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LABORATORY BIOASSAYS OF SANDOZ 415 (BACILLUS THURINGIENSIS BERLINER) AGAINST SPODOPTERA EXIGUA (HBN.)

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LABORATORY BIOASSAYS OF SANDOZ 415
(BACILLUS THURINGIENSIS BERLINER)
AGAINST SPODOPTERA EXIGUA (HBN.)

by

Michael R. Bell

A Thesis Submitted to the Faculty of the
DEPARTMENT OF ENTOMOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

1985
STATEMENT BY AUTHOR

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS ................................................. v
LIST OF TABLES ............................................................. vi
ABSTRACT ................................................................. vii

INTRODUCTION ............................................................. 1
MATERIALS AND METHODS ................................................ 12

Formulations of B.t. ...................................................... 12
Rearing of BAW ............................................................ 12
Dosage--Mortality of SAN 415 against BAW ......................... 13
Feeding Time--Recovery Experiment .................................. 14
Teratological Effects of SAN 415 on BAW ............................ 15
Relative Toxicities of SAN 415 and Thuricide® HPC ............... 15

RESULTS AND DISCUSSION ............................................. 17

Dosage--Mortality of SAN 415 against Beet
Armyworm (BAW) ....................................................... 17
Feeding Time--Recovery Experiment .................................. 17
Teratological Effects of SAN 415 on BAW ......................... 21
Relative Toxicities of SAN 415 and Thuricide® HPC
Against BAW ............................................................. 33

CONCLUSION .............................................................. 37

APPENDIX A: LIMA BEAN DIET FOR BAW .......................... 39
APPENDIX B: DOSAGE--MORTALITY RELATIONS FOR SAN 415
INGESTED BY NEONATE BAW ......................................... 40
APPENDIX C: MORTALITY OF BAW VS. TIME AFTER REMOVAL
FROM (24 HOUR) LC$_{50}$ ................................................. 41
APPENDIX D: DOSAGE--MORTALITY RELATIONS, INITIATION OF
PUPATION, AND PERCENT EMERGENCE FOR 11 DAY OLD
LARVAE FED SAN 415 (TERATOLOGICAL EFFECTS) ............... 42

LIST OF REFERENCES .................................................. 43
# 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 lines for SAN 415 ingested by neonate BAW</td>
<td>19</td>
</tr>
<tr>
<td>2.</td>
<td>Mortality of BAW vs. time after removal from the 24 hr. LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>Dosage-Mortality curves for 11-day-old BAW fed SAN 415</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>Missing proboscis that occurred when SAN 415 was incorporated into larval diet. The wing tip of the pupa on the left appears deformed. Both insects ingested SAN 415 at a rate of 4.3 x 10&lt;sup&gt;4&lt;/sup&gt; IU per ml larval diet</td>
<td>25</td>
</tr>
<tr>
<td>5.</td>
<td>Missing proboscis in pupae from larvae fed SAN 415 (4.3 x 10&lt;sup&gt;4&lt;/sup&gt; IU per ml diet) in larval diet</td>
<td>26</td>
</tr>
<tr>
<td>6.</td>
<td>Numerous deformities in pupa from larva fed SAN 415 (4.3 x 10&lt;sup&gt;4&lt;/sup&gt; IU per ml diet) in larval diet</td>
<td>27</td>
</tr>
<tr>
<td>7.</td>
<td>Missing sternite in pupa from larva fed SAN 415 (4.3 x 10&lt;sup&gt;4&lt;/sup&gt; IU per ml diet) in larval diet</td>
<td>28</td>
</tr>
<tr>
<td>8.</td>
<td>Deformities in mouthparts and legs of pupa from larva fed SAN 415 (4.3 x 10&lt;sup&gt;4&lt;/sup&gt; IU per ml diet) in larval diet</td>
<td>29</td>
</tr>
<tr>
<td>9.</td>
<td>Numerous severe deformities in pupa from larva fed SAN 415 (8.6 x 10&lt;sup&gt;4&lt;/sup&gt; IU per ml diet) in larval diet</td>
<td>30</td>
</tr>
<tr>
<td>10.</td>
<td>Incompletely sclerotized mouthparts in pupa from larva fed SAN 415 (1.7 x 10&lt;sup&gt;5&lt;/sup&gt; IU per ml diet) in larval diet</td>
<td>31</td>
</tr>
<tr>
<td>11.</td>
<td>Missing legs and mouthparts in pupa from larva fed SAN 415 (6.9 x 10&lt;sup&gt;5&lt;/sup&gt; IU per ml diet) in larval diet</td>
<td>32</td>
</tr>
<tr>
<td>12.</td>
<td>Dosage-Mortality lines for neonate BAW (SAN 415 vs. Thuricide&lt;sup&gt;®&lt;/sup&gt; HPC)</td>
<td>34</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. LC₅₀ and LC₉₅ values with confidence intervals for SAN 415 ingested by neonate BAW</td>
<td>18</td>
</tr>
<tr>
<td>2. LC₅₀ and LC₉₅ values with confidence intervals for SAN 415 and Thuricide® HPC ingested by neonate BAW</td>
<td>36</td>
</tr>
</tbody>
</table>
ABSTRACT

Laboratory bioassays were conducted to determine the susceptibility of *Spodoptera exigua* (Hbn.) to SANDOZ 415 (SAN 415), an unregistered preparation of *Bacillus thuringiensis* var. *kurstaki* (3a, 3b). When assayed against neonate larvae of *S. exigua*, the 24, 48 and 72 hour LC$_{50}$ values for this sample were 1,110, 640, and 470 μL aqueous concentrate per ml of larval diet. In comparison, the 24, 48 and 72 hour LC$_{50}$ values for Thuricide® HPC against neonate *S. exigua* were 190, 100, and 80 μL aqueous concentrate per ml diet.

