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# Production and Evaluation of Two Strains of the Bacterium, *Bacillus thuringiensis* Berliner, as Biological Insecticides for Bertha Armyworm, *Mamestra configurata* (Walker)

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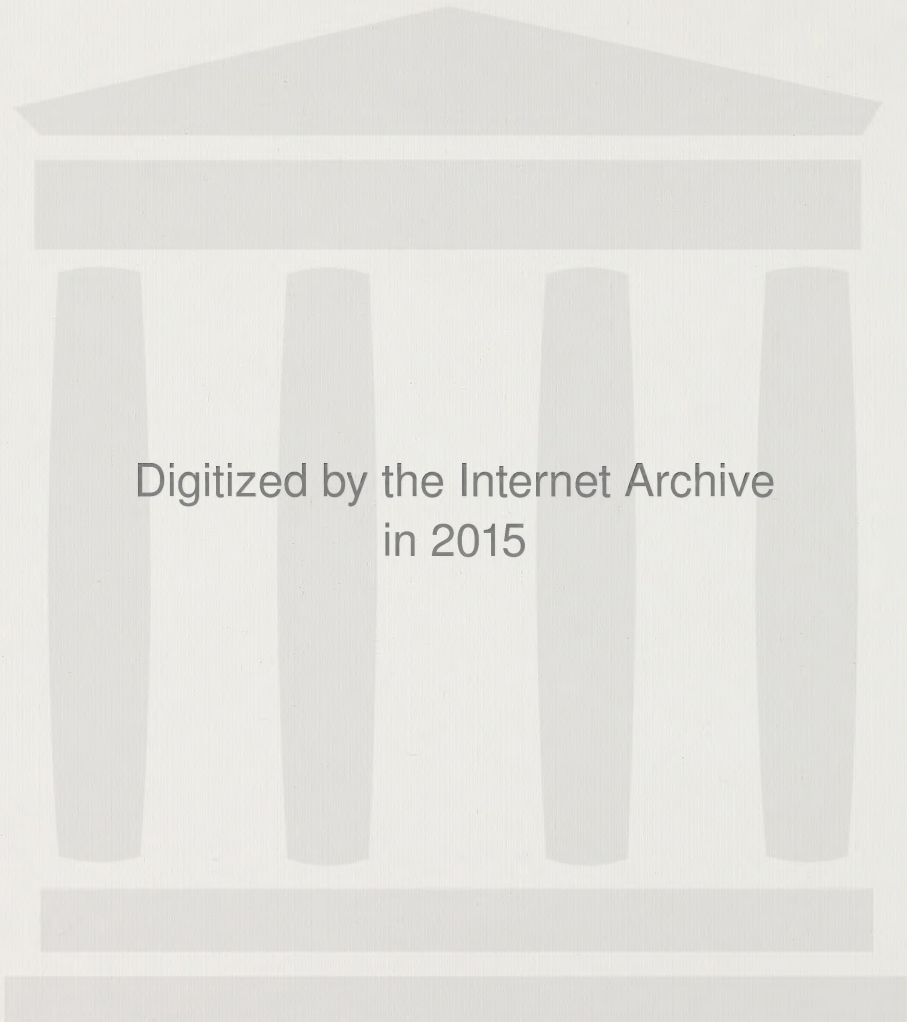
ENVIRONMENTAL CENTRE



# PRODUCTION AND EVALUATION OF TWO STRAINS OF THE BACTERIUM, *BACILLUS THURINGIENSIS* BERLINER, AS BIOLOGICAL INSECTICIDES FOR BERTHA ARMYWORM, *MAMESTRA CONFIGURATA* (WALKER)

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TABLE OF CONTENTS

PAGE

LIST OF TABLES . . . . . iv

ACKNOWLEDGMENTS . . . . . v

SUMMARY . . . . . vi

1 INTRODUCTION . . . . . 1

2 METHODS AND MATERIALS . . . . . 2
2.1 Production of BT in Shake-flasks and Laboratory-scale Fermenters . . . . . 2
2.2 Harvest of the Spore-crystal Preparation . . . . . 4
2.3 Culture of BT in a Pilot-scale Fermenter . . . . . 5
2.4 Formulation of BT for Trials Against Bertha Armyworm . . . . . 6
2.5 Laboratory Rearing of *M. configurata* . . . . . 7
2.6 Determination of Exotoxin Activity . . . . . 7
2.7 Laboratory Bioassays . . . . . 8
2.8 Greenhouse Studies . . . . . 9
2.9 Small Plot Field Studies . . . . . 10
2.10 Larger-scale Field Study . . . . . 10
2.11 Data Analysis . . . . . 11

3 RESULTS . . . . . 12
3.1 Production of BT in Laboratory-scale Fermenters . . . . . 12
3.2 Pilot-scale Culture of BT . . . . . 14
3.3 Exotoxin Assay . . . . . 14
3.4 Laboratory Bioassays . . . . . 14
3.5 Greenhouse Tests . . . . . 17
3.6 Small Plot Studies . . . . . 17
3.7 The Larger-scale Field Study . . . . . 20

4 DISCUSSION . . . . . 20

5 REFERENCES . . . . . 25

LIST OF TABLES

PAGE


Table 1. Production parameters of *Bacillus thuringiensis* Strains A1.2/72 and GE-13.2, and the efficacy of Strain A1.2/72 for inducing mortality of *Mamestra configurata* larvae in leaf-dip bioassays. . . . . 13

Table 2. Results of laboratory bioassays conducted to determine whether BT Strains A1.2/72 and GE-13.2 exhibited exotoxin activity. Third-instar larvae of *Mamestra configurata* were placed on artificial rearing diet in which treatments had been incorporated. . . . . 15

Table 3. Results of laboratory bioassays of fourth-instar larvae of *Mamestra configurata* (20 larvae per treatment) supplied with leaf material of canola (*Brassica rapa*) that had been dipped in solutions containing various treatments. . . . . 16

Table 4. Mean numbers ± SE per plant and percent mortality (in **bold**) of *Mamestra configurata* larvae on canola plants maintained under greenhouse conditions and receiving various treatments on 27 October 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons. . . . . 18

Table 5. Mean numbers ± SE per plant and percent mortality (in **bold**) of *Mamestra configurata* larvae on canola plants maintained under greenhouse conditions and receiving various treatments on 8 December 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons. . . . . 19

Table 6. Mean numbers ± SE per plant and percent mortality (in **bold**) of *Mamestra configurata* larvae on 0.5 $m^2$ field plots receiving various treatments on 16 July 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons. . . . . 21

Table 7. Mean numbers ± SE of *Mamestra configurata* larvae per 1 $m^2$ sample, and percent population reductions (in **bold**) in Stony Plain, Alberta field plots receiving various treatments on 17 August 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons. . . . . 22

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## SUMMARY

Laboratory, greenhouse, and field studies were conducted to evaluate the effectiveness of the spore-crystal preparation of a new strain of *Bacillus thuringiensis* Berliner var. *kurstaki* (Strain A1.2/72) for reducing populations of bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae) on canola plants. For comparative purposes, all production and efficacy trials also were conducted with another strain (Strain GE-13.2) with no special activity against bertha armyworm. The test material was produced by submerged culture of the bacteria in shake-flasks and laboratory-scale (10 L) fermenters at the Alberta Environmental Centre, Vegreville. The preparation was harvested, washed by centrifugation or cross-flow microfiltration, lyophilized, and formulated for spraying on test plants.

