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

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INTRODUCTION

The African armyworm causes great losses to graminaceous crops such as corn, sorghum, rice, millet, rye, oat, wheat, barley and forage grasses. The pest is mainly distributed in the eastern portion of Africa (Kenya, Tanzania, Uganda, Ethiopia, Sudan and down to South Africa), but it had also been reported in central and western parts of the continent. This pest is also present in south east Asia, as well as in Indonesia, New Zealand and Australia (Haggis 1987) (Fig. 1.).

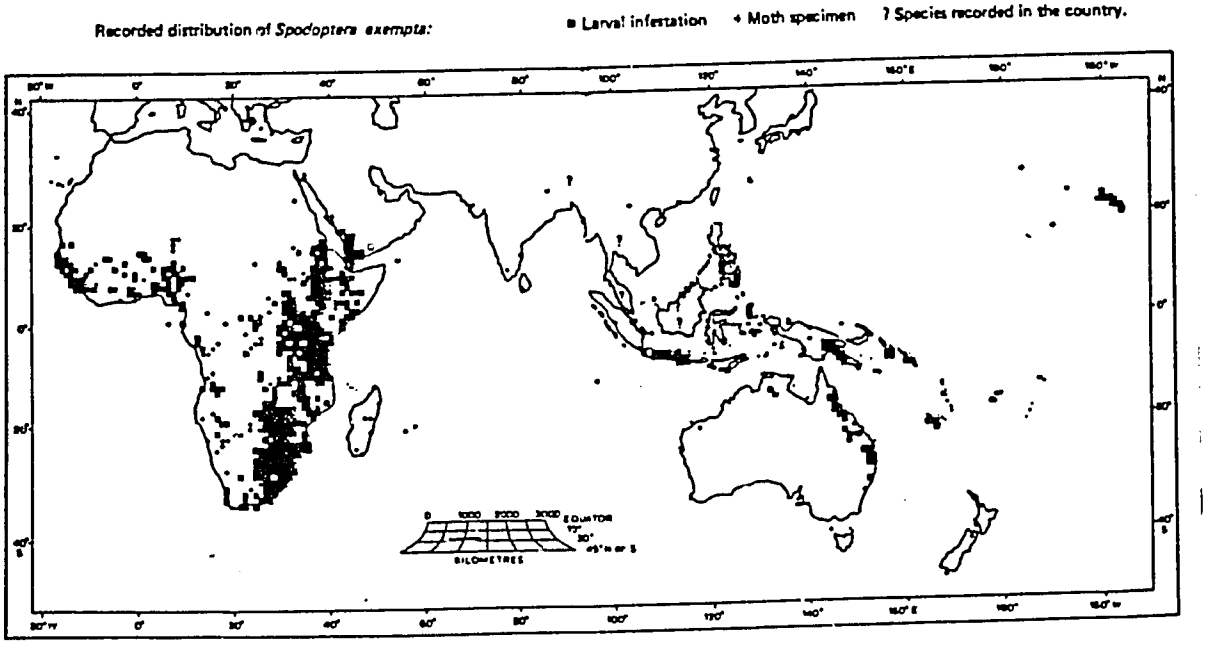


Fig. 1. Distribution of the African armyworm, Spodoptera exempta in Africa, and in other countries in the Southern hemisphere.

Biology.

Unlike S. littoralis, which populates fields in relatively close locations and whose migration is relatively moderate, the African armyworm, S. exempta has a distinct migratory pattern of distribution. The insect breeds in the center of East Africa around Mozambique - Zimbabwe and causes a progression of outbreaks northward from Tanzania to Ethiopia, and southward to South Africa. Generally, the population dynamics o S. exempta is described in three main phases. The first phase, is characterized by a very low population density of larvae, which may be analogous to the "solitary" phase of the desert locust. The second phase, includes a sudden appearance (and simultaneously sudden disappearance) of the analogous "gregarious" phase larvae. It is difficult to predict the locations of such outbreaks before great losses occur. In cases where effective control measures are not taken, a third migrant phase will infest more areas and cause additional damage. After 7-8 generations, the insect will spread up to Yemen in the Arabian peninsula.

One of the main factors determining the population dynamics of this insect is the presence of green cereal leaves. The Armyworm populations, survive and breed in low densities during the dry season in areas where grasses remain green, such as in the highland areas and more especially, the coastal areas of Kenya, Tanzania and possibly, Somalia where it is hot and there are periodic showers during the dry season. These low density populations form the source for the first outbreaks (primary outbreaks) when the rains arrive at the end of the dry season. The size of armyworm populations may increase rapidly, particularly through a high rate of survival of young larvae on new flashes of grasses. Female moths may lay 1000 eggs each. Populations may increase 10000 fold in 2 months (2 generations) even when 90% mortality occurred. It shows that the potential for rapid population increase in grasslands is from one solitary larva/1000m^2 to 10 gregarious larvae/1m^2 after 2 generations.

The conditions that bring to heavy outbreaks of the armyworm have been intensively studied by the research group headed by Dr. Rose in DLCO EA Nairobi (Haggis 1987, Pedgley et al. 1987, Rose et al.

1988). Within Eastern Africa, the armyworm populations are at their lowest numbers in October. Poor rains during the short wet season from October to December in Eastern Africa favor a subsequent population increase and first outbreaks of the season often occur in Tanzania or Kenya during November, December and January, frequently downwind from the coast by the hills and the high grounds where early seasonal rainfall occurs and moths are concentrated. Moths which emerge in large numbers from these outbreaks are carried downwind and contribute to the spread of infestations causing further outbreaks (secondary outbreaks) in subsequent generations at roughly monthly intervals.

The moths fly downwind during the hours of darkness. This migration may last for several nights before they lay their eggs. Within an armyworm outbreak area moths emerge from their pupae in large numbers over a period of up to 12 nights. The newly emerged moths fill the trees in an outbreak area with thousands of moths/tree. Some migrate already at the same night, but most hide in shaded and moist places throughout the first day. They will fly off on migration on the next night. Due to the flying off on migration at different times during 12 nights, the moths are dispersed in time, thus not forming swarms as can be seen with locusts.

When on migration, they may reach hights of several hundred meters, where they meet strong winds which, because they are weak fliers, carry the moths off in a downwind direction. The distance that the moths travel depends on: the speed of the winds, the length of time the moths fly in the winds on any night, the number of nights individual moths fly before stopping to mate and lay eggs, and the weather, especially the rainfall and wind conditions which may not allow the moths to travel any further.