When second-instar larvae of *S. exigua* were allowed to feed on SAN 415-treated diet, mortality increased sharply with the time of exposure. The capacity for recovery following a 6 hour exposure to a 24 hour LC$_{50}$ level of SAN 415 was shown by larvae.

Teratological effects were observed in moths and pupae that were exposed to SAN 415 as eleven day old larvae. Most teratological effects involved mouthparts and legs of affected pupae and moths.
INTRODUCTION

The larva of the beet armyworm (BAW), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is a major pest of vegetable crops throughout most of the United States. Besides beets, this species also feeds on cotton, lettuce, asparagus, beans, cole crops, alfalfa, peas, peppers, corn, and a number of weeds (Wilson 1934). Larvae of the BAW are foliage feeders and attack seedling plants shortly after emergence from the soil. The life history and habits of the BAW were discussed by Wilson (1934). Fye and McAda (1972) conducted extensive laboratory experiments on the development of this pest. The nature of the damage caused by the larvae was described by Blickenstaff (1978) and Hills (1963).

In the southwestern deserts of the U.S., entire stands of lettuce are sometimes lost overnight from damage caused by the BAW (Vail et al. 1972). Wene and Sheets (1961) gave a detailed account of heavy field infestations of the BAW on cotton in Arizona. Many of the most severe infestations of the BAW follow repeated applications of chemical insecticides for the control of other pests (Werner et al. 1982, Wene and Sheets 1961).

Because of the danger of such damaging outbreaks, attempts have been made to develop alternative BAW control measures which do not disrupt the action of natural enemies. One such possibility is biological control with the spore-forming bacterium *Bacillus*...
of which several commercial preparations exist. However, many preparations of B.t. are highly specific, having a relatively narrow host-range of activity. Although new formulations of B.t. are currently being developed for use against the BAW, no available formulation has yet shown good potential for BAW control in the field.

In an early attempt to determine the susceptibility of some insect pests to Bacillus thuringiensis, Hall and Dunn (1958) performed laboratory bioassays with preparations formulated in 1952 from the Mattes strain of B.t. The results of these crude tests (no standard was yet available) indicated that the beet armyworm, Laphygma exigua (Hbn.) (= Spodoptera exigua) was only slightly susceptible. Because of the low mortality achieved in these laboratory tests, the beet armyworm was not listed among pests showing potential for control in the field with B.t.

This prediction was confirmed the following year when Hall and Andres (1959) reported that no control of the beet armyworm was obtained in a field test with commercially produced B.t. preparations. The authors concluded that there was a need for applications of chemical insecticides or development of microbial materials which control resistant pests such as the beet armyworm.

Afify et al. (1968) isolated a bacterium from diseased larvae and malformed pupae of the beet armyworm (called the lesser cotton leafworm) collected from fields at Giza, Egypt. Spore-forming bacilli from the larval gut fluid and gut extract and from pupal body
extract were cultured in the laboratory for identification. This bacterium was identified as *Bacillus thuringiensis* var. *sotto* according to the key of Heimpel (1967). A spore-containing powder was prepared from this culture and tested for its pathogenicity. This preparation when ingested with foliage of Egyptian clover (*Trifolium alexandrium*) produced no infection in BAW larvae. However, it was highly toxic to *Bombyx mori*. Only *B.t.* var. *thuringiensis*, isolated from the pink bollworm, *Pectinophora gossypiella* (Saund.), proved to be highly virulent to *S. exigua*.

Thirteen *Bacillus* preparations (2 commercial formulations of *B.t. var. thuringiensis* Berliner and 11 laboratory preparations of different varieties and species) were assayed against *S. exigua* by Afify et al. (1969). Only three preparations were able to produce high mortality, i.e. above 80%, seven days after voluntary ingestion of treated Egyptian clover foliage for 24 hours. The three most virulent preparations, Biospore 2802 R (a commercial preparation based on *B.t. var. thuringiensis*), *B. entomocidus subtoxis* Heimpel & Angus (#1179), and *B.t. sotto* Ishiwata, gave mortality rates of 84%, 83.3% and 80%, respectively.

Afify and Merdan (1969) studied the response of laboratory-reared BAW larvae to two preparations of *B.t. var. thuringiensis*. Of the two samples tested, Biospore 2802 R was found to have a stronger "initial effect" and greater overall activity than a laboratory preparation, *B.t.* 2172 (provided by Northern Regional Research Laboratories, Illinois). The maximum response of *S. exigua* to both
preparations was shown by 8-9 day old larvae. However, this high response might have occurred as the result of differences in development among larvae approaching molting at the time of treatment. Such large variations in bioassays of larvae with \textit{B.t.} were later demonstrated by Dulmage (1973).

Vail et al. (1972) conducted field tests on fall lettuce in Arizona to evaluate the microbial control of lepidopterous larvae. Of the materials tested, a commercial preparation of strain HD-1, \textit{Bacillus thuringiensis} var. \textit{kurstaki} (3a, 3b) DeBarjac (WP, Abbott Laboratories) when used alone gave the best control of beet armyworm larvae. However, recovery of beet armyworm larvae collected from plots treated with 2 lbs. = $2.7 \times 10^{10}$ IU/ha \textit{B.t.} (HD-1) was 100%. All beet armyworm larvae collected survived to pupate.

The effects of the $\beta$-exotoxin of \textit{Bacillus thuringiensis} on several lepidopterous larvae were described by Ignoffo and Gregory (1972). Prevention of adult mouthpart development after larval feeding on an artificial diet surface treated with $\beta$-exotoxin was demonstrated in \textit{S. exigua}. Reduction or loss of mouthparts was observed among moths of all treated BAW larvae.