In laboratory bioassays, six-day post-treatment mortality of fourth-instar larvae of *M. configurata* that consumed leaf material containing Strain A1.2/72 was 65%, compared with only 20% for control larvae that consumed untreated leaf tissue. Mortality of larvae that consumed leaves treated with Strain GE-13.2 was also high (50%), but mortality of larvae exposed to the formulation[1] alone (without BT) was low (15-30%) and similar to the controls.

In two efficacy tests conducted in the greenhouse on canola plants infested artificially with *M. configurata* larvae, population densities following treatment with high (1.25 $mg \cdot mL^{-1}$) and low (0.31 $mg \cdot mL^{-1}$) dosages of Strains A1.2/72 and GE-13.2 were similar to those observed on the untreated control plants. However, treatment of plants with Decis® 5 EC (deltamethrin; 5 g $a.i. \cdot ha^{-1}$) caused dramatic population reductions that were significant statistically ($P < 0.05$) in both trials.

Small plot (0.5 $m^2$) studies were inconclusive for determining the efficacy of the strains for reducing larval populations of *M. configurata*. Statistically significant ($P < 0.05$) population reductions were observed following treatment with the high (1.25 $mg \cdot mL^{-1}$) and low (0.31 $mg \cdot mL^{-1}$) dosages of Strains A1.2/72 and GE-13.2, and with the formulation alone; however, similar reductions also were observed on control plots treated only with water.

A larger-scale field study was conducted in a portion of a canola field near Stony Plain, Alberta naturally infested with larvae of *M. configurata*. Three days after treatment, significant


[1]Formulation in this study refers to the mixture of chemical compounds used as a carrier for the active agent.

reductions ($P < 0.05$) of 79 and 61% were recorded for plots treated with Strains A1.2/72 and GE-13.2, respectively. These reductions were comparable to those observed using Decis® 5 EC (68%), the commercially available insecticide commonly used for control of bertha armyworm populations. By seven days after treatment, however, significant population reductions were observed on all study plots, including the control, but this was likely caused by a combination of treatment-induced mortality and movement of the population cohort to pupation sites beneath the soil surface.

It is evident from this study that further research is needed to improve the formulation. Although laboratory bioassays indicated that both strains of *B. thuringiensis* have activity against bertha armyworm larvae, enhanced residual activity could improve their effectiveness under natural field conditions.

# 1 INTRODUCTION

In recent years, considerable success has been achieved in controlling outbreaks of several insect pest species through the use of *Bacillus thuringiensis* Berliner (BT), a naturally occurring bacterium. When cultured under appropriate conditions, BT sporulates and produces a crystalline, proteinaceous, parasporal body known as the delta-endotoxin which, along with the endospore, can then be released from the bacterial cell when it lyses (Morris et al. 1986). Following ingestion of the crystal, damage occurs to the gut tissue that can eventually result in death of the insect larva. Of the four subspecies of BT that have been commercialized, subsp. *kurstaki* has been used most extensively and successfully to control pest species of Lepidoptera. It is an important component of many integrated pest management programs because it kills only some lepidopteran leaf-feeding species when ingested and is therefore much less damaging to non-target organisms than chemical insecticides. Moreover, its novel mode of action has enhanced its importance in circumventing problems of resistance in insects to chemical insecticides (Starnes et al. 1993).

The sequence of events following ingestion of the BT crystal and leading to the death of the insect begins when the high pH of the insect gut causes the protoxin to be proteolized to form an activated toxic protein that binds to receptors on the midgut epithelium. This causes gut paralysis and the insect stops feeding. A pore or lesion develops in the epithelium, leading to disruption of the potassium ion gradient, and swelling and destruction of the microvilli. The integrity of the gut is compromised and its contents enter the hemocoel. Spore germination occurs and death ensues from septicemia (Lambert and Peferoen 1992; Starnes et al. 1993).

Larvae of the bertha armyworm, *Mamestra configurata* Walker (Lepidoptera: Noctuidae), can cause severe economic losses to canola producers in western Canada (Bracken and Bucher 1984; Turnock and Philip 1977), but are naturally resistant to formulations of *B. thuringiensis* subsp. *kurstaki* available commercially (Morris 1986). Consequently, the only method available for reducing economically damaging infestations of *M. configurata* is to apply broad-spectrum chemical insecticides. During the outbreak of 1980 to 1985, approximately 70,000 ha were treated with insecticides in western Canada at a cost to producers of approximately $1.1 million (Madder and Stemeroff 1988). Although the outbreak of *M. configurata* from 1989 to 1992 was less widespread in western Canada and affected mainly Alberta, approximately 57,872 ha were sprayed with chemical insecticides to reduce bertha armyworm populations at a cost to producers

of about $1.0 million (Dolinski 1992). Chemical insecticides are very effective for reducing population densities of bertha armyworm larvae, but there are several problems associated with their use. In addition to the risks they pose to human health, chemical control agents are nonselective and kill a wide range of insects, including beneficial species such as canola pollinators, and predators and parasites of bertha armyworm. Destruction of natural control agents can cause a resurgence of pest insect populations to densities greater than those observed before treatment (Dent 1991).

In 1982, research initiated by O.N. Morris of Agriculture Canada, Winnipeg, to find effective strains of BT against bertha armyworm resulted in the discovery of strains that were highly toxic to larvae in laboratory bioassay studies (Morris 1988, 1991; Trottier et al. 1988). The objective of this research was to produce, formulate, and evaluate the effectiveness of one of these strains (Strain A1.2/72) under field and greenhouse conditions with a view to providing the basic efficacy data needed as prerequisite information for stimulating commercial production of the strain. Such development could provide canola producers with an alternative to chemical pesticides that is effective yet far less damaging to agricultural ecosystems.

## 2 METHODS AND MATERIALS

### 2.1 Production of BT in Shake-flasks and Laboratory-scale Fermenters

Stock cultures of test strains were maintained on Difco Plate Count Agar (PCA) slants incubated at 30°C and stored at 4°C until needed. A standard protocol was adopted leading up to fermenter runs for production of BT spore-crystal preparations:

1) Approximately 65 hours before needed, several PCA plates were inoculated and then incubated at 30°C.
2) 500 mL Erlenmeyer flasks containing 100 mL of Difco Tryptose Phosphate Broth (TPB) were inoculated with a loopfull of the above BT culture; flasks were then incubated on a gyratory shaker at 30°C and 300 rpm for 8 hours.
3) Two mL of the TPB culture were then added as inoculum to each of several 500 mL Erlenmeyers containing 100 mL of production medium (Morris 1992) made up to the following composition per litre:

| | |
|---|---|
| GNWBPC* | 10.00 g |
| Bacto Peptone | 2.00 g |
| Yeast Extract | 1.50 g |
| $MgSO_4 \cdot 7H_2O$ | 0.30 g |
| $FeSO_4 \cdot 7H_2O$ | 0.02 g |
| $CaCO_3$ | 1.00 g |
| NaCl | 5.00 g |
| Dextrose | 15.00 g |
| Tween 60 | 1.00 mL |

* Great Northern White Bean Protein Concentrate from Parrheim Foods, Saskatoon, Saskatchewan (45% protein)

The medium was adjusted to pH 7.0 before autoclaving (121°C for 30 minutes). Flasks were agitated at 300 rpm and 30°C for 16 hours. This second generation shake-flask culture was used to inoculate the fermenter.