Because the moths disperse in space and time downwind, for further outbreaks to be caused, the moths need to be concentrated so that they may lay many eggs in one place. This concentration is achieved mainly by moths that being brought together in the air by wind convergences or eddies often associated with rain storms (Fig. 2). Although a proportion of the moths migrating out from an outbreak

emergence area may be re concentrated, large numbers of moths will still be dispersed over a wide area causing low density populations of larvae which, because they are solitary form coloration, are not seen by the farmers. The moths resulting from these low densities may contribute to next outbreaks if they are concentrated later on.

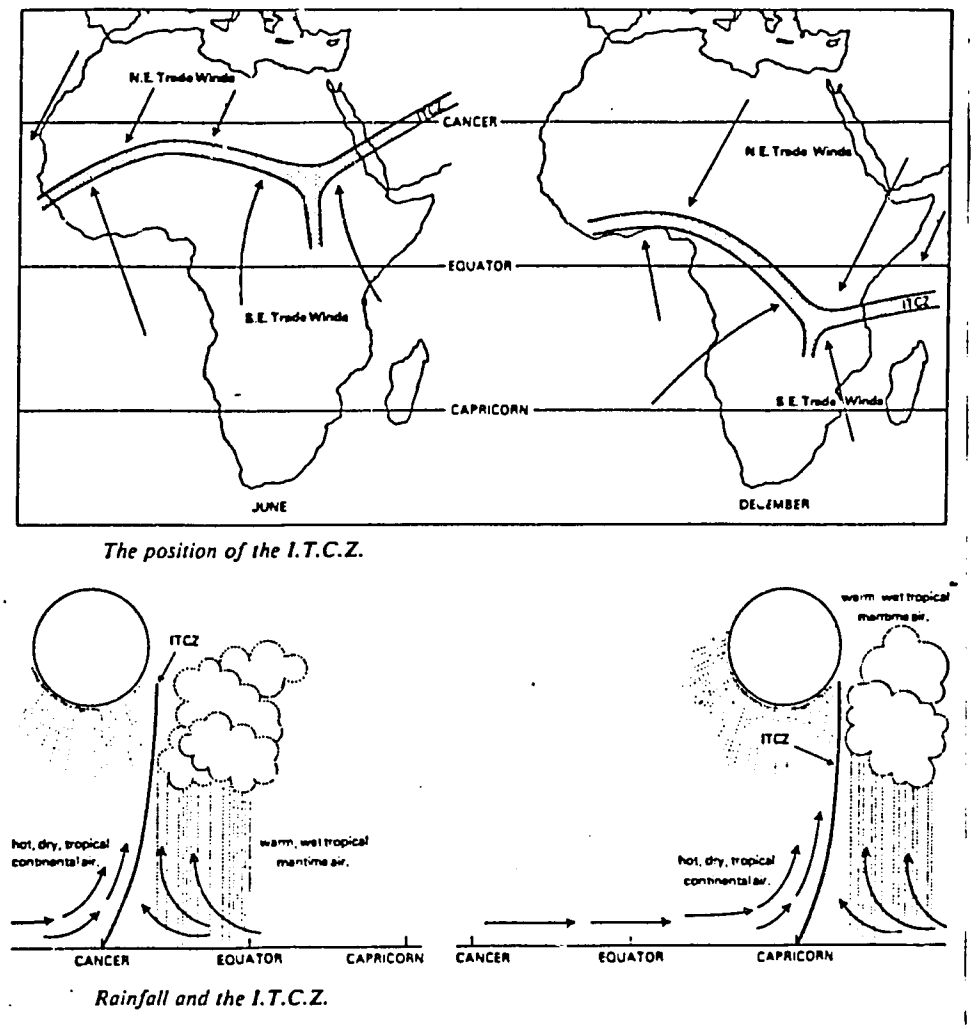


Fig. 2. Position of the Inter Tropical Convergence Zone (I.T.C.Z.) determines the extra heavy rain periods in equatorial regions.

Since the armyworm moths are carried by the winds, the directions of spread of outbreaks are influenced by the seasonal prevailing winds as these are dominant for most nights that the moths are flying. The areas in which further outbreaks occur are usually places downwind from the emergence areas where rain is falling at the time the moths arrive. Since the long rains in East Africa gradually move northward through Tanzania, Kenya, Ethiopia, Somalia and even reaching the Yemen, the monthly generations of armyworm outbreaks tend to show a gradual northward movement. Thus, outbreaks may start in November in Kenya or Somalia, or more commonly, December in Tanzania and begin in the Yemen in June or July the following year, 7 or 8 generations later. The times of year outbreaks are regularly reported in the different DLCO member countries are as follows: Tanzania - Dec. to May; Kenya - Nov. to June; Uganda - Feb. to May; Somalia - Nov to Dec; Ethiopia - April to Sept; Sudan - April to July.

By understanding the seasonal migrations of the armyworm, their underlying causes and factors which lead up to outbreaks, it is possible to issue long- and short- term forecasts with warnings of the areas in which armyworm outbreaks are likely to occur.

Natural enemies.

All stages of the armyworm are subject to attack by many natural enemies which range in size. From microscopic viruses to birds.
The Nuclear Polyhedrosis Virus (NPV). When heavy larval populations exist in the fields, epizootics may kill up to 90% of the larval population. However, The larvae are normally killed after considerable crop damage have already occurred. The Cytoplasmic Virus (CPV), Kills the pre-pupal or pupal stages, usually under stress conditions such as poor food, low temperature and little sunlight.

The fungal pathogen *Nomuraea rileyi* attacks the larvae. For effective control high humidity and temperature are required. This pathogen can seldom control an armyworm outbreak in Africa.

A great number of insects parasitise the armyworm. About 28 different species of flies (Diptera) attack the larvae, and about 25

types of wasps (Hymenoptera) have been recorded to attack its different stages as parasitoids and parasites (endo - and ecto-parasites). Ants may also play an important role in the distraction of both the eggs and the larvae. Certain beetles also consume the armyworm larvae.

Among the vertebrates, the birds are the most obvious predators of the armyworm (White Storks, Abdim's Storks or Marabu Storks). Sometimes, outbreaks may be recognized by watching the concentrations of the birds. Such concentrations may play an important role in the regulation or even control small outbreaks. Some mammals such as baboons shrews and other small animals also feed on the armyworm.

The natural enemies may sometime have a significant effect in controlling small, medium or even large outbreaks, especially if the latter are the result of continued breeding in the same area. Thus, permitting a build up of parasitoid and predator populations. This is particularly true for virus epizootics. However, as the moths are migratory and outbreaks are usually tens or even hundreds of kilometers apart. this type of control with natural enemies is normally unlikely. When severe outbreaks occur and no effective control means are taken, great yield losses occur. This insect is one of the contributors to hunger in East African countries.

Control.

