EFFECT OF BACILLUS THURINGIENSIS VAR. THURINGIENSIS BERLINER ON THE PINK BOLLWORM, PECTINOPHORA GOSSYPTELLA (SAUNDERS)

by

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In Partial Fulfillment of the Requirements For the Degree of MASTER OF SCIENCE In the Graduate College THE UNIVERSITY OF ARIZONA

1970
STATEMENT BY AUTHOR

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APPROVAL BY THESIS DIRECTOR

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Professor of Entomology

Jan. 20, 1978
Date
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS AND MATERIALS</td>
<td>6</td>
</tr>
<tr>
<td>Laboratory Tests</td>
<td>6</td>
</tr>
<tr>
<td>Field Tests</td>
<td>11</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>14</td>
</tr>
<tr>
<td>Laboratory Tests</td>
<td>14</td>
</tr>
<tr>
<td>Field Tests</td>
<td>18</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>25</td>
</tr>
<tr>
<td>REFERENCES CITED</td>
<td>27</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Composition of lima bean medium</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>Mortality of first-instar larvae placed on plants dipped in solutions of Biotrol BTB 183-25W</td>
<td>17</td>
</tr>
<tr>
<td>3.</td>
<td>Mortality of first-instar larvae placed on plants covered with dust mixtures of Biotrol BTB 183-25W</td>
<td>17</td>
</tr>
<tr>
<td>4.</td>
<td>Analysis of variance for dosage treatments on Field 0</td>
<td>21</td>
</tr>
<tr>
<td>5.</td>
<td>Analysis of variance for timing interval treatments on Field T</td>
<td>22</td>
</tr>
<tr>
<td>6.</td>
<td>Treatment differences as indicated by bolls collected from Field 0 on July 9 and held two weeks before examination</td>
<td>23</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dosage-mortality curve at 72 hours for first-instar pink bollworm larvae fed B. thuringiensis incorporated in artificial medium</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>Population growth of the pink bollworm, shown as percent infested bolls</td>
<td>19</td>
</tr>
</tbody>
</table>
ABSTRACT

Laboratory tests were made to determine the susceptibility of the pink bollworm in Arizona to Biotrol BTB 183, a commercial preparation of *Bacillus thuringiensis* var. *thuringiensis*. Field tests were made to determine if this preparation could be used effectively for early season control of the pink bollworm on cotton.

The laboratory tests showed that first-instar pink bollworm larvae are susceptible to Biotrol BTB 183 when fed this material incorporated in artificial medium. Some mortality was obtained by placing first-instar larvae on the leaf surface of treated cotton plants for short periods of time and then returning them to artificial medium. In the field tests, several application rates of both a wettable powder and a dust were compared, but none of these treatments successfully suppressed the pink bollworm populations.
INTRODUCTION

The bacterium, *Bacillus thuringiensis* var. *thur- ingiensis* Berliner, was first isolated by Berliner in 1911 from diseased larvae of the Mediterranean flour moth, *Anagasta kuhniella* (Zell.), received from a flour mill in Thuringia, Germany (Heimpel, 1967). *Bacillus thuringiensis* var. *thuringiensis* is a spore-forming bacillus related morphologically to *Bacillus cereus* Frankland and Frankland, but in addition to forming a resistant spore in the sporangium at the time of sporulation, each vegetative cell produces a proteinaceous crystal of endotoxin pathogenic to many lepidopterous larvae.

Several entomogenous, crystal-bearing, spore-forming bacilli are known, and Heimpel and Angus (1958) proposed a complete and new classification of this group, designating *Bacillus thuringiensis* var. *thuringiensis* Berliner as the type strain. This organism will henceforth be referred to as *B. thuringiensis* in this paper.

The proteinaceous crystal or parasporal body is the principal insecticidal agent in commercial preparations of *B. thuringiensis*. It is a stomach poison for many lepidopterous larvae and must be ingested to be effective. It is water insoluble and may be deposited on the plant
surfaces in a water suspension spray or in a dust formulation. Commercially produced preparations of *B. thuringiensis*, registered with the U. S. Department of Agriculture, are exempt from requirements of a residue tolerance.