4) The majority of the fermenter runs were carried out in New Brunswick Scientific MF-214 Microferm fermenters equipped with 14 L jars. Trials were based on variations of the Morris medium (above); however, for fermenters, 4 mL per litre of an antifoam agent (Polypropylene glycol, Molecular Weight 2000) were added prior to autoclaving. Jars were charged with 10 L of medium and autoclaved for 2 hours at 121°C. Upon cooling, fermenters were inoculated with 2% v/v of broth from the second generation shake-flask culture. Fermenter operating conditions were as follows:

| | |
|---|---|
| Starting pH: | 7.0 |
| Temperature: | 30°C |
| Airflow: | 10 $L \cdot min^{-1}$ |
| Agitation: | 400 rpm |

Because of the tendency to foam in the early stages of the fermenter run, airflow and agitation were cut down to 5 $L \cdot min^{-1}$ and 250 rpm, respectively, for the first 8 hours after inoculation; foam could be controlled during this period but excessive amounts of antifoam would be required which would have had an adverse effect upon harvest procedures later.

At regular intervals, samples were withdrawn aseptically from fermenter jars and examined under the light microscope. Development of the cells could be followed through the logarithmic growth phase, the formation of endospores and parasporal crystals of endotoxin, and finally the stage of cell lysis and the release of free spores and crystals into the medium which signalled that the time for harvest had arrived. (Lysis was also accompanied by a rise in the medium pH.)

Modifications were made to the original recipe in an effort to optimize spore-crystal production in different runs. The different variants of the growth medium recipe were as follows:

1) Normal recipe as above.
2) Double strength - all nutrients doubled except $CaCO_3$, NaCl, and Tween 60.
3) Triple strength - all nutrients tripled except $CaCO_3$, NaCl, and Tween 60.
4) Normal recipe but glucose doubled.
5) Double strength as per #2 plus 1.0 $g \cdot L^{-1}$ $K_2HPO_4$.
6) Double strength as per #2 plus 2.0 $g \cdot L^{-1}$ $K_2HPO_4$.

A rough approximation of productivity in fermentation runs was attempted by viable spore counts of the broth at harvest. Broth samples were heat-treated in a water bath at 80°C for 10 minutes (to kill vegetative cells), and enumerated by a standard method in the Microbiology Laboratory of the Alberta Environmental Centre (Microbiological Methods Manual 1990); results were reported in colony forming units (cfu = viable spores) per mL broth. Although there is a rough equivalency of one endotoxin crystal per endospore, the spore count of BT preparations is not considered a reliable index of efficacy against target insects (Morris 1992; Beegle and Yamamoto 1992).

## 2.2 Harvest of the Spore-crystal Preparation

On a small scale (shake-flasks), spore-crystal preparations could be harvested and washed by centrifugation. Broth from lysed mature shake-flask cultures was centrifuged at 10,000 x g for 20 minutes in a Sorvall RC5B refrigerated centrifuge equipped with an SS-34 rotor. If required, the resulting pellet could be washed by resuspension and repeated recentrifugation in 0.5 Molar saline (NaCl) followed by distilled water.

On a larger scale (fermenter broths of 10 L or more), it was more convenient to use crossflow microfiltration for harvest and washing of BT preparations. A Millipore Pellicon filtration cell equipped with 10 $ft^2$ of 0.45 μm microporous filter membranes was employed for this purpose. Prior to processing in the Pellicon unit, the BT broth was acidified to pH 4.5 with orthophosphoric acid (to protect the endotoxin against bacterial proteases which are less active at low pH), chilled to 4°C, and prefiltered through two stainless steel sieves - first 16 mesh then 100 mesh - to remove larger particulates which might plug the narrow channels (≈ 250 μm) between the filter membranes. Filtration in the Pellicon unit concentrated the particulates in 15% of the original volume. The Pellicon unit was also used to wash the particulates first with four volumes of 0.5 Molar saline and then eight volumes of distilled water.

The final washed slurry of spores, parasporal crystals, and undigested bean meal solids was then lyophilized in the bulk drying chamber of a Virtis Freezemobile 24 freeze dryer. Upon completion of drying, the product was weighed, ground gently with a pestle and mortar to pass through a 60 mesh sieve, and stored at 4°C until required.

## 2.3 Culture of BT in a Pilot-scale Fermenter

Several fermenter runs were performed with Strain A1.2/72 in a larger unit in an effort to derive some scale-up information in case it became necessary to produce significant quantities of product for large-scale field trials. Changes in the operating protocol associated with scale-up were as follows:

1) Initial stages up to and including the first shake-flask generation (TPB) were identical except that more flasks were inoculated.

2) Four hundred mL of combined eight hour TPB broth cultures were transferred aseptically to a New Brunswick Scientific MF-128 28 L fermenter containing 20 L of the usual production medium sterilized in place for one hour at 121°C. This batch was run for 16 hours at 30°C, 300 rpm agitation, and 20 $L \cdot min^{-1}$ air.

3) The contents of the last fermenter were transferred (via a sterilizable transfer line) to a New Brunswick Scientific IF-250 pilot-scale fermenter containing 200 L of production medium sterilized as before. This was run at 30°C, 200 rpm agitation and 150 $L \cdot min^{-1}$ air for the first eight hours until the tendency to foam had declined, and then increased to 300 rpm and 200 $L \cdot min^{-1}$ for the remainder of the run.

The Millipore Pellicon unit was used to harvest the product as before but with only 10 $ft^2$ of membrane in the cell, only 50 L of broth was processed. (The entire 200 L would have taken 24 to 48 hours to harvest and wash.)

## 2.4 Formulation of BT for Trials Against Bertha Armyworm

Part of the current contract was to test a particular formulation from Dr. Morris which had the following composition per litre of formulation:

| | |
|---|---|
| Lactic acid (88%) | 10.0 g |
| o-Cresol (technical grade) | 3.0 g |
| Sodium chloride | 50.0 g |
| Vegetable oil (canola) | 30.0 g |
| Tween 60 | 2.0 g |
| Tween 20 | 6.0 g |
| Carboxymethyl cellulose (medium viscosity) | 10.0 g |
| Polyvinyl alcohol (molecular weight 30-70,000) | 20.0 g |

The polyvinyl alcohol was wetted with 70% alcohol to aid solution. After all the soluble components had dissolved, the oil was added and the formulation emulsified with a sonic probe.

BT powders were formulated in the above mixture at 10% w/w based upon the oven dry weight of the powders. The powders were mixed slowly with the above ingredients in a pestle and mortar to form a smooth viscous suspension which was stored at 4°C until required - at which time they were diluted for spraying.

Another formulation was tried for comparison; this was a modification of a recipe from Beegle et al. (1991) and had the following composition per litre:

| | |
|---|---|
| Glycerol | 50.0 g |
| Sorbitol | 245.0 g |
| Propionic acid | 3.0 g |
| Distilled water | to 1000 mL |

Dry BT powders were formulated as described previously at 10% w/w.