The strategy of control should be first directed to the populations of the primary outbreaks which are critical with regards to their location and timing, so that they are likely to be of major threat to early rainfall regions downwind. By effectively controlling the primary outbreaks, it may be possible to suppress the large upsurges of armyworm which ravage Eastern Africa. Thus, considerably reducing both crop and pasture losses. The majority of primary outbreaks occur in central Tanzania (usually in December) Although the eastern side of the Kenyan highlands (Meru, Embu, Kitui) and sometimes on the coast of Kenya, can be of major importance (usually in November). By recognizing the areas where primary outbreaks are most likely to occur, it is most possible to monitor moth arrivals into

these areas using pheromone traps which gives an early warning of outbreaks and improves the efficiency of control measures.

The detrimental effects of chemical pesticides on the environment, health and especially the natural enemies are well documented. As the armyworm populations colonize forage grasses and other crops, which animals feed on, or small family farms of grain and vegetables, it is dangerous (or impossible) to control this pest with toxic chemical pesticides in these fields. Selective microbial agents may therefore provide safe control means for such a pest. The purpose of the present work is to search for isolates of Bacillus thuringiensis (new isolates or from stock cultures) which will demonstrate an effective insecticidal activity against the African armyworm, and direct the main efforts to control its primary outbreaks in order to suppress the subsequent spread and outbreaks. This will save great yield losses in cereal crops and pastures in East African countries.

Fig 3. The African Armyworm, Spodoptera exempta, life cycle:

A - Egg mass.

B - Hatching larvae (2-4 days after eggs were laid).

C - A larva of the "solitary phase", bright green, hiding on the bottoms of the plants.

D - A larva of the "gregarious phase" (the distractive phase), black, the duration between hatching and pupation is 2-3 weeks.

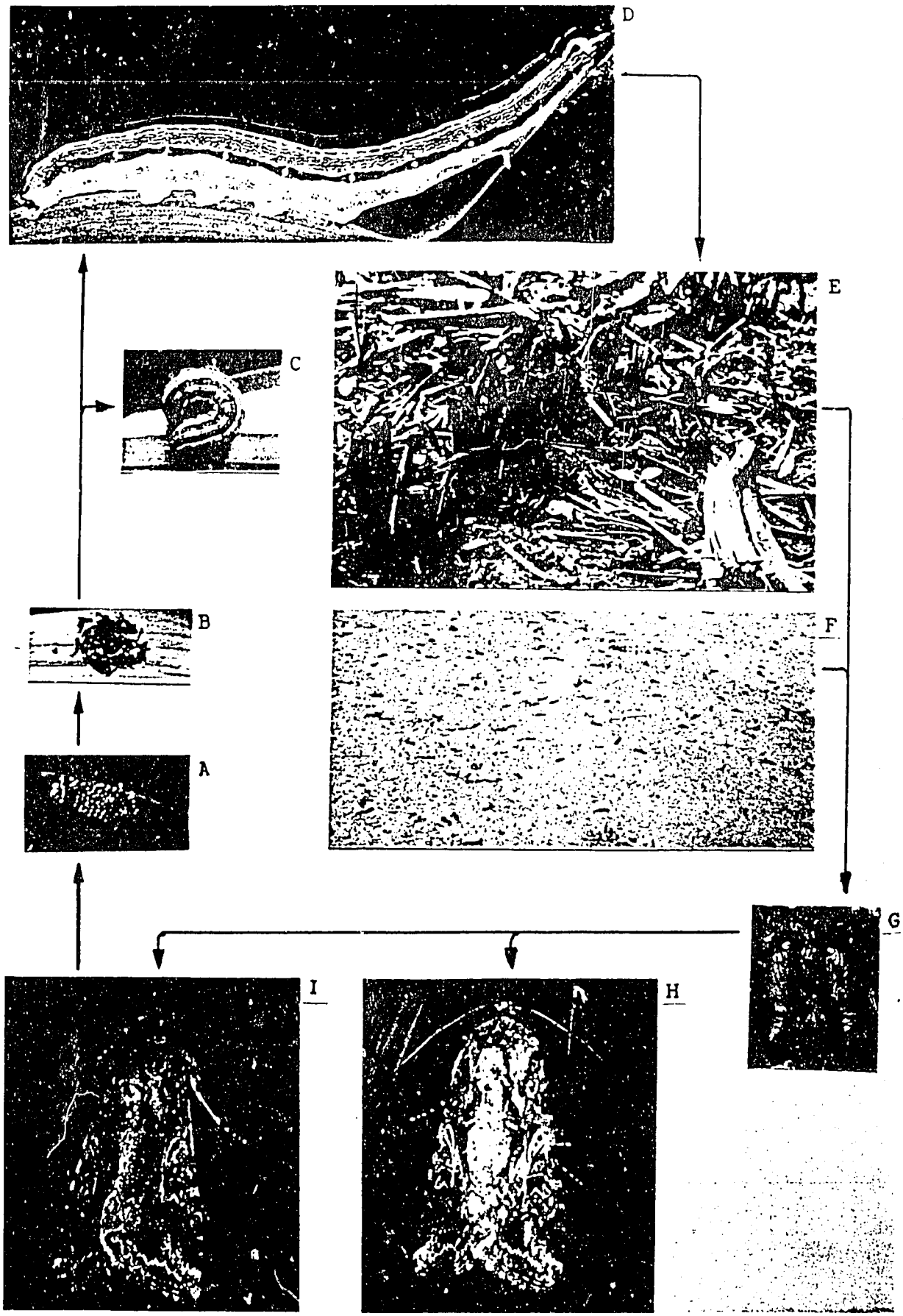
E - An armyworm outbreak in a cereal field causing a complete destruction of the crop.

F - "Marching" of the larvae from the destroyed field to search for another field.

G - Pupae in a cell 1-2cm in soil, time duration from pupation to adult emergence is usually 7-15 days in the outbreak season. when cold, it may take up to 5 weeks.

H - Adult male.

I - Adult female (live 5-10 days, sometimes more when on migration).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MATERIALS AND METHODS

Larvae of the genus Spodoptera are generally more tolerant to the δ-endotoxin of B. thuringiensis than those of other lepidopteran pests (Sneh et al. 1981). The isolates of Bacillus thuringiensis used for screening, were isolated from soil samples collected from various locations in Kenya and Israel (Table 1). In addition, isolates of various H - serotypes obtained from culture collections were also used for screening. (Table 2).

Table 1. Summary of isolations made from soil samples collected from different locations in Kenya and Israel.

