PN-ABM-523

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/30/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C5-175
Semi-annual - July 88

Attachment

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CS-175

Semi annual Report - July 1988
US-ISRAEL AID grant No. DPE-5544-G-5506042-00

Title: DEVELOPMENT OF A MICROBIAL PREPARATION FOR BIOLOGICAL CONTROL OF THE AFRICAN ARMYWORM, *Spodoptera exempta*.

Submitted by:

Prof. BARUCH SNEH and Dr. MEIR BROZA
Institute for Nature Conservation Research
The George S. Wise Faculty of Life Sciences
Tel-Aviv University, ISRAEL.

and

Dr. MICHAEL BROWNBRIDGE
International Center for Insect Physiology
and Ecology (ICIPE) KENYA

Rec'd ... 25 1988

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Moti Hamal continued his isolation and screening of Bacillus thuringiensis against larvae of tne African armyworm in ICIPE, Kenya. He returned to Israel, in the beginning of March 1988, to continue the comparative insecticidal activity of the different isolates against the Egyptian cotton leafworm (Spodoptera littoralis). In mid February 1988, Dr. Meir Broza went over to ICIPE Kenya for a 2 weeks visit, where he became acquainted with the biology and behaviour of the armyworm populations in East Africa, and make the necessary planning and arrangements for the field experiments to control the armyworm larvae with praparations of strains of B. thuringiensis effective against this pest.

In mid March 1988, during the outbreaks of the armyworm populations in Kenya, Ariel Venetzian (a field entomologist from the Israeli group), arrived to ICIPE Kenya for 3 weeks during the outbreak season, with the B. thuringiensis preparations produced in Israel, to set up field experiments. Dr. Michael Brownbridge, who is working in Mbita point Research Station of ICIPE, has joined the project. He worked with Moti Hamal during his stay there. He took over the responsibilities to continue the more detailed isolation and screening work, and the subsequent field work which was initiated together with Ariel Venetzian.

The isolations and screening of new Bt isolates for their comparative insecticidal activities against the African armyworm and the Egyptian cotton leafworm is continued. During this screening, several isolates have demonstrated a higher insecticidal activity against the cotton leafworm than the best strain (Bt_{24}). They are currently evaluated for their insecticidal activity against the armyworm.

Meanwhile, as some isolates have shown good insecticidal activity against the armyworm, it has been decided to make use of the armyworm outbreaks this year to carry out initial field trials with preparations of 2 strains (powder and granule preparations) which were produced in Israel by the BMPI (Backer Microbial Products Israel) fermentation plant. From these applications we intended to get some

idea on the performance of these strains in the field, and to get acquainted with the problems that need to be taken in consideration for our full scale field experiments for the next outbreak season of 1989.

FIELD APPLICATIONS OF *BACILLUS THURINGIENSIS* STRAINS PREPARATIONS AGAINST THE AFRICAN ARMYWORM, *SPODOPTERA EXEMPTA*.

Field experiment No. 1.

Weather: Overcast, windy.
Location: Lambwe valley.
Grass type: Cinadon.

The first experiment was carried out in a short grass field, on mini plots of 1X1m, with three bacterial preparations in three replicates. The powdered preparations were mixed with water and CCSO (crude cotton seed oil), and sprayed with a hand sprayer, approximately .5 cm above ground. The granule preparation was applied by hand. In addition, two preparations were also sprayed in a field with longer grass (about 40cm above ground), on 3X1m plots in 3 replicates. The experimental details are summarized in Table 1.

Larvae were counted using 20X20cm quadrats in the short grass, two counts per replicate. An estimate of larval numbers in the longer grass was made using a sweep net, 5 sweeps for each of five replicate counts, outside of the treated area. Counts were taken before Bt was applied and 4 days post treatments.

Table 1.
Concentrations and application rates of *Bacillus thuringiensis* preparations used for the field experiment.