## 2.5 Laboratory Rearing of *M. configurata*

The laboratory culture of *M. configurata* was derived from larvae field-collected in Alberta in August 1991 and maintained at regulated photoperiod (16 h L : 8 h D) and temperature (21°C : 15°C). Adult moths were kept in screened cages and supplied with water and a nutrient solution of liquid honey. Potted plants of *Brassica napus* L. or *Chenopodium album* L. were added to the cages for oviposition sites. Larvae were reared on an agar-based artificial diet (Velvetbean caterpillar diet, BioServ No. F9795, BioServ, Frenchtown, NJ, U.S.A.) augmented with 1.5% Vanderzant Vitamin mixture and 1% alfalfa meal.

## 2.6 Determination of Exotoxin Activity

A concern when employing any new strain of BT is the possible presence of exotoxins in the BT product (Beegle and Yamamoto 1992; Beegle 1992). Exotoxins are soluble compounds released into the culture broth which have toxicity against target insect larvae. Their presence in fermentation broths, although beneficial to the overall strategy of attacking the larvae, complicates the assay of efficacy of the endotoxin. Presence of exotoxins in the culture medium would necessitate more rigorous washing of the spore-crystal preparation prior to assay of endotoxin activity.

To determine whether the BT strains exhibited exotoxin activity, a trial was set up to test Strains A1.2/72 and GE-13.2 for exotoxin production using a procedure outlined by Beegle (1992). Shake-flask cultures of both strains in production medium were grown to the lysis stage in the usual way. Broth was then centrifuged as before to yield a pellet containing spores and crystals and a supernatant which would contain any exotoxins, if produced. The pellet was then washed by centrifugation as described previously, first with two washes in 0.5 M NaCl, and then three washes in distilled water to remove any residual traces of exotoxin. Finally, the material was resuspended in the original volume of distilled water. The supernatant was filtered aseptically through a 0.45 µm membrane filter to remove any spores or crystals. Supernatants from each strain were next split into two equal parts; one part was autoclaved for 30 minutes at 121°C. The purpose of this was to distinguish between heat-stable and heat-labile exotoxins; both are known from BT. Tenfold ($10^{-1}$) and one hundredfold ($10^{-2}$) dilutions were prepared from all four batches.

A quantity of the normal agar-based diet was made up in only 90% of the final volume; this was then split into fifteen 90 mL batches and maintained in a water bath at 55°C to prevent it from setting. Ten mL of one of the following treatments were added to each of the 90 mL batches:

1) distilled water (control);
2) Strain A1.2/72 - washed pellet;
3) Strain A1.2/72 - undiluted supernatant;
4) Strain A1.2/72 - $10^{-1}$ supernatant;
5) Strain A1.2/72 - $10^{-2}$ supernatant;
6) Strain A1.2/72 - undiluted heat-treated supernatant;
7) Strain A1.2/72 - $10^{-1}$ heat-treated supernatant;
8) Strain A1.2/72 - $10^{-2}$ heat-treated supernatant;
9) Strain GE-13.2 - washed pellet;
10) Strain GE-13.2 - undiluted supernatant;
11) Strain GE-13.2 - $10^{-1}$ supernatant;
12) Strain GE-13.2 - $10^{-2}$ supernatant;
13) Strain GE-13.2 - undiluted heat-treated supernatant;
14) Strain GE-13.2 - $10^{-1}$ heat-treated supernatant; and
15) Strain GE-13.2 - $10^{-2}$ heat-treated supernatant.

After mixing with the diet, the amended diet was dispensed at 10 mL per small plastic cup and allowed to set. Twenty to 29 cups were dispensed for each of the 15 treatments; one third-instar larva of *M. configurata* was then placed in each cup and allowed to feed. Survival and mortality of larvae were recorded eight days following their introduction to the cups.

## 2.7 Laboratory Bioassays

Laboratory bioassays were conducted in petri plates to determine the efficacy of the BT strains on mortality of *M. configurata* larvae. For each treatment, 20 replicate dishes, each containing one fourth-instar larva of *M. configurata*, were supplied with approximately 5 g per dish of excised leaf material of *B. rapa* cv. Colt that was dipped in a solution containing the treatment. Treatments comprised:

1) BT Strain A1.2/72 at 1.25 mg·mL$^{-1}$ using the 'Morris formulation' with caffeine;*
2) BT Strain A1.2/72 at 1.25 mg·mL$^{-1}$ using the 'Morris formulation' without caffeine;*
3) BT Strain A1.2/72 at 1.25 mg·mL$^{-1}$ using the 'Modified Beegle formulation';*
4) BT Strain GE-13.2 at 1.25 mg·mL$^{-1}$ using the 'Morris formulation' with caffeine;*
5) BT Strain GE-13.2 at 1.25 mg·mL$^{-1}$ using the 'Morris formulation' without caffeine;*
6) BT Strain GE-13.2 at 1.25 mg·mL$^{-1}$ using the 'Modified Beegle formulation';*
7) the 'Modified Beegle formulation'* only;
8) the 'Morris formulation'* with caffeine only;
9) the 'Morris formulation'* without caffeine; and
10) distilled water only.

* The original 10% formulations were diluted for this purpose with either distilled water or a 0.1% solution of caffeine as appropriate. (Previous studies by Dr. Morris had indicated that caffeine enhanced BT toxicity.) To this diluted formulation was also added a 'sticker', Ecogen Bond® at 0.125% v/v (Langhorne, Pennsylvania), to help the BT adhere to the leaf surface.

Two and six days after beginning the experiment, the dishes were examined and the numbers of living and dead larvae were counted and recorded.

## 2.8 Greenhouse Studies

Two similar greenhouse studies were conducted under conditions of regulated light (16 h L : 8 h D) and temperature (21°C : 15°C). Canola plants (*Brassica rapa* L. cv. Colt) were maintained in large plastic pots with six plants per pot until they were in early flowering [growth stage 4.2 of Harper and Berkenkamp (1975)]. The experiment utilized 28 pots arranged in a randomized complete-block design with four replications for each of seven treatments. Dilutions of BT formulations (the 'Morris' formulation) were made as described previously for the laboratory bioassays, with 'sticker' and caffeine. In the case of the lower dosage (0.31 mg·mL$^{-1}$), the original 10% BT suspension was diluted 1:3 with formulation before dilution for spraying began; in this way, the concentration of reagents from the formulation remained the same in the sprayed material. The treatments were:

1) BT Strain A1.2/72 at 1.25 mg·mL$^{-1}$;
2) BT Strain A1.2/72 at 0.31 mg·mL$^{-1}$;

3) BT Strain GE-13.2 at 1.25 $mg \cdot mL^{-1}$;

4) BT Strain GE-13.2 at 0.31 $mg \cdot mL^{-1}$;

5) Decis® 5 EC (deltamethrin) applied at the registered, recommended rate for bertha armyworm of 5 g $a.i. \cdot ha^{-1}$;

6) the components of the formulation without BT; and

7) a control sprayed with water only.

Two days before application of the treatments, five third-instar larvae of *M. configurata* were placed onto each canola plant. On each sampling date (two hours before treatment, and three and seven days after treatment), two plants were removed from each pot, bagged individually, and the numbers of living *M. configurata* larvae were counted and recorded.