Prasertphon et al. (1973) tested three different commercial formulations of \textit{B.t.} on third and fourth instar BAW larvae. Although \textit{B.t.} had been thought to sporulate rarely in host insect cadavers, the authors observed that sporulation was frequent in the host cadavers of 3 species of lepidopterous larvae tested. Although no sporulation was observed in dead BAW larvae which had been fed the Biotrol®
preparation (B. t. var. kurstaki), spores and crystals were found in
dead larvae that had fed on Thuricide® (B. t. var. thuringiensis) or
Dipel® (B. t. var. kurstaki). The Dipel® preparation caused greater
mortality than did the other preparations.

Merdan et al. (1975) investigated the influence of plant
species which contained antibacterial substances on the susceptibility
of larvae of several lepidopterous species to three different prepara­
tions of B. t. The level of mortality caused by the three B. t. pre­
parations was markedly lower when the test larvae were taken from
inactive plant foliage. Larvae of S. exigua were comparatively
resistant to the bacterium. Of the three preparations used, Biospore®
(B. t. var. thuringiensis) was the most effective on S. exigua and
S. littoralis larvae, followed by a laboratory preparation of B. t.
var. thuringiensis, and then B. t. var. dendrolimus.

Ignoffo et al. (1977) conducted laboratory bioassays with a
standard preparation of B. t. var. kurstaki (HD-l-S-1971) to determine
the relative susceptibility of six lepidopterous pests of soybeans.
Larvae of the cabbage looper, Trichoplusia ni (Hübner) were used as a
standard for comparing levels of relative susceptibility. Based on
average percentage of mortality and LC₅₀ values, S. exigua was found
to be 2.73 x more resistant than was T. ni. Also, below average
weight gains of larvae exposed to treated diets provided another in­
dication that S. exigua was less susceptible to this strain of B. t.
than was T. ni. The authors estimated that rates of 3.4 - 4.5 Kg/ha,
or the insecticidal equivalent of 22-30 billion IU/acre, would be
needed for satisfactory control of *S. exigua*. In a previous (unpublished) study conducted in 1967, the same authors found that larvae of *S. exigua* were 4.3 x more resistant than *T. ni* to Thuricide® 90 TS (based on *B. t. var. thuringiensis*).

In the development of an integrated pest management program for broccoli in southern California, Wyman and Oatman (1977) investigated the potential of Dipel® (*B. t. var. kurstaki*) to maintain populations of lepidopterous larvae below preestablished levels of 0-1, 2, and 5 larvae per plant. The beet armyworm was observed only in the spring and fall crops. Dipel® was found to be unsatisfactory for control of beet armyworm populations. The authors concluded that a heavy infestation by this pest would require alternative means of control.

Smirnova (1980) described the cultural, biological, and chemical control strategies employed for cotton pest control in the U.S.S.R. The beet armyworm, *S. exigua*, was listed as one of the most harmful and economically significant pests of cotton in the U.S.S.R. The status of this pest was reported to be irregular, with years of heavy outbreaks occurring sporadically alternating with years of depressed populations. Control involved spraying with the microbial preparation Dendrobacillin (based on *B. t. var. dendrolimus*). However, along with the use of Dendrobacillin, the beet armyworm control strategy stipulates the alternation of organophosphorus, carbamate, and organochlorine insecticides.
Salama et al. (1981) screened twenty-nine cultures of \textit{B. t.} (belonging to fourteen serotypes) for their activities against three lepidopterous pests of cotton in Egypt. Using the assay procedure proposed by Dulmage et al. (1971), they found that endotoxin preparations based on \textit{B. t.} var. \textit{entomocidus} had remarkably high potency against \textit{S. exigua}. The strains which gave the lowest LC$_{50}$ values for this pest were var. \textit{entomocidus} (from Institut Pasteur, France), var. \textit{entomocidus} (HD-198 = from the culture of Dr. Howard Dulmage, USDA, Brownsville, TX), and var. \textit{alesti} (Institut Pasteur). When compared to a primary U.S. reference standard for assays of delta-endotoxin formulations, HD-1-S-1971 18,000 IU/mg (Dulmage 1973), the potencies of these samples were calculated to be 79,995 IU, 46,650 IU and 62,077 IU per mg., respectively.

More recently, the same authors studied the effect of mixed cultures of \textit{B. t.} in an attempt to widen the host-range specificity of \textit{B. t.} preparations (Salama et al. 1983). The three cultures selected for study were: var. \textit{entomocidus}, and vars. \textit{kurstaki} HD-1 and HD-73. From results of bioassays designed to test the potency of \textit{B. t.} combinations against \textit{S. exigua}, three combinations showed synergism. Mixtures of \textit{B. t. kurstaki} HD-1 (Serotype 3a, 3b) + \textit{B. t. entomocidus} serotype 6, of \textit{B. t. entomocidus} (serotype 6) + \textit{B. t. entomocidus} HD-198, and of \textit{B. t. kurstaki} HD-73 + \textit{B. t. entomocidus} HD-198 all exhibited a significant synergism as evidenced by the co-toxicity coefficients of the mixtures.
In an attempt to increase sporulation yields and potency of endotoxins produced by *B.t.* var. *kurstaki* HD-251 and *entomocidus* HD-635, Salama et al. (1984a) produced several mutants by means of chemical mutagenesis. However, assays of the resulting endotoxins against *S. exigua* and *S. littoralis* revealed that the sporulation and potencies of the endotoxins produced by the mutants did not exceed those of the wild type strains. Mortality among *S. exigua* larvae treated with endotoxins of mutant *B.t.* strains was the same as that obtained from the wild type strain.

The same authors conducted another study to examine the possibility of improving the longevity of the endotoxins produced by *B.t.* mutants resistant to factors responsible for the decay of these toxins under field conditions (Salama et al. 1984b). Unfortunately, the potency of endotoxins produced by UV-resistant mutants was comparable to that of their wild types when assayed against *S. exigua* and *S. littoralis*. Heat-resistant, and antibiotic resistant mutants produced endotoxins which gave similar results in bioassays. The authors encouraged further studies on this new topic.