TREATMENT	CONCENTRATION OF Bt PREPARATION[a]	APPLICATION RATE	TREATED AREA
SHORT GRASS			
Nontreated			$1m^2$ (X3)
Bt entomocidus powder	2% (W/V) + 1% CCSO[b]	$330ml/m^2$	$1m^2$ (X3)
Bt aizawai powder	1% (W/V) + 1% CCSO[b]	$330ml/m^2$	$1m^2$ (X3)
Bt entomocidus granules (10%Bt)	100ml granules	$100ml/m^2$	$1m^2$ (X3)
LONG GRASS			
Nontreated			1m X 3m (X5)
Bt *entomocidus* powder	2% (W/V) + 1% CCSO[b]	1000ml/plot	1m X 3m (X5)
Bt *aizawai* powder	1% (W/V) + 1% CCSO[b]	1000ml/plot	1m X 3m (X5)

[a] Toxicity of Bt *entomocidus* (Bt_{24}) powder, was 35000 IU (BMPI standard), and Bt *aizawai* powder was 6500 IU. Thus, a 2% concentration of the Bt *entomocidus* vs. a 1% Bt *aizawai* concentrations were used to get approximately the same number of IU/unit area.
[b] Crude cotton seed oil.

Results and descriptions

Table 2.
Effect of preparations of Bacillus thuringiensis on larvae of the African armyworm (Spodoptera exempta) in a Cinadon graas field in Lambwe valley Kenya.

TREATMENT	No. living larvae[a] PRE	POST	%[b]	% OF CONTROL[c]
Short grass				
Nontreated	58	84	145	100
Bt entomocidus	123	264	215	148
Bt aizawai	120	240	200	166
Bt aizawai granules	114	85	75	52
Long grass				
Nontreatred	521	152	29	100
Bt entomocidus	521	186	36	124
Bt aizawai	521	188	36	124

[a] Only living larvae were counted. Larvae in the untreated plots were active, and no cadavers were found, while those in the treated plots were inactive, moribund and cadavers in appreciable numbers were found there.

[b] Percent of post (3 days after) treatmet form pretretment counts.

[c] Percent of % for treated from % of untreated control.

[d] Plots of 1x1m in 3 replicates

[e] Plots of 1X3m in 5 replicates

The results of the first field experiment summarized in Table 2, if viewed purely numerically, indicate that sprayed Bt treatments had no effect in reducing the number of armyworm and protecting the grass. This is very marked in the 1X1m plots. In these plots, the only treatment which gave an actual numeric reduction in larval population

was the granular formulation application (52% survival of the control). There was a reduction in larval numbers in the treated 1X3m plots, but the reduction was significantly larger in the control plots.

However, when seen in the field, the protective effect of Bt sprays was obvious, and in itself reason for the increase in larval density inside the 1X1m treated plots.

The grass in both the granule treated and control plots, was totally consumed by the larvae during the 4 day experimental period. In both the Bt entomocidus and Bt aizawai - treated plots, however, leaves and new growth of grass were evident. This indicated that the larvae fed less on the treated grass. This may have been as a result of direct larval mortality following ingestion of the treated grass, as indicated in the number of cadavers observed (but not counted) inside the sprayed plots, or as a result of larval inactivity following ingestion of the Bt endotoxin. The latter effect was more visible inside the 1X3m plots, where the larvae were very inactive and moribund when compared with those in the untreated control plots.

The increase in larval numbers in the 1X1m plots following treatment may be explained by larval immigration into the treated areas. There were the only areas where fresh grass was available. The protective treatment was apparently maintained up to 10 days post treatment, as dead larvae were continuously observed, and the grass remained fresh unlike the surrounding area where all fresh material has been consumed. This was in spite of heavy rainstorms and long periods of sunshine.

It was thus decided to scale up the field experiment using the Bt aizawai powder only. The use of a larger experimental plot would also reduce the effects of larval movement on the field counts taken.

Field experiment No. 2.

Weather: Slightly overcast
Location: Lambwe valley - short grass.
Grass type: Cinadon.

The larvae observed in the field at this time were in their 4-6th larval instar. Therefore, in order to obtain a better field effect, Bt aizawai was sprayed at a 2% (W/V) concentration with 2% crude cotton seed oil (CCSO) as follows:

Table 3.
Concentrations and application rates of Bacillus thuringiensis powder preparation used for the field experiment.