## 2.9 Small Plot Field Studies

Two small plot field studies were conducted at the Alberta Environmental Centre, Vegreville. Plots measured 0.5 $m^2$ and were seeded on May 13, 1992; each plot was thinned to 25 plants of *B. rapa* cv. Colt. Two days before application of the treatments, four third-instar larvae of *M. configurata* were transferred onto each plant. These larvae were derived from laboratory rearing cultures and had been acclimatized on host plants within outdoor field cages (3.5 m x 3.5 m x 1.8 m high) for one week prior to their placement onto the experimental field plots. The experiment utilized a randomized complete-block design with four replications of the same seven treatments used in the greenhouse study. On each sampling date (two hours before treatment, and three, seven, and 10 days after treatment), five canola plants from each experimental plot were collected, bagged, and labelled individually. The living bertha armyworm larvae from each sample were counted, recorded, and then preserved. Final dilution of the BT formulations for spraying (as per greenhouse trials described above) was completed in the field, just prior to application on the plots. Treatments were applied on 7 and 16 July 1992 using a self-propelled plot sprayer.

## 2.10 Larger-scale Field Study

The study was conducted on a portion of a canola field (*B. rapa* cv. Parkland) on the farm of Mr. Fred Kosch approximately 5 km southwest of Stony Plain, Alberta. The field was infested naturally with *M. configurata*, estimated at 20-30 larvae per $m^2$. The field was subdivided into

five large plots (each approximately 10 m by 50 m), and each plot was subjected to one of five treatments:

1) water only (control plot);
2) formulation ('Morris') only;
3) BT Strain A1.2/72 at 1.25 $mg \cdot mL^{-1}$;
4) BT Strain GE-13.2 at 1.25 $mg \cdot mL^{-1}$; and
5) the insecticide Decis® 5 EC at the registered, recommended application rate for bertha armyworm larvae (5 g $a.i. \cdot ha^{-1}$).

Treatments were applied on August 17, 1992 using a self-propelled plot sprayer equipped with a 3 m side-boom and calibrated for normal field operations. To reduce the impact of solar radiation on the efficacy of the BT strains, treatments were applied late in the day (1930 h). On each sampling date (approximately four hours before treatment, and three and seven days after treatment), population densities of bertha armyworm larvae were estimated by counts from five randomly selected 1 $m^2$ quadrants within each plot.

## 2.11 Data Analysis

Significance of differences in population densities of *M. configurata* before and after each treatment were determined by subjecting data from each greenhouse and field experiment to analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons (SAS Institute Inc. 1985), after performing $\log_{10}(x + 1)$ transformations on the data. Percent population reductions following each treatment were determined using Abbott's formula which corrected for control mortality (Mulla et al. 1971).

Like many studies conducted over large areas, the larger-scale field study did not involve true replication of treatments and was therefore "pseudoreplicated" (Hurlbert 1984). Consequently, statistically significant differences cannot be attributed to treatment effects, but demonstrate that populations did not show parallel trends in the control and treated plots. However, the simultaneous exposure to a particular treatment and departure from parallel trends strongly implies a cause-effect relationship (Dosdall et al. 1991).

# 3 RESULTS

## 3.1 Production of BT in Laboratory-scale Fermenters

Data from some of the fermenter runs with Strains A1.2/72 and GE-13.2 are presented in Table 1. (Many additional runs were performed to optimize conditions but were not harvested; for example, runs were made to determine appropriate antifoam levels.) With the "normal" production medium (see Section 3.1), cell lysis was complete in 48 hours yielding 5.65 g of freeze-dried BT powder and resulting in 76% larval mortality in the bioassay. When nutrient concentration was doubled, the fermentation, as might be expected, took longer; lysis required 72 hours to complete but produced over twice the yield of product with essentially the same activity as the original medium. Increasing nutrient concentration to three times the original recipe, however, did not produce a corresponding yield increase; yield was a little less than the double-strength formula but mortality of *M. configurata* larvae declined to 51%. Since lysis was completed at about the same time as was found for the double-strength recipe, it would appear that the extra nutrients were unused (at least the insoluble bean flour), and served only to dilute the harvested product with inert material giving a lower bioassay titre. Doubling the glucose resulted in little beneficial effect over the original recipe. The premise that phosphate might be limiting in the double-strength medium was not supported by trials where 1.0 and 2.0 $g \cdot L^{-1}$ of phosphate were added; yields were reduced slightly and a dramatic increase in foaming was observed which required the addition of considerable quantities of antifoam to keep under control. Spore counts were not a useful index of activity against larvae as can be seen by comparing the activity of product from BT Strain A1.2/72 at double the nutrient concentration with BT Strain A1.2/72 at triple the nutrient concentration: both had essentially the same spore counts per gram, yet their percent mortality in bioassay tests against bertha armyworm larvae differed considerably (Table 1).

Fewer fermenter runs of Strain GE-13.2 were needed to produce sufficient control material for spraying trials. Yields were comparable to those of Strain A1.2/72, and about the same time was required to complete lysis. Bioassays were not conducted on the control strain.

Table 1. Production parameters of *Bacillus thuringiensis* Strains A1.2/72 and GE-13.2, and the efficacy of Strain A1.2/72 for inducing mortality of *Mamestra configurata* larvae in leaf-dip bioassays.

| Medium | Yield ($g \cdot L^{-1}$) | Spores ($cfu \cdot mL^{-1}$) | EFT[1] at Harvest (hours) | Bioassay[2] Results (% Mortality) |
|---|---|---|---|---|
| BT Strain A1.2/72 "normal" production medium | 5.65 | --- | 48 | 76 |
| BT Strain A1.2/72 double nutrients | 13.70 | $1.2 \pm 0.36 \times 10^9$ | 72 | 74 |
| BT Strain A1.2/72 triple nutrients | 13.00 | $1.3 \pm 0 \times 10^9$ | 76 | 51 |
| BT Strain A1.2/72 "normal" production medium + double glucose | 6.58 | $8.8 \pm 2.9 \times 10^8$ | 48 | 83 |
| BT Strain A1.2/72 double nutrients + 1.0 $g \cdot L^{-1}$ $K_2HPO_4$ | 10.40 | $7.1 \pm 0.7 \times 10^8$ | 72 | --- |
| BT Strain A1.2/72 double nutrients + 2.0 $g \cdot L^{-1}$ $K_2HPO_4$ | 11.90 | $6.6 \pm 0.9 \times 10^8$ | 72 | --- |
| BT Strain GE-13.2 "normal" production medium | 6.15 | $7.4 \pm 1.0 \times 10^8$ | 48 | --- |
| BT Strain GE-13.2 double nutrients | 11.70 | --- | 72 | --- |

[1] Elapsed fermentation time.

[2] A leaf-dip method using a 500 $\mu g \cdot L^{-1}$ suspension of the freeze-dried powder in distilled water and performed at the Agriculture Canada Research Station, Winnipeg according to the protocol of Dr. O.N. Morris.

### 3.2 Pilot-scale Culture of BT

Three test runs of Strain A1.2/72 were performed in the pilot-scale fermenter; two were preliminary runs to determine appropriate conditions, antifoam addition strategies, etc.; the final run was partially harvested (50 L of broth), washed, and dried as before. Runs in the larger fermenter conducted with double-strength medium took only 48 hours to achieve complete cell lysis compared with 72 hours in the laboratory-scale unit. This is probably attributable to the larger inoculum employed in the larger unit, i.e., 10% rather than 4% v/v. An exact figure for the yield in grams per L of freeze-dried powder was not calculated because of technical problems with the harvest, but the yield was estimated to be similar to yields for the smaller units, i.e., in the 11 to 12 $g \cdot L^{-1}$ range. The spore density, too, was similar to previous data: $8.9 \pm 0.1 \times 10^8$ cfu spores per mL.