There are a number of noteworthy studies in which *B.t.* was used against congeners of the beet armyworm, many of which were also relatively non-susceptible to this pathogen. Abdallah (1969) found that the potency of the commercial product Biotrol® BTB-25W was considerably low against the (Egyptian) cotton leafworm, *Spodoptera littoralis* (Boisd.). He reported that massive concentrations were required to obtain high mortality. He also attributed the high
variation in the response of *S. littoralis* to Biotrol® to a nuclear polyhedrosis virus present in the colony at different times. He suggested that *B.t.* provokes the polyhedrosis virus present in the less healthy populations, resulting in a seemingly higher initial toxicity.

The effects of ten different laboratory strains of *B.t.* were tested on larvae of *S. littoralis* in laboratory bioassays conducted by Moore and Navon (1973). Results of tests evaluating the action of a preparation containing the thermostable exotoxin produced by var. *thuringiensis* indicated that spraying this particular toxin for short-term control is not rational. In tests employing spore suspensions of the different *B.t.* varieties, cessation of feeding (the classic symptom elicited by endotoxin ingestion) was never observed. The authors suggested that any mortality above the controls was probably due to a toxin other than the parasporal crystal. Of all the varieties of *B.t.* tested, only var. *darmstadiensis* (Institut Pasteur) was able to produce substantial mortality, but only at a very high dosage. *B.t.* var. *thuringiensis*, which at the time of this study was the only commercially available variety tested, was found to kill *S. littoralis* at too low a mortality rate for short-term economic control.

Salama and Foda (1982) conducted laboratory bioassays with 17 varieties of *B.t.* to determine their potency against *S. littoralis*. Most of the preparations of different varieties and serotypes had low activity against this pest. However, *B.t.* var. *entomicidus* proved to be of high potency to *S. littoralis*. The potency of this variety (as compared to HD-1-S-1971 18,000 IU/mg) was 62,520 IU/mg.
Working with the tobacco cutworm, *Spodoptera litura* F., Asano et al. (1973) evaluated the effectiveness of Thuricides® A, B, and C in laboratory and field tests. All products were found to be insufficient to produce high mortality among larvae of this pest in both laboratory and field tests.

Mixed results have been reported on the effectiveness of B.t. against the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). A field test which demonstrated the failure of B.t. to control this pest was conducted by DeLima et al. (1976). In corn plots treated with Dipel® (B.t. var. kurstaki) WP at 0.5 Kg AI/ha, mortality of fall armyworm larvae did not exceed 56%. However, in heavily infested adjacent fields treated with chemical insecticides, mortality of fall armyworm larvae ranged between 77-96%.

Hofmaster and Francis (1978) investigated the use of combinations of B.t. and synthetic pyrethroids for control of the fall armyworm on sweet corn. Although applications of synthetic pyrethroids alone gave 90% or better undamaged ears, an increase in control was achieved by combining 0.28 kg Dipel® with 0.006 kg Pydrin® (fenvalerate)/ha.

Among several insecticides tested by Janes (1973) for control of the fall armyworm on sweet corn, Dipel® + corn oil gave 95% or higher injury-free ears when applied at 2-3 day intervals at a rate of 1.1 kg/ha.
In another field test employing a wettable powder formulation of B.t. var. *alesti*, as little as 0.11 Kg/ha. gave good control of the fall armyworm on cabbage (Creighton et al. 1972).

As most of the previous attempts to control the BAW with B.t. have failed, the present laboratory study was conducted to evaluate the potential of a newly developed formulation of B.t. for control of the beet armyworm. This unregistered strain of B.t. was evaluated in laboratory bioassays for dosage-mortality relationships, effect of feeding time, teratological effects, and acute toxicity relative to the commercial B.t. preparation, Thuricide HPC."
MATERIALS AND METHODS

Formulations of B.t.

The unregistered formulation of *Bacillus thuringiensis* var. *kurstaki* (3a, 3b) was supplied by Sandoz, Inc., San Diego, CA. This strain of B.t., developed for use against the beet armyworm, was designated as SAN 415 SC 75 lot T 16-3. One quart of this aqueous concentrate (8 billion IU/quart) has a potency (against the cabbage looper, *Trichoplusia ni* (Hbn.)) similar to 2 quarts Thuricide-HPC$, also manufactured by Sandoz, Inc. The Thuricide-HPC$ sample used for comparison in the present study, also formulated as an aqueous concentrate, had a potency (against *T. ni*) of 4,000 IU per mg., equivalent to 4.0 billion IU per quart.

Rearing of BAW

Neonate larvae of the beet armyworm were obtained from the laboratory colony of the USDA Biological Control of Insects Laboratory, Tucson, Arizona. The procedures used to maintain this culture were described by Patana (1969, 1977) and Petterson and Deboît (1975, 1976). Emerging larvae were introduced into paper-capped 29.6 ml. (1 oz.) clear plastic cups containing a modified lima bean diet (Patana 1977, Appendix A). The larvae, five per cup, were reared in an environator programmed to maintain conditions at 25 ± 1°C, and 12:12 (L:D) cycle.
Dosage—Mortality of SAN 415 against BAW

This study was designed to determine the effects of SAN 415 on the BAW. All tests were conducted at the Entomology Laboratory, University of Arizona, Campus Agricultural Center, Tucson. The assays were carried out under controlled laboratory conditions to avoid variability in results often caused by variable field conditions.