TREATMENT	CONCENTRATION	APPLICATION RATE	TREATED AREA
Nontreated			10X10m
Bt aizawai powder	2% (W/V) + 2% (V/V) CCSO	10L/100m²	10X10m

Larvel population density was estimated by taking 20 randomly selected counts within each of the plots, using 20X20 quadrats. Living larvae only were counted. Larvae not responding to physical stimulation were considered to be dead. The populations of both treated and untreated control plots were counted every 24h for 6 days. Counts were taken more in the centers of the plots, rather than along the borders to negate the effect of larval immigration on population counts.

Results and descriptions

Table 4.
Effect of application of *Bacillus thuringiensis* var *aizawai* powder preparation, on cinadon grass in the field,Son larval populations on *Spodoptera exempta*.

TIME AFTER TREATMENT DAYS	NONTREATED CONTROL NUMBER LARVAE[a]	NONTREATED CONTROL % OF INITIAL COUNT	Bt AIZAWAI NUMBER LARVAE[a]	Bt AIZAWAI % OF INITIAL COUNT	% OF CONTROL[b]
0	133	100.0	135	100.0	101.5
1	110	82.7	53	39.2	47.4
2	170	127.8	155	114.8	89.8
3	165	124.0	115	85.2	68.7
4	123	92.5	59	43.7	47.2
5	51	38.3	36	26.7	69.7
6	43	32.3	26	19.2	59.4

[a] Numbers of surviving larvae were counted in 20 quadrants of 20X20cm each.

[b] percentage was calculated as % of initial count of the treated from that of the nontreated control.

For the data summarized in Table 4, the following observations were made with each count:

One day after treatment application. In the treated area, many dead and moribund individuals were observed. Very little larval movement on the ground in the treated plot, except at the border of the area where immigration was evident. In the untreated control plots, the larvae were very active and there was much movement throughout the area.

Two days after treatment, the Bt - treated plots were apparently much greener the the control ones. The plants (grass) were in much better condition with many full and new leaves in evidence. Very little preenery left in the control plots and the whole of the surrounding area.

In the treated plot, many moribund and dead larvaewere again observed. The population increase may have been due to the immigration of larvae into the treated area, where food was available, from the surrounding area, where all grass has been consumed and plants devastated.

Three days after treatment, many dead larvae observed in the treated plots. treated area apparently re-infested from the surrounding area where no grass was available. All grass in the control plots had been consumed. Plants in the treated plots were very green and much new leaf growth was evident. Larvae in the control plots were very healthy and active. Although many larvae were observed in the treated plots, at least 50% (were not definitely counted), were dead or moribund. The Bt treatment thus seems to offer protection and continue to kill the armyworm larvae.

Four days after treatment, the treated area was very distinct from the control plots, with healthy grass growing well. In the control area, all greenery had been consumed and large active larvae were observed. The treated plots where many dead larvae were vi dent in the vegetation.

There was also a significant decrease in the larval population in the treated plots. The 2 effects, good growth of grass and reduction in larval population density appear to provide good evidence for the protective effect of the Bt tratment. This may have occurred in 2 ways: 1. Bt causing direct larval mortality, although the effective field dose was probably decreasing with time as a result of inactivation by sunlight etc., and washing off the leaves by rain., and 2. Ingestion of the Bt endotoxin might have caused the larvae to cease feeding, even if the dose ingested was not rapidly lethal to the relatively large larvae. Both effects would permit the grass to grow as was clearly observed.

Five days hours after treatment, after heavy raifall in the orevious night, the control plots were stripped bare, resulting in a massive migretion of larvae out of the ntreated area in search for food, or into the softended ground to pupate. In the treated plot, a

further reduction in larval population density and the large number of the dead larvae observed, was further evidence of continuing The activity of the Bt endotoxin.

The final observation (6 days after treatment) was taken following second consecutive night of very heavy rain, which made the ground very soft. Many larvae were thus thought to have entered the ground to pupate, as indicated by the relatively low numbers of larvae observed throughout the area. Thus the differences between the treaated and untreated plots are actually much greater than indicated in Table 4. The treated plots were the only green areas in an otherwise totally stripped grassless plain.

