coax	0.500 + 0.250	1.16	1.03	0.90	0.69	0.56
Dipel	1.000	1.11	1.01	0.86	0.62	0.44
Dipel + Coax	1.000 + 0.250	1.23	1.18	0.96	0.76	0.62

1/ L.S.D. = 0.14

APPENDIX D

ARCSIN TRANSFORMATION OF SURVIVAL OF SECOND-INSTAR TOBACCO BUDWORM LARVAE EXPOSED TO VARIOUS DIPEL FEEDING PERIODS.

Dipel Rate (lb/A)	Feeding Period (hrs)	Arcsin of mean survival for indicated developmental period		
		Inoculation to 3rd-instar	3rd-instar to pupation	pupation to adult emergence
0	6	1.29 [1/]	1.20	1.20
	18	1.32	1.22	1.17
	30	1.31	1.20	1.17
0.50	6	1.14	1.06	1.03
	18	0.90	0.86	0.86
	30	0.79	0.72	0.70
1.00	6	1.08	1.00	0.97
	18	0.84	0.77	0.74
	30	0.73	0.68	0.67

1/ L.S.D. = 0.15 for either column.

APPENDIX E

ARCSIN TRANSFORMATION OF PERCENT SURVIVAL OF FIRST-INSTAR TOBACCO BUDWORM LARVAE EMERGING FROM DIPEL-TREATED EGG.

Dipel Rate (lb/A)	Percent survival at indicated residue period		
	1-Day	2-Days	3-Days
0	1.26 [1/] [2/]	1.24	1.22
0.125	0.34	0.51	0.62
0.250	0.25	0.40	0.45
0.500	0.20	0.36	0.36
1.000	0.00	0.22	0.30

1/ Survival based on that occuring at 6 days after eclosion.

2/ L.S.D. = 0.08

APPENDIX F

ARCSIN TRANSFORMATION OF PERCENT SURVIVAL OF FIRST-INSTAR TOBACCO BUDWORM LARVAE TREATED WITH VARIOUS COMBINATIONS OF DIPEL AND *GEOCORIS PUNCTIPES* FOR INDICATED STUDIES.

Table F_1. Greenhouse Studies

Dipel Rate (lb/A)	Residue Period (days)	*Geocoris* Levels[1/] I	II	III	IV	V
0	0	1.01[2/]	0.84	0.80	0.49	0.26
	2	1.01	0.87	0.82	0.50	0.26
	4	0.98	0.87	0.81	0.48	0.25
	6	0.98	0.84	0.79	0.50	0.25
0.125	0	0.36	0.33	0.30	0.23	0.20
	2	0.67	0.63	0.45	0.36	0.22
	4	0.87	0.79	0.74	0.47	0.27
	6	0.96	0.84	0.79	0.50	0.27
0.250	0	0.30	0.23	0.25	0.11	0.12
	2	0.53	0.43	0.42	0.27	0.22
	4	0.71	0.72	0.70	0.44	0.26
	6	0.89	0.83	0.82	0.50	0.29
0.500	0	0.30	0.23	0.23	0.11	0.14
	2	0.50	0.41	0.38	0.27	0.20
	4	0.74	0.63	0.62	0.40	0.27
	6	0.91	0.81	0.81	0.48	0.26
1.000	0	0.27	0.23	0.18	0.11	0.0
	2	0.45	0.40	0.36	0.26	0.20
	4	0.68	0.56	0.59	0.39	0.23
	6	0.89	0.79	0.79	0.47	0.25

1/ Geocoris level: I = 0, II = 1 per 4 pots/row, III = 1 per 3 pots/row, IV = 1 per 2 pots/row, V = 1 per 1 pot/row.

2/ L.S.D. = 0.06

Table F_2. Field Studies

Dipel Rate (lb/A)	Geocoris[2/] Levels	Residue Period 0-day	Residue Period 2-days
0	1	.78[1/]	.80
	2	.58	.65
	3	.53	.52
0.125	1	.37	.70
	2	.32	.57
	3	.21	.47
0.250	1	.30	.59
	2	.23	.49
	3	.15	.40
0.500	1	.26	.51
	2	.15	.42
	3	.11	.35
1.000	1	.23	.45
	2	.05	.38
	3	.00	.26

1/ Geocoris levels: 1 = 0, 2 = one per 4 ft., 3 = one per 2 ft.

2/ L.S.D. = .12 (0.05 level of probability)

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