Country	Location	Marking initials	Number of soil samples	Number of Bt isolates
Kenya				
	Central	A, L, K	45	30
	Mfangana island*	MF	4	6
	Northwest	BU	16	6
	Coast	-	20	0
	Kakamaga forest	-	13	0
	Massai Mara	-	17	0
	Mbita*	-	10	0
	Rusinga island*	-	10	0
Israel				
	Gilboa	GL	25	32
	Kfar Saba	M, MR	10	16
	Petah Tiqua, Lod	S	59	13
	Galilee	MG	2	5
	Negev	-	5	0

* The region of lake Victoria.

Table 2. List of *Bacillus thuringiensis* isolates from our stock used in the present work.

Isolate No.	Serotype	Source
HD-1	3a3b kurstaki	Dulmage
HD-2	1 thuringiensis	Dulmage
HD-23	1 thuringiensis	Dulmage
HD-73	3a3b kurstaki	Dulmage
HD-120	1 thuringiensis	Dulmage
HD-129	5a5b galleriae	Dulmage
HD-133	7 aizawai	Aizawa
HD-146	10 darmastadiensis	Dulmage
HD-151	5a5b galleriae	Dulmage
HD-155	5a5b galleriae	Dulmage
HD-220	5a5b galleriae	Dulmage
HD-227 (HA-3)	7 aizawai	Aizawa
HD-228 (IH-A)	7 aizawai	Aizawa
HD-229 (T63-L4)	7 aizawai	Aizawa
HD-263	3a3b kurstaki	Dulmage
HD-305	5a5b galleriae	Dulmage
HD-498	10 darmastadiensis	Dulmage
HD-593 (Ao-7)	7 aizawai	Aizawa
HD-610	10 darmstadiensis	Dulmage
HD-695	1 thuringiensis	Dulmage
Berliner 1715	1 thuringiensis	Dulmage
7f	7 aizawai	Aizawa
Ao-17	7 aizawai	Aizawa
Ao-18	7 aizawai	Aizawa
NRD-12	3a3b kurstaki	Dubois
Bt24	6 entomocidus	Sneh (1981)
B17	17	Aizawa
B18	18	Aizawa
Bt1 to Bt60	ND	Sneh (1981)
6105	ND	Abbott

ND - not determined.

Isolation procedure of B. thuringiensis from soil samples and larvae.

Before the new isolation procedure developed by Travers et al. (1987) was published, the following procedure was used: One hundred grams of soil samples were collected from various fields in Israel and Kenya. Each sample was well mixed and sieved through a 2m sieve. One gram of each sample was transferred to 20 ml. of 0.5% peptone solution in a 100 ml erlenmyer flask for enrichment of Bacillus spp. After 48 h. incubation at 32°C in a rotary shaker, the suspension was passed through 3 layers of sterile gauze. one ml of the culture broth was transferred to a new erlenmyer flask containing 20 ml of 0.5% peptone solution and 50µg/ml ampicillin. After incubation for an additional 48 h, aliquots of 2ml suspension were transferred to sterile test tubes which were immersed in a water bath at 55°C for 5 min to kill the non-spore forming bacteria.

Aliquots of 0.2ml of the soil suspensions were spread on L-agar plates using a Drigalski rod. After 48 h incubation at 32°C the Bacillus type colonies were observed under the microscope for crystal formation. The crystal forming colonies were streaked twice on L-agar plates for confirming the isolation, and maintained in stab cultures in screw cup tubes with (1% agar) nutrient agar.

A modification of the isolation method was also used:
Soil sample (2g) placed in a plate with 7ml of 0.5% peptone solution containing 100µg/ml ampicillin. After incubation at 32°C for 48h, 2ml of the supernatant were transferred to a test tube and placed in a water bath at 55°C for 5min. The suspension was diluted to 10^{-4} and aliquots of 0.1ml were spread on NA plates with a Drigalski rod. Colonies of Bacillus spp. were inspected under the microscope for the presence of crystals and isolated as previously described.

When available, the method for selective isolation of Bacillus thuringiensis developed by Travers et al. (1987, Martin et al. 1985) as used. The procedure is based on two major selective principles:
1. The resistance of Bacillus spp. spores to high temperature levels while the other microorganisms are not.

2. Inhibition of spore germination of B. thuringiensis in sodium acetate solution (0.25M), while the spores of other Bacillus spp. are not inhibited. The vegetative cells derived from the germinated spores are killed by exposure to high temperatures while the Bt spores may now be isolated on a suitable agar medium.

Soil samples of 0.5g were added to 10ml L broth buffered with 0.25mM sodium acetate in 100ml erlenmyer flasks. The flasks were incubated in a rotary shaker at 250 rpm for 4h at 30°C. Aliquots of 1ml were transferred to test tubes and heated (in a water bath) to 70°C for 10 min. and plated on L agar (0.2 ml/plate). After incubation for over night at 30°C, colonies were transferred on T3 medium and allowed to sporulate at 30°C. Cultures were then examined for the presence of crystals, confirming that they belong to Bt. The T3 media allows germination and good growth of Bt colonies and inhibits the growth of other Bacillus spp.

Media composition:

L agar : tryptone - 10g; yeast extract - 5g; NaCl - 5g; agar - 15g; H_2O - 1000ml.

T3 agar : Tryptone - 3g; $MnCl_2$ - 5g; agar - 15g; 0.05M Sodium Phosphate buffer pH 6.8 - 1000ml.

Growing bacterial isolates for bioassays.

Each isolate was grown in 2ml tryptic soy broth (20g Difco tryptic soy powder/liter) and incubated for over night at 30°C in a rotary shaker at 110 rpm. The cultures were transferred to 35ml BTSB medium (Dubois 1968, Sneh et al 1981) in 100ml erlenmyer flasks for 70h incubation in a rotary shaker at 110 rpm. Numbers of viable cells in the culture broth (cfu) were counted on L-agar plates using the drop plate method (Reed and Reed 1948). The spore crystal suspensions were harvested by centrifugation at 12000 rpm at 4°C for 10 min and washed with sterile distilled water (to discard soluble toxins other than the endotoxin). The pellets were kept in the freezer until use and re-suspended to the original volume when larvae were available for bioassay.

Bioassay procedure for evaluation of the insecticidal activity of isolates of Bacillus thuringiensis on larvae of Spodoptera spp.

For the first screenings, the pellets were diluted with saline solution and 2ml aliquots of the dilutions were incorporated into 18ml of the molten (kept at 50°C) diet to give a 45 fold dilution of the original culture broth (about 2-4 x 10^9 cfu/ml were counted in the original culture broths). The isolates inducing relatively high larval mortality were tested in a dosage response assay, using the following concentrations: $3x10^6$, 10^7, $3x10^7$, 10^8 cfu/ml artificial diet. For determination of LC 50 values 5-6 concentrations were used. As mortality results were variable using suspensions grown at different times and different larvae, the most effective isolates were grown in relatively large amounts (2 liters) and powdered preparations were made by lyophilization. These powders were used for comparison on both larval species in both countries at concentrations of either 5, 10, 20, 40, and 60µg/ml diet for S. littoralis, or 20, 40, 80, 160, 320 and 640 µg/ml diet for S. exempta.