The results obtained thus, indicate that the strain (Ao7) of Bt aizawi is very effective in reducing the damage of the armyworm larvae also when they are in their advanced development instars. The population density in the treated plot was constantly lower than that in the untreated control. (Fig. 1), and population falls generally significant, probably as a result of the entomocidal activity of the Bt endotoxin. The continuing growth of the grass within the sprayed plot also suggested that the endotoxin remained active throughout the experimental period.

Field experiment No. 3.

Weather: Calm and slightly overcast
Location: Lambwe valley - short grass.
Grass type: Cinadon.

Two 15X15m plots were used in this experiment. Due to the age of the larvae (most 4-6th instars) a 2% (W/V) concentration of Bt aizawai + 2% CCSO in water was used for spray application.

Table 5
Concentrations and application rates of Bacillus thuringiensis powder preparation used for the field experiment.

TREATMENT	CONCENTRATION	APPLICATION RATE	TREATED AREA
Nontreated			15X15m
Bt aizawai powder	2% (W/V) + 2% (V/V) CCSO	15L/225m^2	15X15m

Larval density was estimated by counting the living larvae within 20X20cm quadrats in 25 randomly selected counts in each plot. Population counts were taken every 24h for 4 days, with sampling carried out at least 2m in, from the border area, to negate the effect of larval movement which could influence the results.

Results and descriptions

For the data summarized in Table 6, the following observations were made with each count: One day after treatment, some rainfall on previous evening, but ground was still too dry for the larvae to enter and pupate. Also, ample food available to facilitate further growth and development. Rain may however, have washed some of the spray deposits from the leaves.

Table 6.

Effect of application of *Bacillus thuringiensis* var *aizawai* powder preparation, on cinadon grass in the field, on larval populations on *Spodoptera exempta*. (see also Fig. 2).

TIME AFTER TREATMENT DAYS	NONTREATED CONTROL		Bt AIZAWAI		
	NUMBER LARVAE[a]	% OF INITIAL COUNT	NUMBER LARVAE[a]	% OF INITIAL COUNT	% OF CONTROL[b]
0	200	100.0	167	100.0	83.5
1	177	88.5	59	35.3	39.9
2	130	65.0	32	19.2	29.5
3	81	40.5	34	20.3	50.1
4	43	21.5	16	9.6	44.7

[a] Numbers of surviving larvae were counted in 25 quadrants of 20X20cm ach.

[b] percentage was calculated as % of initial count of the treated from that of the nontreated control.

In the treated plots, many dead and moribund larvae, and little movement of the surviving ones were observed on the ground. In the untreated control plots, large and very active larva were observed on the ground. Only late instar (V-VI) larvae were observed alive in the area, presumably as a larger dose or longer feeding time are required to incite mortality of large larvae.

Two days after treatment, dead and moribund larvae were observed in the treated plots. Again, there was little larval activity within the treated plots and high activity in the untreated controls.

Three days after treatment, Slight overnight rain. Softer ground could account for some of the reduction in larval population density in the control plots, with larvae going into the ground to pupate. Also, the poor state of the grass in the control plots would induce the larvae to migrate out of the area in search for food.

In the treated plot, larval cadavers were in evidence. The grass also appeared to be much more healthy than in the control plot, with much new leaf growth observed.

Four days after treatment, after heavy overnight rain, the ground was very soft and favorable for larval entry for pupation. This was probably the main reason for the reduction in larval density in the control. Larvae ingesting sublethal concentrations of the endotoxin, develop slower and pupate after an extended period of time.

Overall, Bt appeared again to effectively protect the treated grass. Whilst there was a significant reduction in larval numbers, through the toxic activity of the bacterial preparation. The treatment also appeared to reduce larval activity and feeding. This allowed the grass to grow and it was very evident in the treated plot that much new leaf growth had occurred. This is especially important at the time of outbreak which coincides with periods of rain and sunshine, and is a time of peak plant growth and leaf production. Therefore, if protection can be provided over this period, then a good grass crop will be available for grazing cattle etc.

GENERAL OVERVIEW OF OUTBREAK SITUATION IN LAMBWE VALLEY, AND EXPERIMENTAL RESULTS.