Steinhaus (1951) collated much of the literature on *B. thuringiensis* and showed in laboratory and field tests that infestations of the alfalfa caterpillar, *Colias eurytheme* Boisd., can be reduced below economically important levels with proper applications of a bacillus preparation. Since then, tests of a variety of formulations of *B. thuringiensis* have been conducted against many lepidopterous pests in both laboratory and field. This work is reviewed by Heimpel and Angus (1960), Franz (1961), Jaques (1964) and Heimpel (1967). One of the most successful uses of *B. thuringiensis* has been against the cabbage looper, *Trichoplusia ni* (Hubner) (Hall et al., 1961).

Heimpel and Angus (1959) divided the responses of various Lepidoptera to the crystalline endotoxin into three main types. In types I and II the endotoxin crystals are quickly broken down by the alkaline gut contents and mid-gut paralysis occurs a few minutes after ingestion. Type I larvae are then stricken by a general paralysis caused by an increase in hemolymph pH. This increase in hemolymph pH is thought to be caused by leakage of the alkaline gut
contents, through the damaged gut wall, into the poorly buffered hemolymph. Type II larvae suffer no increase in hemolymph pH and die in 2 to 4 days without general paralysis. Most susceptible larvae, including the pink bollworm, fall into this category. Septicemia may occur, with large numbers of vegetative cells multiplying in the body cavity when a mixture of spores and crystalline material is ingested. Type III is represented solely by the larva of *Anagasta kuhniella* which dies in 2 to 4 days without symptoms of general paralysis, but is susceptible only when mixtures of spores and crystalline material are ingested.

Disease organisms were isolated from the pink bollworm as early as 1936, when White and Noble (1936) isolated a bacillus and several other organisms from sick larvae in a culture.

Ignoffo (1962a) injected spores of *B. thuringiensis* directly into the hemocoel of non-feeding, fourth-instar pink bollworm larvae and found that there is a positive linear relationship between probit-percent mortality and log-spore concentration for both diapausing and non-diapausing larvae. Ignoffo (1962b) also demonstrated that the usual temperatures and humidities in the field should not adversely affect spore stability and effectiveness of *B. thuringiensis*. 
Ignoffo and Graham (1967) conducted laboratory tests to determine whether first-instar pink bollworm larvae could be controlled with simulated foliar applications of a commercial \textit{B. thuringiensis} preparation. The larvae were exposed to various dust concentrations of Thuricide S9-517 diluted with lactose, and then placed in individual vials containing a synthetic diet and held for observation. An LC$_{50}$ for the larvae was estimated at $25 \times 10^7$ spores per gram of material.

Bullock and Dulmage (1969) conducted tests in Texas in which Thuricide S8-141, in a solution containing $60 \times 10^{12}$ spores per gallon, was applied early in the season and evaluated for control of the pink bollworm on cotton in field cages. After six weekly applications, 80% of the bolls in the control cages were infested compared with 40 and 10% infested bolls in cages treated with 1 and 2 quarts per acre, respectively.

The pink bollworm has been a pest of cotton in Arizona since 1926, and is now an economically important pest in almost all cotton producing areas in the state (Watson and Fullerton, 1969). Certain cultural practices are helpful in reducing the spring emergence of moths, but insecticidal treatments are still necessary in many areas for suppression of populations, particularly in the latter part of the growing season. These treatments are expensive.
and may cause outbreaks of other pests by disruption of
the ecological balance between the pests and their natural
enemies.

The purpose of this study was (1) to determine
under Arizona conditions how susceptible the pink bollworm
is to B. thuringiensis, and (2) to determine whether a
commercial preparation of B. thuringiensis can be used
effectively for early season control of the pink bollworm.
It was thought that if early season applications could
significantly reduce populations the need for later chemical
insecticide applications might be reduced. Since the insec
ticidal action of B. thuringiensis is selective, the
beneficial insect populations would not be disrupted.
METHODS AND MATERIALS

Laboratory Tests

Attempts have been made by various workers to use viable spore counts as a means of estimating insecticidal activity or potency of formulations of *B. thuringiensis*. The activity of the pathogen against insects does not always correlate with spore count, however, since the proteinaceous crystal and other by-products as well as the viable spore may have an effect on a susceptible host (Heimpel and Angus, 1960). Variations in activity can also occur between strains of *B. thuringiensis* (Heimpel, 1967).