The artificial diet for S. exempta (Bot 1967) consisted of: agar - 1.8g; wheat germ- -10.4g; dry Brewer's yeast - 10g; vitamin-free casein - 1.6g; ascorbic acid - 1.0g; nipagin (methyl-paraben) - 0.52g; choline chloride - 80mg; cholesterol - 40mg; inositol - 40mg; and H_2O - 100ml.

The artificial diet for S. littoralis (Yawetz et al. 1983) consisted of: agar - 2g; sucrose - 2.5g; yeast extract - 1g; alfalfa meal - 1.5g wheat germ - 3g; cholesterol - 40mg; sorbic acid - 100mg; nipagin
(methyl- paraben) - 100mg; chloramphenicol - 10mg; and H_2O - 100ml.

Aliqouts of 1.8ml of the artificial diet were distributed into small test tubes, kept in a heating block at 45°C. The spore crystal suspension was diluted as desired and 0.2ml aliquots were mixed well with the 1.8ml diet in the tubes and poured on plastic plates. After solidified, each sample was dividedation into 4 portions and placed on 2 layers of Whatman No. 4 filter paper, moistened with 0.5 ml solution of nipagin and sorbic acid (100µ/ml each). Egg masses were left to

hatch on maize leaves for S. exempta, and on Castor leaves for S. littoralis. Second instar larvae (2-4 days old) previously starved for 3-4h, were placed in each plate (40 larvae/treatment). After 4 days incubation at 25°C and 60% R.H., mortality counts were made. as described by Yawetz et al. (1983).

Rearing larvae of Spodoptera exempta and S. Littoralis.

Table 3. Composition of the rearing diets for Spodoptera exempta (Bot 1967) and S. littoralis.

Ingredient	S. exempta	S. littoralis
Dry beans	-	200g
Wheat germ	104g	-
Cotton meal	-	45g
Milk powder	-	18g
Dry brewers yeast	100g	55g
Vitamin free casein	16g	-
Agar	24g	15g
Ascorbic acid	10g	8g
Nipagin	5.2g	5g
Choline chloride	0.8g	-
Cholesterol	0.4g	-
Inositol	0.4g	-
Sorbic acid	-	5g
Chloramphenicol	-	5g
Formalin	-	5ml
H_2O	1000ml	1000ml

For S. exempta: The wheat germ, yeast and casein were thoroughly mixed. The nipagin was dissolved in 100ml acetone : ethyl ether (3:1 and mixed with the wheat germ yeast casein mixture. It was then spread in a thin layer to ensure the evaporation of the solvents. The agar was heated in 800ml water to boiling point. The mixture was added to

the agar solution with continuous stirring and boiled again. The ascorbic acid, choline and inositol were dissolved in 200ml water and added to the hot agar mixture. The diet was poured in 250ml plastic cups. After solidified the diet was kept at 4°C until needed for routine use (Table 3).

For S. littoralis: The beans were soaked in water for 48h at 4°C. Then, strained and blended in a food processor. The ingredients (except for the agar and yeast, were mixed with the beans. The yeast in 200ml and the agar in 500ml were autoclaved for 10 min. and added to the mix. The diet was poured into 250ml plastic cups and kept at 4°C until needed for routine use (Table 3).

In order to prevent viral epidemics, all the items used for rearing were disinfected with 1% formaldehyde solution. Egg masses and pupae were also surface disinfected with 0.1% formaldehyde solution. The larvae were placed in 30x15x15cm aerated plastic boxes. The diet was placed on a half plastic Petri dish. For young larvae until the 4th instar the bottom of the boxes was covered with towel paper. The larger larvae secrete excess moisture which has to be removed. In order to prevent over crowding and accumulation of moisture, the larvae were transferred at the 4th instar stage to new boxes (65/box). The bottom of the boxed was covered with a layer of sawdust. After pupation, the pupae were removed, surface disinfected. The pupae were transferred after 7 days to plastic boxes covered in their inside with towel paper. A 5% honey solution was added for feeding the adult moths. The egg masses were collected daily.

FIELD EXPERIMENTS

1

Field experiments, in which powdered preparations of selected isolates of *Bacillus thuringiensis* were used to control larval populations of *S. exempta* were carried out during its population outbreaks of: a. preliminary very small scale (1x1m and 3x1m plots) experiments in spring of 1988, b. preliminary small scale (10x10m and 15x15m plots) in the summer of 1988 and c. larger scale (small private farms of 2000 and 3700m^2 plots) in the spring of 1989.

Monitoring field infestations with the larvae was made with the aid of the research group of Dr. Rose from DLCO EA in Nairobi. During 1988 it was not profoundly organized. Therefore, when larval infestation was detected, the larvae have already been developed, resulting in higher larval resistance to the control agent and the damage to the crop was already significant before treatment. During 1989, the monitoring was better organized. A network of pheromone traps was placed in 9 farms covering an area of 625km^2. Searches for egg masses were carried out in fields where masses of trapped moths were counted (2 days after the peak). Egg masses were marked with plastic bands to facilitate re-counting larval numbers and after Bt applications.

Spring 1988

The first experiment was carried out in Lambwe valley in a short cinadon grass field, on mini plots of 1X1m, with three bacterial preparations in three replicates:

1. Bt_{24} (*entomocidus*) 2% (w/v) + CCSO (crude cotton seed oil) 1% (v/v) 330ml/m^2.
2. IH-A (*aizawai*) 1% (w/v) + CCSO 1% (v/v), 330ml/m^2.
3. Bt_{24} granular, 100ml/m^2.

Toxicity of the Bt_{24} powder, was 35000 IU (BMPI standard), and that of the IH-A, was 6500 IU. Thus, a 2% concentration of the Bt_{24} vs. a 1% of the IH-A concentrations were used to get approximately the same number of IU/unit area. The powdered preparations, produced by Becker Microbial Products, Israel (BMPI), 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. Bt_{24} 2% (w/v) + CCSO 1% (v/v) and IH-A 1% (w/v) + CCSO 1% (v/v), each was applied at 1000ml/plot.

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

The second experiment was carried out in a short cinadon grass field in Lambwe valley. The larvae observed in the field at this time were in their 4-6th instars. Therefore, in order to obtain a better field effect, Bt aizawai (IH-A) was sprayed at a 2% (W/V) concentration with 2% CCSO. The plots were 10x10m, and 10 liter suspensions were sprayed/plot.