The 1988 outbreak of the African armyworm (*Spodoptera exempta*) in the Lambwe valley was spectacular in the sheer numbers of larvae and the destruction to large areas of grass and crop land which occurred as a result of their voracious feeding activity. Many of the small larvae located in the area suffered as a result of the armyworm, with maize, sorghum and finger millet crop being totally consumed by larvae which had migrated from the grass in search for an alternative food source. Few farmers can afford to spray their crop to protect against the armyworm. Their crops were totally lost and the area under cultivation had to be replanted. Those farmers spraying their fields, had farms over 5 hectars and can be considered wealthy in the general subsistance - type farming which is practiced in the Lambwe valley.

The data obtained with Bt indicate that the bacterial preparation has a good potential for crop protection against the armyworm. Unfortunately, the trials could not be carried out in farmers fields due to the lack of government permit giving permission to use the bioinsecticide. (now we have already obtained the permit for our future experiments). However, the response of the growe s was very positive to the possible use of their fields for experimental purposes in the next year. Full cooperation will be given, so a number of growers were for possible extension of the field work during the next outbreak. Now that we have the required permission, we plan larger scale field experiments to be performs in the armyworm outbreaks expected in 1989. The potent preparation of Bt aizawai, as a reference strain and more effective local strains that are currently screened for their insecticidal activity against both the African armyworm and the Egyptian cotton leafworm will be used in these field application experiments. Now we have a new soil isolate of Bt that has a 4-5 fold insecticidal activity against the Egyptian cotton leafworm that the highest effective strain Bt_{24} (entomocidus).

ACKNOWLEDGEMENTS

We would like to thank Moti Hamal for his important share in carrying out the experimental work in Israel and In Kenya, to Robinson for the technical assistance in rearing the larvae and lab. work in Kenya, to Silvia Schuster for her assistance in the lab. in Israel and to Ariel Venetzian for his work in the field experiments.