Menn (1960), Splittstoesser and McEwen (1961) and Mechalas and Dunn (1964) describe bioassay procedures by which effectiveness of the collective factors involved in causing mortality can be measured directly. Methods developed by these workers were employed in the present study.

The pink bollworm larvae used in these tests were taken from a culture established by Rush (1969) from moths obtained from field-collected bolls grown on the University of Arizona Agricultural Experiment Station Farm at Mesa, Arizona. The culture was maintained by the methods described by Rush, except that the wheat-germ diet was replaced with a lima bean diet modified from Shorey (1963).
A technique, described by Splittstoesser and McEwen (1961) and by workers of Nutrilite Products, Inc. (Anonymous, 1968), in which various dosage levels of microbial material are uniformly dispersed in an artificial medium, was used to establish an LC₅₀ for first-instar pink bollworm larvae. The lima bean diet was used as the medium or substrate for evaluating the dosage levels. The microbial material used was Biotrol BTB 183-25W, a wettable powder produced by Nutrilite Products, Inc. with a concentration of $25 \times 10^9$ viable spores per gram of material.

A stock solution was prepared by adding 1 gm of microbial material to 100 ml of distilled water and mixing for 2 minutes in a Custom Osterizer. The material was then allowed to soak for 30 minutes, blended again for 3 minutes, and allowed to stand 5 minutes to settle out large particles of impurity. Dosage levels were formulated by pipetting measured amounts of stock solution and thoroughly blending these with weighed amounts of artificial medium. For example, 20 ml of stock solution blended with 180 gm of medium would give a dosage of 100 mg of microbial material per 100 gm of medium.

For preparing the medium (see Table 1) 300 gm of dry lima beans were soaked in warm water for about 12 hours until maximum water absorption was reached. The soaked beans, ascorbic acid (L), methyl-p-hydroxybenzoate, and
Table 1. Composition of lima bean medium.\textsuperscript{a}\

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lima beans (soaked)</td>
<td>600 gm</td>
</tr>
<tr>
<td>Ascorbic acid (L)</td>
<td>6 gm</td>
</tr>
<tr>
<td>Methyl-p-hydroxybenzoate</td>
<td>6 gm</td>
</tr>
<tr>
<td>Brewer's yeast</td>
<td>60 gm</td>
</tr>
<tr>
<td>Agar</td>
<td>26 gm</td>
</tr>
<tr>
<td>Tap water</td>
<td>1200 ml</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Modified after Shorey (1963) to fill approximately 150 individual portion cups.

Yeasts were blended with 600 ml of tap water in a C-5 Waring blender until smooth. The agar was mixed with 600 ml of warm tap water and heated until boiling. When the agar began to thicken, the blender was started again and the agar added to the mixture. Portions of the medium were then weighed out in 200 ml dixie cups, and the dosage levels of \textit{B. thuringiensis} were formulated and blended before the agar hardened. Individual one-ounce portion cups were then half-filled with the medium plus microbial material. The temperature of the medium at the time of blending was about 115-120\textdegree F. and the pH was about 6. Angus (1956) showed that crystals of endotoxin suspended
in water are not inactivated when exposed to 194°F. for 1 hour, and remain stable in solutions as acidic as pH 4.

Single first-instar larvae, reared under uniform conditions and of uniform age, were placed in each one-ounce cup, and the cups were sealed with cardboard pull lids. The cups were held at 83°F. and about 50% R.H. and were checked for mortality after 24, 48, 72, and 96 hours.

After range-finding studies, five concentrations were chosen to yield mortalities between 20 and 90%. The greatest increment in mortality was recorded at 72 hours, with little increase in mortality after this time. The 72-hour interval was therefore selected as the time for recording final mortality. A control group was used, in which larvae were placed on untreated medium, and Abbott's formula (Abbott, 1925) was used to correct for natural mortality. The test was repeated four times, using 25 cups of larvae per dosage level per replication, for a total of 100 larvae per dosage for the entire study.

A dosage-mortality line was plotted on log dosage-probit scales, and the LC$_{50}$, slope and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (1949) for the evaluation of dose-effect experiments.