Dilutions of SAN 415 were mixed into the lima bean diet prepared according to Patana (1977) except that formalin was omitted. Stock suspensions of SAN 415 in water were mixed using a magnetic stirrer. These SAN 415 suspensions were pipetted into divided portions of the diet to obtain mixtures with the concentration of SAN 415 in each dilution being \( \frac{1}{2} \) the previous dilution. The test doses used were assigned letters, which ranged from A through O, with A being half the concentration of B, etc. The concentrations of A and O were 88.2 and \( 1.44 \times 10^7 \) IU per ml of diet, respectively, representing a 16,000-fold range. Each mixture was then blended separately in a Waring\textsuperscript{®} blender for 2 minutes at high speed. A control medium, without SAN 415, but otherwise identical, was also prepared. Mixtures were dispensed into 29.6 ml plastic cups at approximately 10 ml per cup. For each age group tested, five larvae were placed in a cup which was then capped with a paper lid. Larvae were reared to the various ages on untreated diet. There were 15 cups with 5 larvae per replicate (in each cup) for each dose. Seventy-five larvae maintained on non-infected media served as controls for each test. After the larvae were placed on the medium,
cups were held at 25 ± 1°C. Observations of mortality were made at 24 hour intervals through 72 hours. If there was no response when prodded with a blunt probe, larvae were judged as dead. If there was any response, no matter how feeble, larvae were judged as alive. Larval mortalities were recorded and analyzed using a computer program for probit analysis (Daum and Killcreas 1966; Finney 1962), designed by Brian Maurer and Ted Chester (Center for Quantitative Studies, University of Arizona).

Feeding Time—Recovery Experiment

This experiment was designed to investigate the influence of exposure time to infected media upon subsequent mortality or recovery. Neonate BAW larvae were introduced into uninfected media cups (5/cup) and held for 96 hours at 25°C, 12 h L:D. After 96 hours, these mid 2nd-Instar larvae (avg. wt. 2.48 mg) were transferred to SAN 415 infected media cups, 5 larvae per cup. Larvae were allowed to voluntarily feed on the infected diet, which contained a dose approximating the 24 hour LC50 (67,000 IU/ml diet) for larvae of this age. One hundred larvae (20 cups of 5 larvae each) were used for each exposure time-treatment. One hundred 4-day-old larvae reared on uninfected diet served as a control. Larvae from groups designated as 1, 2, 3 and 4 were removed from infected media cups and transferred to uninfected media cups after 6, 12, 18 and 24 hours, respectively. Larval mortality counts were made at the time of removal, and at 6, 12, 24, 48 and 72 hours thereafter. Graphs of percent mortality vs. time
after removal were prepared for each group. Surviving pupae and adults were inspected for deformities with a dissecting microscope. Percentage of adult emergence was recorded.

**Teratological Effects of SAN 415 on BAW**

Fifth instar larvae, approaching pupation, were used as test insects. After being reared for 11 days at 25°C, the larvae were divided into six equal groups. Thirty larvae were kept on an uninfected control medium. The five remaining groups of thirty larvae each were kept on media containing $4.3 \times 10^4$, $8.6 \times 10^4$, $1.7 \times 10^5$, $3.4 \times 10^5$, and $6.9 \times 10^5$ IU SAN 415 per ml media, respectively. In each treatment, there were 15 media cups with 2 larvae per cup. All larvae were held at 25°C, 12:12 (L:D) cycle during treatment. Counts of larval mortality and percent pupation were made at 24, 48 and 72 hours after treatment. After completion of pupation, all test insects were examined for deformities with a dissecting microscope. Photographs of teratological effects among pupae were taken 72 hours after treatment began. Also, the percentage of moth emergence was determined on the basis of the number of pupae.

**Relative Toxicities of SAN 415 and Thuricide® HPC**

This experiment was designed to determine the relative potencies of SAN 415 and Thuricide HPC® against the BAW. A preliminary rangefinding bioassay was conducted to determine appropriate test doses of Thuricide HPC® for first instar BAW. Five doses were then selected which gave mortalities in the range needed to compute an
$\text{LC}_{50}$ and $\text{LC}_{95}$. A bioassay was conducted using these five Thuricide® doses (which bracketed the $\text{LC}_{50}$), as well as the five doses of SAN 415 which were found to produce similar mortality rates in neonatal BAW. Seventy-five neonatal BAW larvae were used for each dosage tested, for both B.t. preparations. Mortality counts were made at 24, 48 and 72 hours of exposure. Probit analysis was used in comparing the $\text{LC}_{50}$ and $\text{LC}_{95}$ for each B.t. formulation.
RESULTS AND DISCUSSION

**Dosage-Mortality of SAN 415 Against the Beet Armyworm (BAW)**

The 24 hour LC\(_{50}\) for SAN 415 against neonate BAW was 10,900 IU/ml diet (Table 1, Figure 1, Appendix B). The 24 hour LC\(_{95}\) of 65,100 IU/ml diet is almost 6 times higher. However, the ratios of LC\(_{95}\) to LC\(_{50}\) at 48 and 72 hours are only 4.3 and 3.4, respectively. This is due to the difference in slopes of the dosage-mortality regression lines for 24, 48 and 72 hours. The slopes of 2.12, 2.57 and 3.05 for the 24, 48 and 72 hour SAN 415 dosage-mortality regression lines are similar to slopes reported by Dulmage (1973) for bioassays of B.t. against larvae of *Heliothis virescens* (F.).

**Feeding Time—Recovery Experiment**

The progression of mortality of BAW larvae following removal from the 24 hour median lethal dose is illustrated in Figure 2. The B.t. induced mortality of BAW larvae increased sharply with the length of exposure period. Extension of the exposure period beyond 6 hours produced a striking difference in the progression of mortality (Figure 2). As expected, about half (44%) of the larvae exposed for 24 hours were dead at the time of removal from the diet treated with the 24 hour LC\(_{50}\) (as determined in dosage—mortality section above) for SAN 415. However, all survivors of this exposure period were observed to be dead 36 hours after being transferred to SAN 415-free
Table 1. LC$_{50}$ and LC$_{95}$ values with confidence intervals for SAN 415 ingested by neonate BAW.