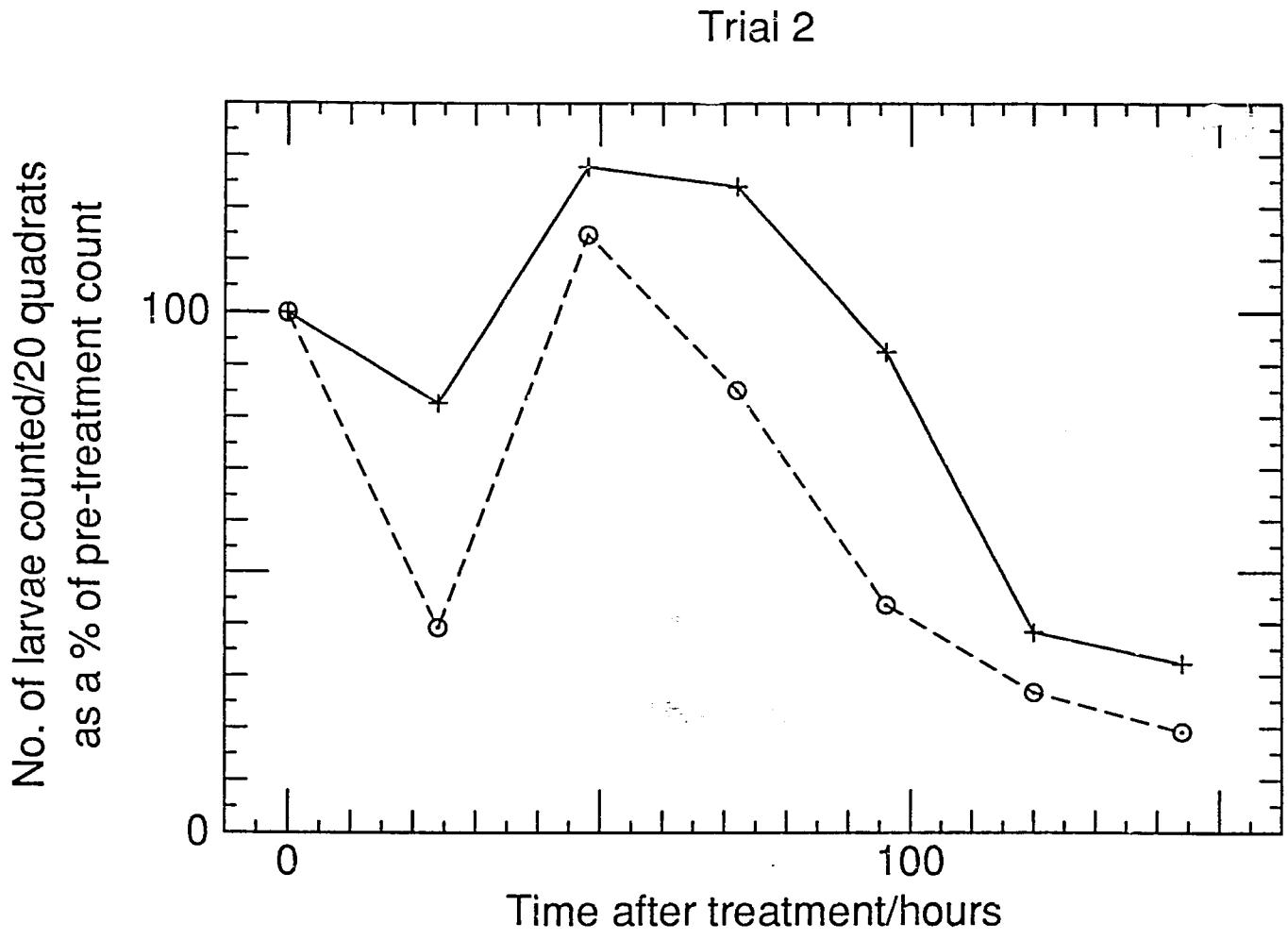

Figure 1. *S. exempta* larval density in experimental plots taken from 20 quadrat counts and expressed as a per cent value of the pre-treatment count (taken at time 0 prior to application of *B.t.*) with time. 10 x 10 m plots. +———+ control plot, o – – – o treated plot, 2% *B.t. aizawai* powder.