Larval population density was estimated by taking 20 randomly selected counts within each of the plots, using 20X20cm squares. 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.

The third experiment was carried out in a short cinadon grass field in Lambwe valley. 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% (v/v) CCSO in water was used for spray application. Fifteen liters were sprayed/225m^2. Plots of 15x15m were used. One for the Bt treatment and the other for the untreated control. Larval density was estimated by counting the living larvae within 20X20cm squares 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.

Summer 1988

Following encouraging results obtained from initial field trials with *Bacillus thuringiensis* (Bt) var. *aizawai* against an outbreak of the armyworm in the Lambwe valley region, South Nyanza District, Western Kenya, a second series of experiments was undertaken to:

1. Obtain information on the effects of bacterial concentrations on larval mortality in the field.
2. Further test the Bt *entomocidus* powder preparation on a larger field scale.

Outbreak area and outbreak site.

The armyworm outbreak was detected in a 40 acre wheat field at Nagungugu farm, Rongai, Nakuru, Rift Valley, in Kenya. The wheat variety being grown, as a pasture crop, was Kenya Fahari.

The armyworm larvae observed were largely in their second (the highly susceptible stage to Bt) and third instar stages, although some later instars were also present. Plant damage, owing to the nature of the crop, was difficult to assess. At the time of initial treatment, however, despite there being high numbers of larvae, the crop appeared to be relatively healthy, with only a few leaves of the seedlings consumed. This may have been a result of the early developmental stages of the larvae, the greatest crop damage being caused by the feeding activity of later instar larvae (4th-6th).

The experimental site allocated, was approximately 2 hectares in area, located in a narrow portion of the field. An earlier brief survey of larval numbers within the field indicated a relatively homogeneous population throughout the crop. However, lower numbers were observed along the edges of the field, which were bounded by hedges, possibly as a result of a feeding activity of insect predators, birds, small mammals etc. inhabiting these environments.

Furthermore, the outbreak seemed to be confined to the wheat field, with no armyworm observed in the surrounding fields which contained either fallow grass or maize.

Owing to the high larval population density, The farm had no option other than to spray the crop in order to prevent damage. A pyrethroid insecticide was used to control them. However, a 1m boundary was maintained between the experimental area and the limit of the insecticide treatment. Also, the wind direction was such at the time of spraying that insecticide drifts into the treated areas could be considered negligible. The insecticide treatment gave approximately 100% larval mortality within 48 hours.

The Bt powder preparations were the ones used in the previous experiment. The Bt aizawai (IH-A) powder was applied by spraying (Achelis hand pumped pressurized knapsack sprayer) at three different rates: 0.5%, 1.0% and 2.0% (w/v) suspensions. All suspensions were prepared by thorough mixing in 10L of water in which crude cotton seed oil (CCSO) had been added to obtain a 1% v/v concentration. Each treatment was replicated three times, Ten L suspension were applied to each of three 15x15m randomized plots.

Larval counts were taken within each of the plots before treatment and every 24 hours, if possible, following treatment. Twenty counts were recorded from 20x20cm. squares to give an indication of treatment effect on larval population density. Counts were taken at least 2m. inside the boundaries to reduce any effect of immigration /emigration on the larval population, although owing to the young status of the larvae and the abundant food supply, such movement was considered to be negligible.

Unfortunately, as a result of heavy rainfall, at the time of spraying, only two concentrations could be applied on the first day, while the 2.0% concentration had to be applied on the following day. This accounts for the shorter experimental period. Furthermore counting on the third day after spraying had to be abandoned as a

result of a torrential downpour, so no figures are available for two of the treatments.

The Bacillus thuringiensis entomocidus (Bt_{24}) powder was applied in one 15x15m. plot only at a 2.0% w/v suspension in 10L water containing 1% v/v CCSO. Larval counts were taken in a similar manner as outlined above.

The untreated population was also monitored throughout the experiment to evaluate any possible adverse effects of rain etc. on larval population density. This was achieved by counting the larvae in untreated plots between the sprayed plots and along the experimental border. Samplings were performed at randomly chosen sites in a 20x20cm squares. Thirty counts were taken each time to obtain data on a widespread larval population.

One day following an initial assessment of the outbreak site, the first treatments were applied in overcast conditions at 3^{30} pm The second batch of treatments were applied on the next day in overcast conditions, at 10^{00} am. Heavy rain fell during the first, second and fourth day following Bt entomocidus applications. The experiment was concluded on the fifth day after Bt application. A longer experimental period was not possible, as the farm manager wished to spray the allocated area where larval numbers remained high in the untreated crop, and risk of damage and and subsequent crop loss was too great.

Spring 1989

Monitoring field infestation with the African armyworm.

This outbreak season was used for carrying out the concluding field experiments using conclusions from all the knowledge that we had accumulated throughout the research period. Field infestations with this pest, have been usually noticed only when larval infestations were in massive numbers and quite developed (4th-6th instars). At this point, the crop had already suffered a considerable damage. In order to get efficient control, the application of any kind of insecticide

should be directed to the most vulnerable stage of larval development and before an economic crop damage had occurred.

The emphasis during this season was to design a monitoring network of pheromone traps for male moths of S. exempta and direct the searching and marking the egg masses in the fields to the locations, where the largest numbers of adults had been trapped and two days after the peak trapping. Spray applications with powdered preparations made from the effective isolates of B. thuringiensis, were directed to meet the neonate larvae, a short time after hatching, when they are most vulnerable to the δ-endotoxin.

Pheromone traps were located in nine different farms covering an area of ca. 625km^2 (25x25km) and counts of trapped adult males were recorded 12 times during the period from March 28, to April 9, 1989.

Spray applications of powdered preparations of B. thuringiensis to control the armyworm larvae in the field

According to the pheromone traps, two sites in Makende were chosen for spray application. The farm of Makende site 1, had the following fields from north to south: a) Young cinadon grass (115x32m), b) A relatively tall maize (70cm, 115x28m), c) Young maize (2-4 open leaves) (80x24m) and d) Millet (80x21m). The Makende farm site 2 had a young corn field (5 open leaves), (80x32m).

The first experiment was carried out in Makende site 1. Egg masses were marked with plastic bands, two days after the peak of moth counts in the pheromone traps. When most eggs were either in dark color, or neonate larvae, shortly after hatching. The first spray was applied with a hand sprayer, to cinadon grass on the next day, with 0.2% (only one tenth of the ordinary concentration used) suspension of isolate MF4B-2. The experimental field consisted of 4 untreated and 4 Bt-treated randomized 3x3m plots. Numbers of surviving larvae were recorded in 7-15 areas of 0.5m^2 around marked egg masses/plot, 5 days after spray application.