<table>
<thead>
<tr>
<th>Measured at$^a$</th>
<th>Mortality Level</th>
<th>LC$^b$</th>
<th>95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>50</td>
<td>10,900</td>
<td>8,900</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>65,100</td>
<td>44,800</td>
</tr>
<tr>
<td>48 hours</td>
<td>50</td>
<td>4,600</td>
<td>4,100</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>20,000</td>
<td>16,500</td>
</tr>
<tr>
<td>72 hours</td>
<td>50</td>
<td>3,200</td>
<td>2,500</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>11,000</td>
<td>8,500</td>
</tr>
</tbody>
</table>

$^a$75 BAW larvae per dose, 10 doses
$^b$IU SAN 415 per ml diet
Figure 1. Dosage Mortality lines for SAN 415 ingested by neonate BAW.

Figure 1. Dosage Mortality lines for SAN 415 ingested by neonate BAW.
Figure 2. Mortality of BAW vs. Time after removal from (24 hr.) LC50.
diet. All larvae exposed for 12 and 18 hours were observed to be dead at 163 and 78 hours after removal respectively. Only larvae having been exposed for 6 hours were able to recover and grow normally. Observations on larvae held to emergence (10%) indicated that recovery from SAN 415 was complete. This was indicated by successful mating among survivors of this exposure period.

**Teratological Effects of SAN 415 on BAW**

The average weight of the 11-day-old larvae used in this study was 193 mg. Within 24 hours after treatment, larvae were observed burrowing into the media and initiating pupation. There was a general increase in the delay of onset into pupation with an increase in SAN 415 concentration in the media of treated larvae (Appendix D). Both the 48- and 72-hour dosage-mortality curves from this experiment were notably different from all others obtained in this study in that they did not indicate a linear response. Initially, there was a linear increase in mortality, after which mortality decreased with increasing concentrations of SAN 415 (Figure 3, Appendix D). However, after one week, there were no survivors among insects treated with the highest two doses, while some larvae treated with the lower doses survived to emerge as adults. This suggests that within the range of very high B.t. (SAN 415) concentrations used in this study, there exists a threshold in concentration, above which the initial symptom of feeding inhibition occurs before a lethal dose can be consumed. Presumably, the course of mortality is delayed in larvae exhibiting rapid and
Figure 3. Dosage Mortality curves for 11 day old BAW fed SAN 415.

Figure 3. Dosage Mortality curves for 11 day old BAW fed San 415.
complete inhibition of feeding. In the time required to heal the gut of such a larva before feeding can resume, larvae ingesting media treated with lower B.t. concentrations may accumulate a lethal dose and exhibit a more rapid course of mortality. In a recent study on the effect of exposure time to B.t. on mortality of the spruce budworm, Choristoneura fumiferana (Clem.), Fast and Regniere (1984) reported that even at very high doses, feeding inhibition induced by B.t. is temporary. They also observed a cycle of feeding inhibition and recovery from B.t. for this insect. It appears that among BAW treated with very high doses of SAN 415, the recovery period is long enough to delay the onset of mortality.

Very high concentrations of SAN 415 were used in this experiment in hopes of observing teratological effects among survivors. B.t.-induced malformations were first described by Burgerjon and Biache (1967) in Diptera and Lepidoptera. This effect was later attributed to the insecticidally active β-exotoxin of B.t. (Ignoffo and Gregory 1972). Malformed moths of the cotton leafworm, Spodoptera littoralis (Boisd.) were observed by Abdul-Nasr and Abdallah (1970) among insects exposed to B.t. as larvae. Wolfenbarger et al. (1970) studied the teratological properties of a water insoluble formulation (IMC 10,001) of the β-exotoxin of B.t. They were unable to demonstrate any visible teratological effect on the tobacco budworm, Heliothis virescens (F.), when larvae less than 4 days old were exposed to exotoxin-treated diet. Younger larvae either died or recovered to become normal moths. However, a reduction in length or absence of the proboscis was
observed among pupae that were exposed as 4 to 8 day old larvae. A pupa with a missing proboscis was pictured as an example of β-exotoxin induced teratological effects (Wolfenbarger et al. 1972). Similar B.t.-induced malformations of BAW were reported by Ignoffo and Gregory (1972). They observed deformities in pupae and adults from BAW larvae which were reared to maturity and then exposed to β-exotoxin treated diet. Exposure of larvae within 24-48 hours of pupation prevented mouthpart development, resulting in deformities identical to those reported in Pieris brassicae L. by Burgerjon and Biache (1967).

In the present study, the survival rate was very low for exposed larvae (Appendix D). However, deformities similar to those reported by Burgerjon and Biache (1967), Wolfenbarger et al. (1972), and Ignoffo and Gregory (1972) were common among survivors. The percentages of deformities observed in pupae reared from larvae treated with $4.3 \times 10^4$, $8.6 \times 10^4$, $1.7 \times 10^5$, and $3.4 \times 10^5$ IU/ml diet were 80%, 50%, 0%, and 50%, respectively. Most of the observed deformities involved the lack of sclerotization of pupal mouthparts and legs. Pupae with unsclerotized or incompletely sclerotized labial palpi, maxillae and probosci were most common. Other deformities included a lack of sclerotization in sternites, malformed and reduced wings, and blackened, malformed meso- and meta-thoracic legs (Figures 4-11). Among the few deformed pupae that emerged as moths, adult deformities observed included a short uncoiled deformed proboscis, a lack of proboscis, and a missing meta-thoracic leg. Incomplete emergence was also
Figure 4. Missing proboscis (right) that occurred when SAN 415 was incorporated into larval diet. -- The left wing tip of the pupa on the left appears deformed. Both insects ingested SAN 415 at a rate of $4.3 \times 10^4$ IU per ml larval diet.
Figure 5. Missing proboscis in pupae from larvae fed SAN 415 (4.3 x 10^4 IU per ml diet) in larval diet.
Figure 6. Numerous deformities in pupa from larva fed SAN 415 (4.3 x 10^4 IU per ml diet) in larval diet. -- Note reduced (right) wing, and necrotic tissue around legs and mouth-parts.
Figure 7. Missing sternite in pupa from larva fed SAN 415 (4.3 x $10^4$ IU per ml diet) in larval diet.
Figure 8. Deformities in mouthparts and legs of pupa from larva fed SAN 415 (4.3 x 10^4 IU per ml diet) in larval diet.
Figure 9. Numerous severe deformities in pupa from larva fed SAN 415 (8.6 x 10^4 IU per ml diet) in larval diet.
Figure 10. Incompletely sclerotized mouthparts in pupa from larva fed SAN 415 (1.7 x 10^5 IU per ml diet) in larval diet.
Figure 11. Missing legs and mouthparts in pupa from larva fed SAN 415 (6.9 x 10^5 IU per ml diet) in larval diet. -- Note color of diet, which is due to the high concentration of SAN 415.
observed in a deformed pupa. Among the control group, all larvae that survived to pupate (29 larvae = 97%) emerged as normal-looking adults.