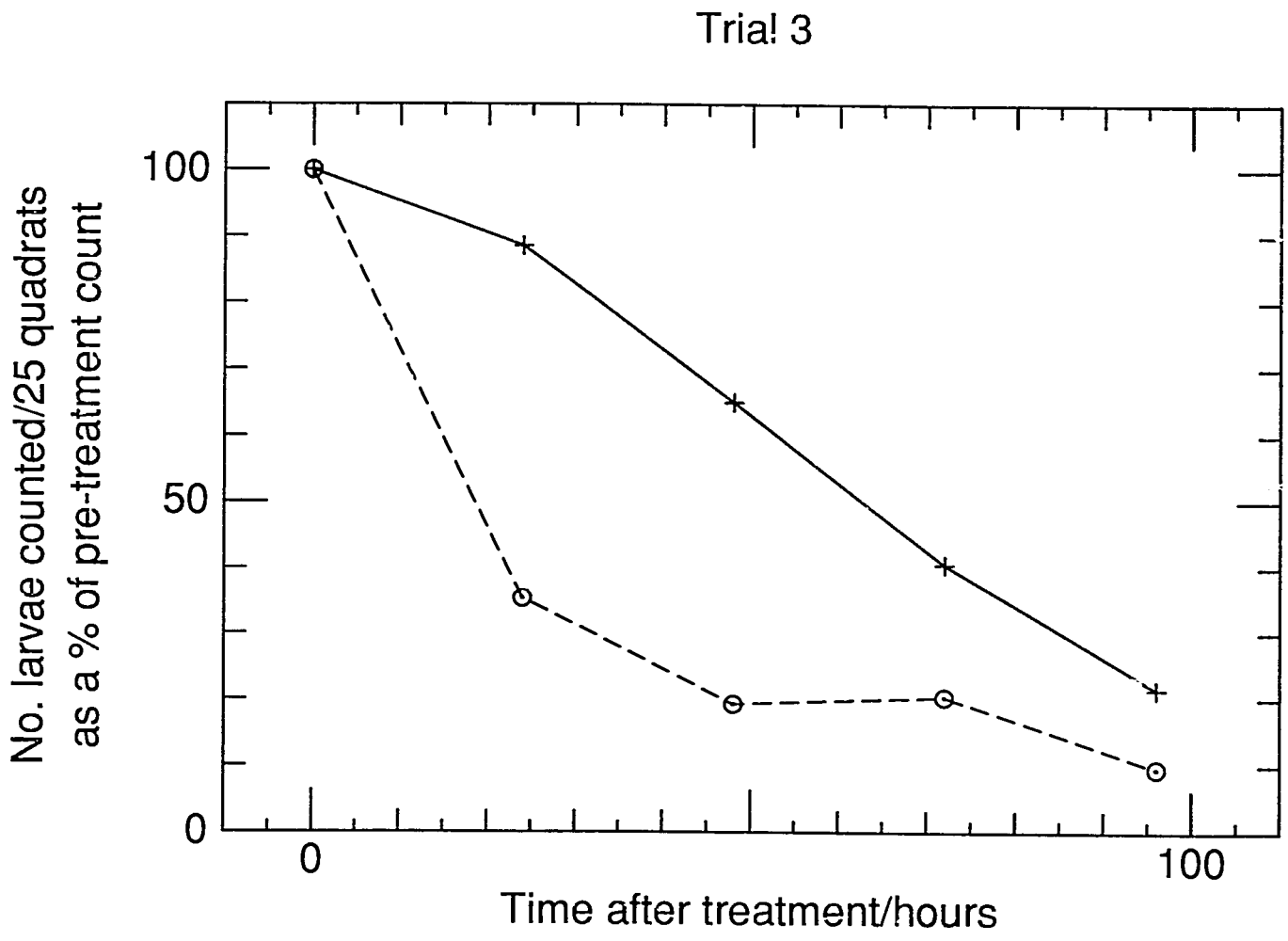

Figure 2. *S. exempta* larval density in experimental plots taken from 25 quadrat counts and expressed as a per cent value of the pre-treatment count (taken at time 0 prior to application of *B.t.*) with time. 15 x 15 m plots. +——+ control plot, o – – – o treated plot, 2% *B.t. aizawai* powder.

Fig. 3. Damage to young maize plants in the field caused by the gregarious larvae of the African armyworm, Spodoptera exempta. note the great number of larvae on and around the plants on the grooound.

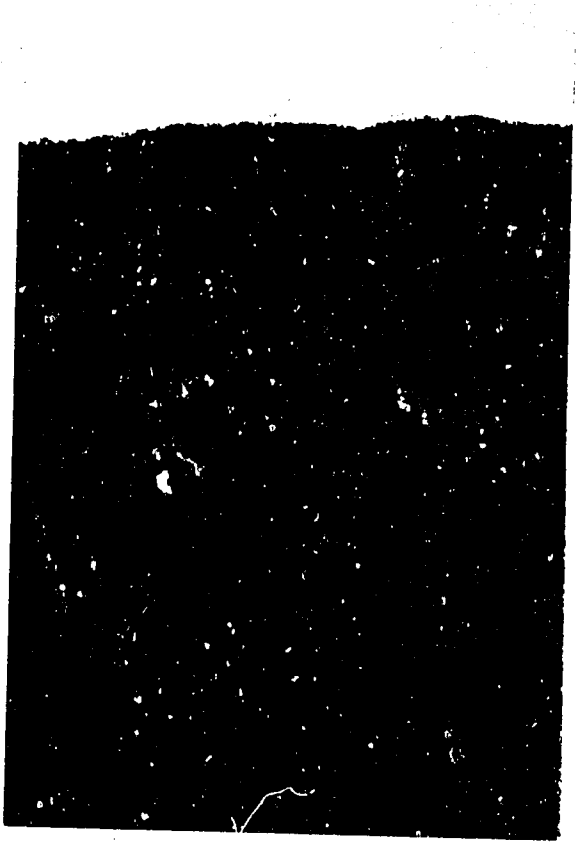

Fig. 4. Effect of Bacillus thuringiensis on prevention of damage to cinadon grass caused by gregarious larvae of the African armyworm Spodoptera exempta. The green plots in the center were sprayed with Bt aizawai (left) and entomocidus (right), while the front and the back plots were untreated control plots.