In bioassay procedures using foliage-eating insects, green plant foliage can be used as the substrate on which
the preparation to be evaluated is deposited (Mechalas and Dunn, 1964). One of the problems involved in the control of the pink bollworm with insecticides is that the larvae feed within the squares or bolls. The larvae are thus protected against insecticidal applications except for the short period before the newly-hatched larvae penetrate into the squares or bolls. Since the proteinaceous endotoxin produced by *B. thuringiensis* is a stomach poison, the larvae must ingest a lethal dose before or during penetration if control is to be obtained.

Newly-hatched larvae were placed on the leaf surface of uniform-aged cotton plants dipped in various concentrations of Biotrol BTB 183-25W to determine whether any mortality would occur after the larvae had moved about over the plant surfaces. Cotton plants (at the 4 leaf stage) were dipped in various concentrations, prepared by diluting a stock solution such as the one described above, plus a wetting agent, Triton X-100.

The larvae were placed on the leaf surfaces with a camel's hair brush, left for 30 minutes, and returned to medium cups and checked for mortality at 24, 48, 72 and 96 hours. Lukefahr and Griffin (1961) state that newly-hatched larvae usually move over the plant surface for about 30 minutes before entering a square or boll. The test was repeated three times, using four concentrations plus a
control, with five plants per concentration per test and five larvae placed on each plant.

Another test was conducted, using the above described techniques, by dusting plants (at the 4 leaf stage) with dust formulations of various spore concentrations. The dust formulations were made by diluting Biotrol BTB 183-25W with pyrophyllite. The plants were treated on both the upper and lower leaf surfaces with a small hand duster. The test was repeated two times, using three concentrations plus a control, and using five plants per concentration per test with five larvae placed on each plant.

Field Tests

Field tests were conducted during the summer of 1969, at the University of Arizona Experiment Station Farm at Yuma, Arizona. This area was chosen for the tests because of a high incidence of pink bollworm in Yuma County during the 1968 season. The microbial materials tested were Biotrol BTB 183-25W and Biotrol BTB 183-2.5D, a dust with $2.5 \times 10^9$ spores per gram of material. The effectiveness of wettable powder and dust formulations was compared at various application rates and timing intervals between applications. The tests were conducted on plots of Delta Pine 16 cotton planted on March 24.
The wettable powder and dust formulations were compared in a 5 acre field (Field 0) using rates of 2, 4 and 6 pounds of wettable powder, and 20, 40 and 60 pounds of dust per acre. Triton X-100 was added to wettable powder formulations as a wetting agent. Each treatment, including a check, was replicated four times in a randomized complete block design. The first applications were made on May 21, just after the first squares began to appear, and applications were continued at 5 to 6 day intervals until July 24, after the first mature bolls appeared.

Another 3 acre field (Field T) was used to test timing intervals of 7 and 14 days between applications, using 4 pounds of wettable powder and 40 pounds of dust per acre. Each treatment, including a check, was again replicated four times in a randomized complete block design. Applications were first made on May 21, and continued until July 21.

It was originally planned to use a tractor-drawn Bean sprayer and a tractor mounted duster to make all applications to treatment plots 6 rows wide and 300 feet long. Due to difficulties encountered in calibrating the machinery and because of problems anticipated in getting a tractor into the fields after irrigations, it was decided to use a Hudson hand sprayer and a Hudson rotary hand duster on smaller plots located in the middle 100 feet
of the center two rows of the original six row plots, leaving the remaining area of the original plots untreated.

The treatments were evaluated by making weekly examinations of 25 squares per plot through July 1. Ten bolls per plot were also collected and examined on July 1. One hundred bolls per plot were collected on July 9 from Field 0 and on July 10 from Field T. Fifty bolls per plot were examined immediately, and 50 bolls per plot were held two weeks before examination to insure accurate counts since some of the newly-hatched larvae might be missed in the bolls examined immediately. One hundred bolls per plot were collected from both fields on July 18, and examination of bolls performed as described above. Fifty bolls per plot were collected from Field 0 on July 25 and examined immediately.