**Relative Toxicities of SAN 415 and Thuricide® HPC Against BAW**

Due to industrial competition, the value of % A.I. of SAN 415 (expressed as dry weight of B.t./g aqueous concentrate) was not available at the time of writing. Therefore, the potencies of the two B.t. samples used in this study were compared on the basis of relative volumes of aqueous concentrate per ml diet required for each preparation to produce a 50% and 95% mortality rate in the test insects. In this study, the relative equally effective volumes of the two B.t. preparations were the most relevant index for comparing their toxicities. This is because their respective values of international units (IU) become meaningless when assaying insects other than the cabbage looper, *Trichoplusia ni* (Hbn.), the insect for which standardization of B.t. preparations is based.

The dosage-mortality regression lines for SAN 415 and Thuricide® HPC are presented in Figure 12. The difference in susceptibility of the BAW to the two preparations is represented by the separation of the SAN 415 and Thuricide® HPC dosage-mortality lines. This separation, with respect to the abscissa (dose), is most apparent at lower mortality rates. The difference in tolerance variances exhibited by the BAW to the two preparations is represented by a markedly high slope of the regression lines for SAN 415 and much lower slopes for those of Thuricide® HPC. The slopes of the 24, 48 and 72
Figure 12. Dosage Mortality lines for neonate BAW (SAN 415 vs. Thuricide HPC).

Log Dose (μL aqueous conc./ml diet) vs. Mortality (Probits).
hour dosage-mortality lines for SAN 415 were 2.10, 2.94, and 3.85, respectively. In comparison, the slopes of the Thuricide® regression lines at 24, 48 and 72 hours were 1.35, 1.35, and 1.45, respectively. Therefore, it can be said that for the BAW, the variation in tolerance to SAN 415 is less than the variation in tolerance to Thuricide® HPC. This difference in the distribution of tolerance "spread" to the two B.t. preparations makes the interpretation of their comparative effects difficult. For example, any increase or decrease from the median lethal dose (or field application rate) for these two B.t. preparations would be expected to produce a greater change in BAW mortality for SAN 415 than for Thuricide® HPC. Therefore, in this case, the term "relative toxicity" has limited meaning, and depends on the rate of mortality at which SAN 415 and Thuricide® HPC are compared.

In comparing the 72 hour LC$_{50}$ values (Table 2), Thuricide® HPC was found to be 5.9 times as toxic to the BAW as SAN 415 (by volume). However, if the concentrations required to produce a 95% mortality rate at 72 hours are compared, both preparations are about equally effective. Because of the intersection of the 72 hour dosage-mortality lines for SAN 415 and Thuricide® HPC, the order of toxicity reverses above the 95% mortality level (Figure 12).

It can be demonstrated that the error in calculating lethal concentrations is least when the level causing 50% mortality is determined (Finney, 1962). However, in the laboratory bioassays discussed here, evaluation of the two B.t. preparations at high mortality rates may have greater relevancy to the field efficacy of these products.
<table>
<thead>
<tr>
<th>B.t. Sample</th>
<th>Measured at</th>
<th>Mortality Level</th>
<th>LC&lt;sub&gt;b&lt;/sub&gt;</th>
<th>95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAN 415</td>
<td>24 hours</td>
<td>50</td>
<td>1,110</td>
<td>930 - 1,310</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>6,710</td>
<td>4,660 - 9,650</td>
</tr>
<tr>
<td></td>
<td>48 hours</td>
<td>50</td>
<td>640</td>
<td>550 - 740</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>2,310</td>
<td>1,790 - 2,960</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>50</td>
<td>470</td>
<td>400 - 530</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>1,200</td>
<td>1,030 - 1,640</td>
</tr>
<tr>
<td>Thuricide</td>
<td>24 hours</td>
<td>50</td>
<td>190</td>
<td>140 - 245</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>3,010</td>
<td>1,720 - 5,270</td>
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<tr>
<td></td>
<td>48 hours</td>
<td>50</td>
<td>100</td>
<td>70 - 130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>1,570</td>
<td>920 - 2,670</td>
</tr>
<tr>
<td></td>
<td>72 hours</td>
<td>50</td>
<td>80</td>
<td>60 - 110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95</td>
<td>1,110</td>
<td>780 - 1,830</td>
</tr>
</tbody>
</table>

<sup>a</sup> 75 BAW larvae per dose, 5 doses for each B.t. preparation

<sup>b</sup> μL aqueous concentrate per ml diet
CONCLUSION

This investigation demonstrated that the mortality of BAW increases sharply with the length of time that the larvae are exposed to SAN 415. The potential for recovery has important implications for larvae that may escape death after initial exposure in the field, where normal applications of B.t. have a short effective residual life, and do not cover plants completely.

Severe teratological effects were observed in BAW pupae and moths following exposure to SAN 415 as mature larvae. Although little if any immediate control is achieved by treating larvae approaching pupation, the delayed teratogenic effects produced by B.t. preparations such as SAN 415 may prove to contribute to field control by preventing successful reproduction. This consideration would be most important for pest species that require feeding as adults before reproduction, as most of the teratogenic effects observed in this study involved the mouthparts.