### 3.3 Exotoxin Assay

No evidence of exotoxin activity was observed in culture fluids of either strain of BT (Table 2). No deleterious effects on survival of *M. configurata* occurred with supernatants, whether heat-treated or not. As expected, the washed "pellets" (i.e., spore-crystal preparations) of both strains were lethal to larvae when incorporated into diet. For example, washed "pellets" of Strains A1.2/72 and GE-13.2 caused 73 and 89% mortality to *M. configurata* larvae, respectively, and surviving larvae failed to develop beyond the third instar - their growth stage at the beginning of the experiment.

### 3.4 Laboratory Bioassays

Two days following exposure to leaves dipped in various treatments, mortality of *M. configurata* larvae was low; in all cases only one or no larvae were dead (Table 3). Six days after treatment, however, mortality of larvae that consumed leaf material containing Strain A1.2/72 with and without caffeine was 65% compared with only 20% for the controls. Mortality of larvae that consumed leaves with Strain GE-13.2 with and without caffeine was also comparatively high (50 and 45%, respectively), but mortality was low and comparable to controls for larvae that consumed leaves containing either the 'Morris' formulation without caffeine (15%), the 'Morris' formulation with caffeine (20%), or the 'Beegle' formulation (30%). Mortality of larvae that consumed leaf material containing Strain GE-13.2 in the 'Beegle'

Table 2. Results of laboratory bioassays conducted to determine whether BT Strains A1.2/72 and GE-13.2 exhibited exotoxin activity. Third-instar larvae of *Mamestra configurata* were placed on artificial rearing diet in which treatments had been incorporated.

| Treatment | #Surviving/ Total | Percent Survival | Surviving Instar (%) 3 | Surviving Instar (%) 4-6 | % Treatment Induced Mortality[1] |
|---|---|---|---|---|---|
| Control 10 ml water added | 15/20 | 75 | | 100 | N/A |
| BT Strain A1.2/72 washed pellet | 4/20 | 20 | 100 | | 73.3 |
| BT Strain A1.2/72 undiluted supernatant | 21/24 | 87.5 | 9.5 | 90.5 | -16.7 |
| BT Strain A1.2/72 $10^{-1}$ supernatant | 18/20 | 90 | | 100 | -20.0 |
| BT Strain A1.2/72 $10^{-2}$ supernatant | 21/24 | 87.5 | | 100 | -16.7 |
| BT Strain A1.2/72 undiluted supernatant - heat treated | 21/24 | 87.5 | | 100 | -16.7 |
| BT Strain A1.2/72 $10^{-1}$ supernatant - heat treated | 21/24 | 87.5 | 9.5 | 90.5 | -16.7 |
| BT Strain A1.2/72 $10^{-2}$ supernatant - heat treated | 21/22 | 95.5 | | 100 | -27.3 |
| BT Strain GE-13.2 washed pellet | 2/24 | 8.3 | 100 | | 88.9 |
| BT Strain GE-13.2 undiluted supernatant | 22/24 | 91.7 | | 100 | -22.2 |
| BT Strain GE-13.2 $10^{-1}$ supernatant | 25/26 | 96.2 | | 100 | -28.2 |
| BT Strain GE-13.2 $10^{-2}$ supernatant | 23/24 | 95.8 | | 100 | -27.8 |
| BT Strain GE-13.2 undiluted supernatant - heat treated | 29/29 | 100 | | 100 | N/A |
| BT Strain GE-13.2 $10^{-1}$ supernatant - heat treated | 23/24 | 95.8 | 8.7 | 91.3 | -27.8 |
| BT Strain GE-13.2 $10^{-2}$ supernatant - heat treated | 22/24 | 91.7 | | 100 | -22.2 |

[1] Treatment-induced mortalities were calculated on Day 8, using Abbott's formula (Mulla et al. 1971) to correct for control mortality. Negative values indicate that reductions in larval numbers were not related to treatment effects.

Table 3. Results of laboratory bioassays of fourth-instar larvae of *Mamestra configurata* (20 larvae per treatment) supplied with leaf material of canola (*Brassica rapa*) that had been dipped in solutions containing various treatments.

| Treatment | Number of Surviving Larvae Following Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 days | | | | 6 days | | | |
| | Instar | | | % Mortality | Instar | | | % Mortality |
| | 4 | 5 | 6 | | 4 | 5 | 6 | |
| BT Strain GE-13.2 (1.25 mg·mL$^{-1}$) + 'Morris' Formulation with caffeine | 10 | 6 | 3 | 5 | 0 | 2 | 8 | 50 |
| BT Strain GE-13.2 (1.25 mg·mL$^{-1}$) + 'Morris' Formulation without caffeine | 4 | 15 | 0 | 5 | 0 | 5 | 6 | 45 |
| BT Strain A1.2/72 (1.25 mg·mL$^{-1}$) + 'Morris' Formulation with caffeine | 3 | 17 | 0 | 0 | 0 | 0 | 7 | 65 |
| BT Strain A1.2/72 (1.25 mg·mL$^{-1}$) + 'Morris' Formulation without caffeine | 20 | 0 | 0 | 0 | 7 | 0 | 0 | 65 |
| 'Morris' Formulation without caffeine | 0 | 12 | 8 | 0 | 0 | 2 | 15 | 15 |
| 'Morris' Formulation with caffeine | 1 | 9 | 9 | 5 | 0 | 3 | 13 | 20 |
| 'Beegle' Formulation | 1 | 4 | 15 | 0 | 1 | 3 | 10 | 30 |
| BT Strain GE-13.2 (1.25 mg·mL$^{-1}$) + 'Beegle' Formulation | 11 | 6 | 3 | 0 | 2 | 2 | 3 | 65 |
| BT Strain A1.2/72 (1.25 mg·mL$^{-1}$) + 'Beegle' Formulation | 6 | 8 | 6 | 5 | 3 | 5 | 5 | 35 |
| Control (water only) | 1 | 6 | 13 | 0 | 0 | 1 | 15 | 20 |

formulation was higher (65%) than for larvae that consumed leaves with Strain A1.2/72 in the 'Beegle' formulation (35%).

### 3.5 Greenhouse Tests

In the first greenhouse trial (27 October 1992), no statistically significant changes were observed in mean numbers of *M. configurata* larvae per control plant for any of the sampling dates ($P > 0.05$) (Table 4). Similarly, for plants treated with the high and low dosages of BT Strains A1.2/72 and GE-13.2, and for plants treated with the formulation, no statistically significant population reductions were observed among pre- and post-treatment sampling dates ($P > 0.05$). For plants treated with Decis® 5 EC, however, statistically significant population reductions were observed between pre- and post-treatment sampling dates ($P < 0.05$). By seven days after treatment with Decis® 5 EC, *M. configurata* populations were 97% lower than those recorded before treatment.

In the second greenhouse trial (8 December 1992), larval populations on control plants did not differ significantly between the pre-treatment and three days post-treatment sampling dates ($P > 0.05$); however, a statistically significant population reduction was observed between the three and seven days post-treatment sampling dates ($P < 0.05$) (Table 5). No statistically significant population reductions were recorded between pre- and post-treatment sampling dates for plants treated with the high and low dosages of BT Strains A1.2/72 and GE-13.2, and plants treated with the formulation ($P > 0.05$). Following application of Decis® 5 EC, however, populations were reduced by 100%, and this reduction was significant statistically on both post-treatment sampling dates ($P < 0.05$).