The second experiment was carried out in Makende site 2. Young maize plants (5 leaves). Four treatments and 4 replicate plots (3x3m) were arranged in a Latin square. Three different concentrations of isolate K26-21 were applied on 2nd to 3rd instar larvae. As the egg masses are laid in the lower side of the lower leaf of the maize plants, each plant was separately sprayed and special attention was paid to direct the spray to this area. Counts of surviving larvae were made 2 days after spray application from 303 separate untreated plants and 216, 275 and 207 plants treated with 0.4, 0.8 and 1.6% (w/v) Bt concentrations respectively.

The third experiment was carried out in the same plot as the second experiment. Before spraying the maize field, the third leaf had already been damaged by the larvae while the 4th leave was intact. These leaves were removed for evaluating the effect of Bt application (isolate K26-21, at 1.6% w/v level of the previous experiment) on prevention of damage. A total of 12 leaves/treatment (4 replicates, 3 leaves/replicate). The fresh weight and the dry weight of each leaf were recorded and the difference between treated vs. untreated ones were calculated.

The fourth experiment was carried out in Makende site 2, in the millet field (80x25m), sprayed with a suspension (2% w/v) of a preparation of isolate IH-A (Bt aizawai) except for four random plots (4x4m) left as untreated controls. Larval stage at spraying time was 3rd instar. Larval counts were recorded from 12 random squares (0.5m^2 each), one day before and 2 days after spray application.

The fifth experiment was carried out in Makende site 1, in the cinadon grass band. As larvae developing on the grass around the crops provide masses of hungry larvae which immigrate to the adjacent crops, it was important to control them there before their "marching". The cinadon grass band 115x32m (3700m^2) was sprayed with a suspension (2% w/v) of a preparation of isolate IH-A (Bt aizawai), while 4 randomized plots (4x4m) were left as untreated controls. Larval development stage at the time of spray application was 4th (some were also 3rd or 5th) instar. Larval counts were made with the Californian sweep net, using

10 sweeps of 180°/count. Counts were made once before spray application, and after 2, 4, and 8 days after application.

For the control of large larvae (5th and 6th Instars) a bait preparation was prepared. The mixture contained 25% millet meal, 60% maize meal, 10% molasses and 5% Bt powder of isolate IH-A. Forty larvae (5th instar) were placed in plastic boxes with the bait for 2 hours and then, the bait was replaced with a maize leaf. The leaves were changed every day. Diet which did not contain Bt served as control. Mortality was recorded daily until pupation.

In the field, ants tend to collected the dry bait. Therefore it was considered for use as a barrier between grass bands and the crops to control the marching larvae to the crops.

Sweeping out

In small farms when effective control measures are not taken, especially in the grass bands, after consuming the green grass, the large larvae (4th and 5th instars) move in great masses to green adjacent crops. A grass band (140x20m) around crops was swept with the Californian sweep net for 40 min. in each of 3 consecutive days. The larvae were then drowned in a detergent solution in a bucket. Larval counts were made of ten samples (10 sweeps of 180° each).

RESULTS AND DISCUSSION

From 135 and 101 soil samples collected from various locations in Kenya and Israel, 42 and 66 isolates of B. thuringiensis were isolated respectively (Table 1). These isolates together with additional ones from the culture stock (Table 2), were tested for their insecticidal activity against second instar larvae of S. exmpta in Kenya, and S. littoralis in Israel.

The preliminary screenings were carried out using only one Bt concentration for each isolate. Isolate Bt_{24}, which was our best one against larvae of S. littoralis (Sneh et al. 1981) was used as a reference. The first screening of new 88 isolates against S. littoralis indicated that 22 isolates have demonstrated a higher insecticidal activity than isolate Bt_{24}. The best ones, were isolates K26-21 (310%), MF4B-2 (288%), MR1-37 (220%) and K28-30 (177%) (Table 4). Comparative insecticidal activities of: 50-98% of Bt_{24}, were caused by 18 of the isolates, 20-40% - by 37 isolates, and 0-19% - by 31 isolates (Table 4).

For more accurate determination of the relative insecticidal activity of the better isolates, LC_{50} values have been determined from bioassays made according to pellet weight of the fermentation broth/ml diet The best isolates were 26-21 (244% of Bt_{24}), MF4B-2 (180%), MR1-37 (163%), and K26-8 (145%) (Table 5). Final determinations were made from lyophilized powder preparations of the concentrated spore crystal suspensions (Tables 6 and 7). Because there were differences in the insecticidal activities in the bioassay experiments carried out in different times, the experiments were repeated several times (Table 6). The means for the relative insecticidal activities recorded in the repeated experiments have consistently indicated the isolate K26-21 was superior to all the others against S. littoralis (Tables 7 and 8).

Table 4. Preliminary screening of new isolates of *Bacillus thuringiensis*, for their relative insecticidal activities (compared to Bt_{24}) against second instar larvae of *Spodoptera littoralis*

Relative insecticidal activity*							
Isolate No.	% of Bt_{24}	Isolate No.	% of Bt_{24}	Isolate No.	% of Bt_{24}	Isolate No.	% of Bt_{24}
K26-21	310	MF4B-2	288	MR1-37	220	K28-30	177
K26-8	149	GLB-14	147	K37-10	138	K30-3	136
K4-1a	129	K10-25	120	K4-2	110	K40-31	106
Bt24	100						
MG12-39	98	M3	96	M15	93	GLB-15	90
M12-	90	GLB-12	89	MR1-38	80	K26-18	80
M14	78	K40-33	75	K36-9	74	MF3a-1	70
MR1-36	62	GL13-2	60	M21	54	GLB-11	54
K37-17	54	GLB-13	50	S44	49	GL13-3	43
Bu1-1	47	S45	47	S47	45	GL13-1	44
K4-1b	4[illegible]	K26-3b	43	GL13-4	40	M16	38
GLB-16	37	K10-26	35	M23	35	K30-31	34
K40-34	31	GLB-7	30	GLB-5a	26	K28-28	26
M1	26	M7	26	M8	26	M9	26
S46	26	K26-3a	25	BU5-12	24	BU8-22	23
MF4B-1	23	GL20-42	23	K28-29	22	M5	22
M6	22	M10	22	M11	22	MR1-35	22
GL8-5b	21	K26-19	21	MG9-4b	21	GL20-43	19
Bu8-24	18	MF1-3	18	MF1-4	18	K10-24	17
K10-27	17	K26-20	17	M13	17	S44-1	17
BU5-3-7	12	BU5-14	12	MF28-3	12	GL20-41	24
M2	11	M4	11	MG12-40	11	S39-1	11
M20	10	BU1-2	6	K40-32	5	S31-1	5
S31-2	5	S31-3	5	S52-1	5	GLB-6	0
GL20-4	0	GL24-1	0	M26	0	MG9-4a	0
S32-1	0	S39-2	0				

* The bioassays were carried out using three dilutions (1, 3 and 10%) of the original concentration in the culture broth (40 larvae/isolate).