Data concerning the relative effectiveness of the two B.t. preparations tested indicated that the tolerance variance of the BAW to SAN 415 is less than that for Thuricide® HPC. If this relationship proves to hold true in field conditions, deviations from required application rates would be expected to be less critical for SAN 415 than for Thuricide® HPC.
When used at low concentrations in larval diet, it appeared that Thuricide® HPC produced higher mortality in BAW than SAN 415 (Figure 12). However, this study revealed an interesting property of SAN 415 that may merit further investigation. Unlike Thuricide® HPC, SAN 415 produced substantially increased mortality rates with increasing exposure time, as evidence by the increasing slope of the regression lines for SAN 415 from 24 to 72 hours of exposure (Figure 12). It would be of interest to determine whether this results from a difference in pathogenicity between the two samples of B.t., or from a difference in their formulations.
APPENDIX A

LIMA BEAN DIET FOR BAW

Dry ingredients:

12 g ascorbic acid
12 g methyl paraben
1 tblsp Coax®
½ tsp L-tryptophan
1 tsp DL-Methionine

Vitamin solution (1L):

15.6 g inositol
12.0 g calcium pantothenate
6.0 g niacin
3.0 g riboflavin
3.0 g folic acid
1.5 g thiamine HCl
1.5 g pyrodoxine HCl
120 mg biotin
6 mg B₁₂

Six hundred grams of baby lima beans were soaked in 2,000 ml of water for 15 minutes. The dry ingredients, 12 ml of the vitamin solution, 12 ml of 15% choline chloride, and 6 ml of formaldehyde were combined with the lima beans and mixed in a Waring blender for four minutes at high speed. A boiling mixture of Gelcarin® (35 g of Gelcarin® in 1200 ml of water) was then added to the lima beans and blended for another minute. The diet was then dispensed into 1 oz. (29.6 ml) plastic cups.
APPENDIX B

DOSAGE–MORTALITY RELATIONS FOR SAN 415 INGESTED BY NEONATE BAW

<table>
<thead>
<tr>
<th>Dose (^a)</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>2400</td>
<td>8.0</td>
<td>38.7</td>
<td>53.3</td>
</tr>
<tr>
<td>2700</td>
<td>12.0</td>
<td>20.0</td>
<td>29.3</td>
</tr>
<tr>
<td>4800</td>
<td>20.0</td>
<td>46.7</td>
<td>65.3</td>
</tr>
<tr>
<td>5400</td>
<td>32.0</td>
<td>53.3</td>
<td>72.0</td>
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<tr>
<td>9600</td>
<td>30.7</td>
<td>77.3</td>
<td>93.3</td>
</tr>
<tr>
<td>10800</td>
<td>60.0</td>
<td>82.7</td>
<td>96.0</td>
</tr>
<tr>
<td>19200</td>
<td>60.0</td>
<td>96.0</td>
<td>100.0</td>
</tr>
<tr>
<td>21600</td>
<td>80.0</td>
<td>96.0</td>
<td>100.0</td>
</tr>
<tr>
<td>38400</td>
<td>89.3</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>43200</td>
<td>90.7</td>
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</tr>
<tr>
<td>Control (^c)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

\(^a\) IU SAN 415 per ml diet
\(^b\) 75 BAW larvae per dose (10 doses)
\(^c\) 150 BAW larvae served as the control
APPENDIX C

MORTALITY OF BAW VS. TIME AFTER REMOVAL FROM
(24 HOUR) LC$_{50}$

<table>
<thead>
<tr>
<th>Time after removal</th>
<th>0 hours</th>
<th>6 hours</th>
<th>12 hours</th>
<th>18 hours</th>
<th>24 hours</th>
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<tbody>
<tr>
<td>control</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>14</td>
<td>44</td>
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<tr>
<td>exposure</td>
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<td>0</td>
<td>16</td>
<td>50</td>
<td>57</td>
</tr>
<tr>
<td>exposure</td>
<td>0</td>
<td>8</td>
<td>48</td>
<td>64</td>
<td>82</td>
</tr>
<tr>
<td>exposure</td>
<td>0</td>
<td>32</td>
<td>73</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>exposure</td>
<td>2</td>
<td>43</td>
<td>95</td>
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<tr>
<td>exposure</td>
<td>3</td>
<td>47</td>
<td>97</td>
<td>99</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$100 BAW larvae per exposure time.
APPENDIX D

DOSAGE—MORTALITY RELATIONS, INITIATION OF PUPATION, AND PERCENT EMERGENCE FOR 11 DAY OLD LARVAE FED SAN 415 (TERATOLOGICAL EFFECTS)

<table>
<thead>
<tr>
<th>Dose a (IU SAN 415 per ml diet)</th>
<th>Percent Pupation at 24 hours b</th>
<th>Percent Emergence</th>
<th>Mortality (%) 48 hours</th>
<th>Mortality (%) 72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>43,000</td>
<td>46.6</td>
<td>5.0</td>
<td>43.0</td>
<td>66.6</td>
</tr>
<tr>
<td>86,000</td>
<td>43.3</td>
<td>0.0</td>
<td>63.3</td>
<td>86.6</td>
</tr>
<tr>
<td>172,000</td>
<td>33.3</td>
<td>3.3</td>
<td>83.3</td>
<td>93.3</td>
</tr>
<tr>
<td>344,000</td>
<td>26.6</td>
<td>0.0</td>
<td>76.0</td>
<td>96.6</td>
</tr>
<tr>
<td>688,000</td>
<td>33.3</td>
<td>0.0</td>
<td>56.0</td>
<td>80.0</td>
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<tr>
<td>Control</td>
<td>90.0</td>
<td>96.7</td>
<td>3.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

a IU SAN 415 per ml diet
b 30 BAW per dose
LIST OF REFERENCES