### 3.6 Small Plot Studies

Approximately five hours after transferring larvae of *M. configurata* from the acclimatization cages onto plants for the first small plot trial (7 July 1992), it rained at the study site and scattered showers continued for the next two days. Although the weather was clear when application of treatments began, by the time the applications were completed about two hours later, heavy rain occurred almost immediately afterwards. Determinations of larval populations on plants collected before treatment indicated that almost no larvae were present; consequently, sampling for the first small plot trial was discontinued.

Table 4. Mean numbers ± SE per plant and percent mortality (in **bold**) of *Mamestra configurata* larvae on canola plants maintained under greenhouse conditions and receiving various treatments on 27 October 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons.

| | Mean no. individuals/plant, pre- and post-treatment (days) | | |
|---|---|---|---|
| Treatment | Pre-treatment | 3 | 7 |
| Control (water only) | 4.3 ± 0.8 a | 3.6 ± 0.7 a | 4.9 ± 0.7 a |
| BT Strain A 1.2/72 (1.25 mg·mL$^{-1}$) | 3.4 ± 0.3 a | 3.1 ± 0.7 a<br>**-8.5**[1] | 2.4 ± 0.6 a<br>**38.1** |
| BT Strain A 1.2/72 (0.31 mg·mL$^{-1}$) | 3.0 ± 0.5 a | 2.1 ± 0.7 a<br>**16.7** | 1.9 ± 0.7 a<br>**44.5** |
| BT Strain GE-13.2 (1.25 mg·mL$^{-1}$) | 3.1 ± 0.5 a | 3.0 ± 0.7 a<br>**-15.2**[1] | 2.0 ± 0.2 a<br>**43.5** |
| BT Strain GE-13.2 (0.31 mg·mL$^{-1}$) | 3.5 ± 0.5 a | 4.4 ± 1.3 a<br>**-49.6**[1] | 3.4 ± 0.9 a<br>**14.8** |
| Decis® 5 EC (deltamethrin) (5 g a.i.·ha$^{-1}$) | 2.9 ± 0.6 a | 0.1 ± 0.1 b<br>**95.9** | 0.1 ± 0.1 b<br>**97** |
| Formulation (no BT) | 3.4 ± 0.8 a | 3.1 ± 0.9 a<br>**-8.5**[1] | 2.3 ± 0.8 a<br>**40.7** |

[1] Negative numbers indicate that the cause for the reduction in numbers of larvae is not related to effects of the treatment.

Table 5. Mean numbers ± SE per plant and percent mortality (in **bold**) of *Mamestra configurata* larvae on canola plants maintained under greenhouse conditions and receiving various treatments on 8 December 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons.

| | Mean no. individuals/plant, pre- and post-treatment (days) | | |
|---|---|---|---|
| Treatment | Pre-treatment | 3 | 7 |
| Control (Water only) | 4.6 ± 0.5 ab | 6.6 ± 1.3 a | 3.0 ± 0.9 b |
| BT Strain A 1.2/72 (1.25 mg·mL$^{-1}$) | 4.8 ± 0.9 a | 4.3 ± 1.2 a<br>**37.4** | 2.5 ± 0.9 a<br>**19.9** |
| BT Strain A 1.2/72 (0.31 mg·mL$^{-1}$) | 2.9 ± 0.8 a | 3.6 ± 1.1 a<br>**13.2** | 4.0 ± 1.3 a<br>**-112.2**[1] |
| BT Strain GE-13.2 (1.25 mg·mL$^{-1}$) | 4.4 ± 1.1 a | 2.8 ± 0.7 a<br>**55.5** | 2.5 ± 0.8 a<br>**12.6** |
| BT Strain GE-13.2 (0.31 mg·mL$^{-1}$) | 3.5 ± 0.7 a | 4.1 ± 0.8 a<br>**18.1** | 4.6 ± 1.6 a<br>**-102.2**[1] |
| Decis® 5 EC (deltamethrin) (5 g a.i.·ha$^{-1}$) | 2.9 ± 0.9 a | 0 b<br>**100** | 0 b<br>**100** |
| Formulation (no BT) | 4.4 ± 1.1 a | 3.5 ± 1.2 ab<br>**44.4** | 1.9 ± 0.6 ab<br>**33.6** |

[1] Negative numbers indicate that the cause for the reduction in numbers of larvae is not related to effects of the treatment.

Data from the second small plot trial indicate that larval populations in the control plots declined throughout the study period (Table 6). This reduction was significant statistically between pre- and post-treatment sampling dates ($P < 0.05$), and by 10 days after treatment, no larvae were collected on the control plots. Similar statistically significant larval population reductions were observed between pre- and post-treatment sampling dates for plots treated with high and low dosages of both BT Strains A1.2/72 and GE-13.2, Decis® 5 EC, and the formulation alone without BT ($P < 0.05$).

### 3.7 The Larger-scale Field Study

When treatments were applied to the Stony Plain field on 17 August 1992, most individuals in the population were instars 4 (23.1%), 5 (56.2%), and 6 (14.1%); few specimens were instars 2 (1.8%) and 3 (4.8%) ($n = 502$). Population reductions occurred in all plots throughout the study period, including the controls (Table 7). Between the pre-treatment and three days post-treatment sampling dates, statistically significant population reductions were observed in all treated plots ($P < 0.05$) but not in the control plot. By seven days post-treatment, population reductions relative to the pre-treatment sampling date occurred in all plots (including the control) that were significant statistically ($P < 0.05$). Greatest percent reductions between pre- and seven days post-treatment sampling dates occurred in the plot treated with Decis® 5 EC (100%); population reductions seven days after treatment in plots treated with the formulation, Strain A1.2/72, and Strain GE-13.2 were 60.0, 48.9, and 64.0%, respectively.

## 4 DISCUSSION

Laboratory bioassay studies indicated that both BT Strains A1.2/72 and GE-13.2 affected survival of *M. configurata* larvae. Although little impact on survival was evident two days after larvae were exposed to fresh leaf material containing the BT, by six days following treatment mortality of larvae subjected to leaf dips of 1.25 $mg \cdot mL^{-1}$ of Strains A1.2/72 and GE-13.2 was greater than that for larvae consuming leaves treated with formulation alone or for larvae consuming leaves treated with water only (controls). Greatest mortality in the bioassays occurred when washed "pellets" containing the spore-crystal preparations were incorporated into the artificial rearing diet of bertha armyworm larvae. This is probably due to the greater BT dosage available to larvae when the "pellets" were used. In diet, larvae would be subjected to a

Table 6. Mean numbers ± SE per plant and percent mortality (in **bold**) of *Mamestra configurata* larvae on 0.5 $m^2$ field plots receiving various treatments on 16 July 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons.