The analysis of variance and Duncan's Multiple Range Test (Duncan, 1955) were used in analyzing the results of the treatments.
RESULTS AND DISCUSSION

Laboratory Tests

The dosage-mortality line for first-instar pink bollworm larvae fed on medium incorporated with *B. thuringiensis* is presented in Figure 1. Dosages are expressed as milligrams of Biotrol BTB 183-25W per 100 grams of medium. The $LC_{50}$ was calculated as $5.2 \text{ mg material/100 gm}$ medium with 95% confidence limits of $4.0 \text{ mg to 6.8 mg/100 gm}$. The slope of the line was calculated to be $3.89$ with 95% confidence limits of $2.90$ to $5.21$.

Workers at Nutrilite Products (Anonymous, 1968) established an $LC_{50}$ of $100 \text{ mg Biotrol BTB 183-25W/100 gm}$ medium for both the bollworm, *Heliothis zea* (Boddie), and the beet armyworm, *Spodoptera exigua* (Hbn.), at 6 days. The $LC_{50}$'s are much higher than that established for the pink bollworm larvae in this study, but they are for late instar larvae. However, these comparisons indicate that the first-instar pink bollworm larvae are relatively susceptible to *B. thuringiensis*. The workers gave no slopes for the mortality curves obtained for the bollworm and beet armyworm.

Splittstoesser and McEwen (1961) obtained an $LC_{50}$ expressed as $0.084 \text{ mg microbial material/ml medium and a}$
LC$_{50}$ and 95% Confidence limits:  
5.2 (4.0 to 6.8 mg/100 gm)

Slope and 95% Confidence limits:  
3.89 (2.90 to 5.21)

Fig. 1. Dosage-mortality curve at 72 hours for first-instar bollworm larvae fed B. thuringiensis incorporated in artificial medium.
slope of 4.83 for third-instar cabbage looper larvae fed on medium with incorporated *B. thuringiensis*. Since the lima bean medium used in the present study on the pink bollworm weighs approximately 95 gm per 100 ml, the \( LC_{50} \) for first-instar pink bollworm larvae is slightly less than .052 mg material/ml medium. Thus, the \( LC_{50} \) is less than that for third-instar cabbage looper larvae. The steeper slope obtained by Splittstoesser and McEwen for the cabbage looper larvae indicates that the response of the pink bollworm larvae to *B. thuringiensis* is more variable than that shown by the looper larvae.

The mortality was low for first-instar larvae placed on treated cotton plants (Tables 2 and 3). The mortality was slightly higher than the natural mortality obtained in the controls for larvae placed either on the plants dipped in wettable powder solutions or those covered with dust mixtures. This indicates that some microbial material was ingested. This could have occurred while the larvae were moving around over the plant surface, or the mortality could have been caused by contamination of the medium after the larvae were placed in medium cups. Almost no differences in mortality were obtained for larvae placed on the plants covered with various dust mixtures, differing only in spore counts.
Table 2. Mortality of first-instar larvae placed on plants dipped in solutions of Biotrol BTB 183-25W.

<table>
<thead>
<tr>
<th>Dosage mg/100 ml</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>6.6</td>
<td>6.6</td>
<td>8</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
<td>10.6</td>
<td>10.6</td>
<td>10.6</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>14.6</td>
</tr>
<tr>
<td>100</td>
<td>8</td>
<td>12</td>
<td>18.6</td>
<td>22.6</td>
</tr>
<tr>
<td>200</td>
<td>6.6</td>
<td>12</td>
<td>21.3</td>
<td>21.3</td>
</tr>
</tbody>
</table>

/ Microbial material contained 25 x 10^9 spores per gram.

Table 3. Mortality of first-instar larvae placed on plants covered with dust mixtures of Biotrol BTB 183-25W.

<table>
<thead>
<tr>
<th>Dosage spores/gm</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2.5 x 10^9</td>
<td>4</td>
<td>14</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>12.5 x 10^9</td>
<td>4</td>
<td>12</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>25 x 10^9</td>
<td>8</td>
<td>16</td>
<td>34</td>
<td>34</td>
</tr>
</tbody>
</table>
Ignoffo and Graham (1967) obtained enough mortality to establish an LC$_{50}$ by exposing first-instar larvae to various dust mixtures. They placed 1 gm samples of each of five concentrations in 12-dram vials with about 100 newly-hatched larvae. The vials were stoppered, rolled gently for 30 seconds to insure contact of the larvae with the dust, and then placed upright. The larvae were then removed and placed in vials containing artificial medium. These authors did not speculate on how the larvae were ingesting lethal doses of material.

**Field Tests**

Figure 2 shows the population growth of the pink bollworm in Field 0 and Field T. The population increases did not occur until sizeable bolls began to appear. The temperature data shown in Figure 2, taken from a standard height weather station at the Yuma Experiment Station Farm, indicate no correlation between mean daily temperature and the population increase.

The highest pink bollworm infestation sampled in the squares was 2.2% in Field 0 on June 2. Consequently, no data were obtained to determine how well squares might be protected with applications of *B. thuringiensis*.

The first population increases were detected in bolls collected from Field 0 on July 9 and from Field T
Fig. 2. Population growth of the pink bollworm, shown as percent infested bolls.
on July 10. The last applications before these sample
dates were on July 7 in both fields. Tables 4 and 5 show
that none of the applications were effective in permanently
suppressing pink bollworm populations. There was a sig­
nificant difference, however, between dosage treatments in
bolls collected from Field 0 on July 9 and held two weeks
before examination (Table 4). Table 6 shows that signifi­
cant reductions of infested bolls occurred from treatments
at the two highest application rates of Biotrol BTB 183-
2.5D dust. The differences in percent infested bolls in
the other treatments can probably be attributed to the
random distribution of the pink bollworm population in the
field. The 6 lb/acre rate of wettable powder showed a
slight though insignificant decrease of infested bolls
compared with the check plots.

The 200 bolls per treatment examined immediately
after each sample did not show a significant difference
between treatments. The laboratory tests indicated that
maximum mortality does not occur until after 72 hours, and
since these bolls were examined before 72 hours, this might
explain why the mortality was not evident until examination
of the bolls which were held for two weeks. In all cases,
a boll was not considered infested unless a live larva was
found inside. After the July 9 samples, no significant
Table 4. Analysis of variance for dosage treatments on Field 0.

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Critical value of F statistic</th>
<th>Computed value of treatment F</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 9&lt;sup&gt;b&lt;/sup&gt;/</td>
<td>2.66</td>
<td>2.00</td>
<td>no</td>
</tr>
<tr>
<td>July 9&lt;sup&gt;c&lt;/sup&gt;/</td>
<td>2.66</td>
<td>7.48</td>
<td>yes</td>
</tr>
<tr>
<td>July 18&lt;sup&gt;b&lt;/sup&gt;/</td>
<td>2.66</td>
<td>1.94</td>
<td>no</td>
</tr>
<tr>
<td>July 18&lt;sup&gt;c&lt;/sup&gt;/</td>
<td>2.66</td>
<td>2.02</td>
<td>no</td>
</tr>
<tr>
<td>July 25&lt;sup&gt;b&lt;/sup&gt;/</td>
<td>2.66</td>
<td>0.92</td>
<td>no</td>
</tr>
</tbody>
</table>

<sup>a</sup>/Critical value of F statistic at the 5% level of probability with 6 and 18 degrees of freedom.

<sup>b</sup>/Two hundred bolls per treatment checked immediately for percent boll infestation.

<sup>c</sup>/Two hundred bolls per treatment held two weeks before checking for percent infested bolls.
Table 5. Analysis of variance for timing interval treatments on Field T.

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Critical value of F statistic&lt;sup&gt;a/&lt;/sup&gt;</th>
<th>Computed value of treatment F</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 10&lt;sup&gt;b/&lt;/sup&gt;</td>
<td>3.26</td>
<td>0.31</td>
<td>no</td>
</tr>
<tr>
<td>July 10&lt;sup&gt;c/&lt;/sup&gt;</td>
<td>3.26</td>
<td>2.58</td>
<td>no</td>
</tr>
<tr>
<td>July 18&lt;sup&gt;b/&lt;/sup&gt;</td>
<td>3.26</td>
<td>1.42</td>
<td>no</td>
</tr>
<tr>
<td>July 18&lt;sup&gt;c/&lt;/sup&gt;</td>
<td>3.26</td>
<td>1.44</td>
<td>no</td>
</tr>
</tbody>
</table>

<sup>a/</sup>Critical value of F statistic at the 5% level of probability with 4 and 12 degrees of freedom.