| | Mean no. individuals/plant, pre- and post-treatment (days) | | | |
|---|---|---|---|---|
| Treatment | Pre-treatment | 3 | 7 | 10 |
| Control (water only) | 2.6 ± 0.6 a | 0.6 ± 0.3 b | 0.1 ± 0.1 b | 0 b |
| BT Strain A 1.2/72 (1.25 mg·mL$^{-1}$) | 2.5 ± 0.5 a | 0.6 ± 0.2 b<br>**-4.3**[1] | 0.2 ± 0.1 b<br>**-100**[1] | 0 b<br>*0* |
| BT Strain A 1.2/72 (0.31 mg·mL$^{-1}$) | 2.3 ± 0.4 a | 0.4 ± 0.2 b<br>**24.4** | 0.1 ± 0.1 b<br>**-8.7**[1] | 0 b<br>*0* |
| BT Strain GE-13.2 (1.25 mg·mL$^{-1}$) | 2.4 ± 0.5 a | 0.7 ± 0.2 b<br>**-26.8**[1] | 0.1 ± 0.1 b<br>**-4.2**[1] | 0.1 ± 0.1 b<br>*0* |
| BT Strain GE-13.2 (0.31 mg·mL$^{-1}$) | 2.0 ± 0.5 a | 0.5 ± 0.2 b<br>**-8.7**[1] | 0.2 ± 0.1 b<br>**-150**[1] | 0 b<br>*0* |
| Decis® 5 EC (5 g a.i.·ha$^{-1}$) | 1.6 ± 0.4 a | 0.1 ± 0.1 b<br>**72.9** | 0 b<br>**100** | 0.1 ± 0.1 b<br>*0* |
| Formulation (no BT) | 2.1 ± 0.5 a | 0.5 ± 0.1 b<br>**-3.5**[1] | 0 c<br>**100** | 0 c<br>*0* |

[1] Negative numbers indicate that the cause for the reduction in numbers of larvae is not related to effects of the treatment.

Table 7. Mean numbers ± SE of *Mamestra configurata* larvae per 1 $m^2$ sample, and percent population reductions (in **bold**) in Stony Plain, Alberta field plots receiving various treatments on 17 August 1992. Means in the same row followed by the same letter are not significantly different ($P > 0.05$) using analysis of variance (ANOVA) and Student-Newman-Keuls multiple comparisons.

| | Mean no. individuals/sample, pre- and post-treatment (days) | | |
|---|---|---|---|
| Treatment | Pre-treatment | 3 | 7 |
| Control (water only) | 21.0 ± 5.4 a | 11.8 ± 2.8 a | 4.0 ± 1.8 b |
| Formulation (no BT) | 34.2 ± 11.6 a | 6.2 ± 2.7 b **67.9** | 2.6 ± 1.6 b **60.0** |
| BT Strain A 1.2/72 (1.25 $mg \cdot mL^{-1}$) | 20.6 ± 7.4 a | 2.4 ± 1.2 b **79.2** | 2.0 ± 0.5 b **48.9** |
| BT Strain GE-13.2 (1.25 $mg \cdot mL^{-1}$) | 14.6 ± 6.0 a | 3.2 ± 0.8 b **60.9** | 1.0 ± 0.5 b **64.0** |
| Decis® 5 EC (deltamethrin) (5 g a.i.$\cdot ha^{-1}$) | 5.6 ± 1.5 a | 1.0 ± 0.6 b **68.1** | 0.0 ± 0.0 b **100** |

concentration of spore-crystal preparation of approximately 0.5 $mg \cdot mL^{-1}$ of diet; on leaves, this would be considerably less because only those crystals remaining on the leaf surface after dipping in the 1.25 $mg \cdot mL^{-1}$ solution would be available to larvae.

The efficacy studies conducted under greenhouse conditions did not indicate that BT Strains A1.2/72 and GE-13.2 were effective for reducing larval densities of *M. configurata*. By contrast, the insecticide Decis® 5 EC caused immediate and dramatic population reductions in both trials. It is possible that the toxic activity of the BT crystals was reduced by incident radiation, although both treatments were applied later in the day to minimize this effect.

Interpretation of data from the small plot study is confounded by the decrease in mean numbers of larvae per plant that occurred throughout the study period on all plots, including the controls. Although larvae were derived from laboratory cultures maintained under artificial rearing conditions, they had been acclimatized to field conditions prior to their placement onto the experimental plots; consequently, mortality after transfer should have been minimal. However, while moving *M. configurata* larvae onto individual plants on the study plots, we observed several larvae being parasitized by the ichneumonid wasp, *Banchus flavescens* Cresson. Parasitized larvae then appeared to resume normal feeding. Obviously, parasitism will not result in the immediate mortality of host larvae, and because completion of the first four (of a total of five) larval instars of the parasitoid normally requires 17-18 days (Arthur and Mason 1985), these larvae should still have been present on untreated plots on all post-treatment sampling dates. A small number of larvae of green lacewing (*Chrysopa carnea* Stephen; Neuroptera: Chrysopidae) were also observed on the study plots, and are known to prey on bertha armyworm larvae (Bucher and Bracken 1976). In fact, a single larva of *C. carnea* can destroy as many as five bertha armyworm larvae per day, and consequently *C. carnea* is considered to be an important natural limiting factor of bertha armyworm populations. It is probable, therefore, that the decline in bertha armyworm populations in the small plots throughout the study period was caused by natural predators of bertha armyworm combined with some natural larval mortality.

Results of the field test conducted in the natural bertha armyworm infestation in the Stony Plain canola field were more promising than those obtained from either the greenhouse or the small plot studies. Three days following treatment, population reductions were observed for plots treated with Strains A1.2/72 and GE-13.2 that were comparable to those recorded for plots treated with Decis® 5 EC. However, because the majority of the bertha armyworm population was in

instars 5 and 6 when treatments were applied, by seven days after treatment many larvae had already burrowed into the soil and were in the prepupal or pupal developmental stages. Beneath the soil surface, these individuals were not collected using our sampling method. The decline in larval densities recorded seven days after treatment was then a result of treatment-induced mortality and larval pupation in the soil.

Results of these studies did not demonstrate superior efficacy of Strain A1.2/72 relative to Strain GE-13.2 against *M. configurata* larvae. In laboratory bioassays, greenhouse, and field studies both strains were comparable in their effectiveness against bertha armyworm larvae.

Further research is needed to improve the efficacy of the BT for use under field conditions. Laboratory bioassay studies indicated that both strains can kill *M. configurata* larvae, but at present, the effectiveness of the strains under field conditions is clearly inferior to the insecticide applied most commonly in the field for reducing bertha armyworm populations. Research should be directed toward improving the residual biological activity of BT by limiting exposure of the BT spore-crystal to incident radiation. This could be achieved through the addition of a sunlight screen to the formulation, using a protective carrier cell to deliver the BT, or incorporating the gene for the BT endotoxin in the genome of *Brassica rapa* and *B. napus*. Morris (1991) found that incorporation in the formulation of the ultraviolet sunlight screen, Congo Red, increased the residual activity of BT on canola plants without causing harmful effects to sprayed plants. Residual activity could also be improved through the use of a carrier cell such as the recombinant, plant-colonizing *Pseudomonas* developed by Monsanto and Mycogen for delivery of BT genes (Starnes et al. 1993). BT could be incorporated with the pseudomonad cell, sprayed on the crop, and provide longer residual activity because the BT endotoxin would be protected from environmental degradation. Engineering the BT genes into canola could provide the greatest crop protection against damage by bertha armyworm larvae, but would favor development of resistance to BT by this pest. Other means of improving efficacy could include adding a feeding stimulant to the formulation, or improving the BT application technology by producing more concentrated and finer droplets.

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