<sup>b/</sup>Two hundred bolls per treatment checked immediately for percent infested bolls.

<sup>c/</sup>Two hundred bolls per treatment held two weeks before checking for percent infested bolls.
Table 6. Treatment differences as indicated by bolls collected from Field 0 on July 9 and held two weeks before examination.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate lbs/acre</th>
<th>Percent Infested Bolls&lt;sup&gt;a/&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust 40</td>
<td>40</td>
<td>12       a</td>
</tr>
<tr>
<td>Dust 60</td>
<td>60</td>
<td>17.5     ab</td>
</tr>
<tr>
<td>Wettable powder 6</td>
<td>6</td>
<td>23.5     bc</td>
</tr>
<tr>
<td>Check</td>
<td>--</td>
<td>25       c</td>
</tr>
<tr>
<td>Dust 20</td>
<td>20</td>
<td>25       c</td>
</tr>
<tr>
<td>Wettable powder 2</td>
<td>2</td>
<td>26.5     c</td>
</tr>
<tr>
<td>Wettable powder 4</td>
<td>4</td>
<td>27.5     c</td>
</tr>
</tbody>
</table>

<sup>a/</sup>Duncan's Multiple Range Test; values followed by the same letter are not significantly different at 5% level.

Differences were observed between treatments, probably due in part to the increase in population pressure of the pink bollworm.

The data indicate that Biotrol BTB 183 preparations are not effective in controlling the pink bollworm in bolls. This appears to be due to the limited exposure of the larvae to B. thuringiensis in the field, rather than a lack of susceptibility to the bacterium. Al-Azawi (1964) placed larvae of mixed age of the spiny bollworm, *Earias insulana* Boisd., in petri dishes containing cotton leaves...
dipped in *B. thuringiensis* solutions, and in petri dishes containing bolls dipped in the solution. A mortality of 50% was obtained after 72 hours for larvae which fed on the leaves, but almost no dead larvae were found inside the bolls, although many larvae entered the bolls and fed on the fiber and seeds. He attributed this to the fact that the larvae fed very little on the contaminated surface of the bolls.

The results of these tests are at variance with those of Bullock and Dulmage (1969) using Thuricide S8-141. This might be explained in part by the fact that their test was conducted under controlled conditions inside field cages. They released a limited number of moths inside each cage, after the cages had been treated with methyl parathion one week prior to starting the test. The differences in results could also be due to a difference in the effectiveness of Thuricide and Biotrol. The plant coverage obtained should have been about the same, since their applications were also made with hand equipment.
SUMMARY AND CONCLUSIONS

The pink bollworm is an economically important pest in most of the cotton growing areas of Arizona. Chemical insecticide treatments are necessary in many areas for suppression of populations to sub-economic levels, but these treatments may cause outbreaks of other pests due to a disruption of the ecological balance in the field.

Biotrol BTB 183, a commercial preparation of Bacillus thuringiensis var. thuringiensis, was tested both in the laboratory and in the field to determine if this material could be used successfully for early-season control of the pink bollworm in Arizona.

A dosage-mortality curve was obtained by feeding first-instar larvae various concentrations of Biotrol BTB 183-25W incorporated in an artificial medium. It was shown that some mortality is obtained by leaving first-instar larvae on leaf surfaces of treated cotton plants for 30 minutes and then returning them to the artificial medium.

A field test was conducted at Yuma, Arizona, using application rates of 2, 4 and 6 lbs/acre of Biotrol BTB 183-25W and 20, 40 and 60 lbs/acre of Biotrol BTB 183-2.5D on a 5 to 6 day schedule. Another test was conducted at different timing intervals comparing applications of 4 lbs
of wettable powder and 40 lbs of dust per acre. None of the treatments was successful in suppressing the pink bollworm. The dust treatments of 40 and 60 lbs/acre showed a significant reduction of infested bolls on the first sample date after a population increase occurred in the field. On subsequent sampling dates, no significant differences occurred between treatments.

These data indicate that perhaps better coverage is obtained with dust preparations than with wettable powder solutions. Even if the results of this work had shown Biotrol BTB 183 to be an effective control agent, it is not likely that the higher application rates would be practical because of the high cost of the materials.
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