Table 5. Relative nsecticidal activity (LC_{50} values, compared to Bt^{24})[a] of selected isolates of *Bacillus thuringiensis* on second instar larvae of *Spdoptera littoralis*.

Isolate No.[b]	Serotype[c]	Subspecies	slope	% of Bt_{24}
K26-21[K]	ND	–	2.45	244
MF4B-2[K]	ND	–	1.56	180
MR1-37[I]	MD	–	1.46	163
K26-8[K]	ND	–	1.57	145
HD-593	7	aizawai	1.76	102
Bt24	6	entomocidus	1.55	100
Bt17	ND	–	3.11	87
HD-133	7	aizawai	2.11	83
Bt3	ND	–	1.79	83
K28-30[K]	ND	–	1.66	76
k30-3[K]	ND	–	1.72	61
7f	7	aizawai	1.81	60
Bt57	ND	–	2.53	52
NRD-12	3a3b	kurstaki	1.56	48
Bt40	ND	–	1.83	44
Bt15	ND	–	1.73	39
Bt54	ND	–	2.45	35
Bt53	ND	–	1.53	28
Bt7	ND	–	3.39	11
Bt18	ND	–	2.06	4
HD-151	5a5b	galleriae	2.21	2
HD-120	1	thuringiensis	0.85	0
HD-73	3a3b	kurstaki	0.00	0

[a] Bioassays were made with five dilutions based on the weight of the pellet from the isolates culture broths (µg/ml diet), using 40 larvae/dilution.

[b] [K] = new isolations from Kenyan soils, and [I] = from Israeli soils.

[c] ND = not determined.

Table 6. Relative insecticidal activity (compared to Bt_{24}) of lyophilized powders of selected isolaes of *Bacillus thuringiensis* on second instar larvae of *Spodoptera littoralis*[a]

Isolate number	Relative insecticidal activity (% of Bt_{24}) 1	2	3	4	5	6	7	8	9
K26-21[k]	397	124	140	220	122	194	160	166	270
(slope)	1.91	2.50	1.74	1.62	1.94	2.34	2.22	4.16	2.28
MF4B-2[k]	–	–	–	211	–	–	–	–	165
(slope)	–	–	–	1.81	–	–	–	–	2.49
MRI-37[I]	204	105	124	201	–	–	–	116	186
(slope)	2.72	3.48	2.45	2.09	–	–	–	2.79	2.73
K28-30[k]	148	116	114	–	–	–	–	–	–
(slope)	3.34	3.93	1.67	–	–	–	–	–	–
K26-8[k]	109	–	–	–	83	–	130	–	129
(slope)	1.89	–	–	–	1.24	–	1.32	–	2.39
HD-593	91	–	–	113	–	–	–	88	140
(slope)	2.24	–	–	2.09	–	–	–	2.10	2.07
Bt17	–	–	–	–	97	–	87	–	139
(slope)	–	–	–	–	1.43	–	2.50	–	2.21
HD-133	–	–	–	–	90	–	–	121	–
(slope)	–	–	–	–	1.17	–	–	2.57	–
GLB-14[I]	122	85	102	–	–	–	–	–	–
(slope)	2.20	4.51	2.03	–	–	–	–	–	–
K30-3[k]	–	–	–	–	–	91	–	–	113
(slope)	–	–	–	–	–	1.42	–	–	1.68
Bt24	100	100	100	100	100	100	100	100	100
(slope)	1.89	2.11	1.16	1.36	1.44	1.55	1,80	1.34	3.06
K37-10[k]	133	61	101	–	–	–	–	–	–
(slope)	2.85	4.26	1.05	–	–	–	–	–	–
7f	–	–	–	–	–	–	–	–	94
(slope)	–	–	–	–	–	–	–	–	1.32
IH-A	121	84	75	–	–	–	–	–	–
(slope)	1.97	2.12	1.07	–	–	–	–	–	–
K4-1a[k]	141	66	69	–	–	–	–	–	–
(slope)	2.42	3.44	1.68	–	–	–	–	–	–
Bt3	–	–	–	–	–	82	–	49	–
(slope)	–	–	–	–	–	1.56	–	1.58	–
GL20-43[I]	10	–	–	–	–	–	–	–	–
(slope)	1.14	–	–	–	–	–	–	–	–
GL20-32[I]	2	–	–	–	–	–	–	–	–
(slope)	1.00	–	–	–	–	–	–	–	–
HD-73	0	–	–	–	–	–	–	–	–
(slope)	0.00	–	–	–	–	–	–	–	–

[a] The LC_{50} values for each isolate were evaluated from 5 concentrations (5, 10, 20, 40, and 60μg powder/ml diet).
[k] = New isolates obtained from Kenyan soils and
[I] = from Israeli soils.
The summary of this Table is presented in Table 10.

Table 7. Mean of relative insecticidal activity (from Table 6) of lyophilized powders of isolates of Bacillus thuringiensis, on second instar larvae of Spodoptera littoralis[a].

Isolate number	Serotype	slope	Insecticidal activity (±SE)[b] LC_{50} (µg/ml)	% of Bt_{24}
K26-21[1]	ND	2.19	12.5 (5.2)	199 (83)
MF4B-2[1]	ND	2.15	13.2 (1.6)	188 (23)
MR1-37[1]	ND	2.70	15.9 (4.3)	156 (42)
K28-30[1]	ND	2.98	19.7 (2.5)	126 (16)
K26-8[1]	ND	1.71	22.0 (3.7)	113 (19)
HD-593 (Ao7)	7 aizawai	2.12	23.0 (4.5)	108 (21)
Bt17	ND	2.05	23.0 (4.7)	108 (22)
HD-133	7 aizawai	1.87	23.7 (3.4)	105 (15)
GLB-14[2]	ND	2.91	24.1 (3.2)	103 (15)
K30-3[1]	ND	1.55	24.3 (2.6)	102 (11)
Bt24	6 entomocidus	1.81	24.9 (9.5)	100 (-)
K37-10[1]	ND	2.72	25.4 (7.5)	98 (29)
7f	7 aizawai	1.32	26.4 (-)	94 (-)
HD-228(IH-A)	7 aizawai	1.72	26.7 (5.8)	93 (20)
K4-1a[1]	ND	2.51	27.0 (10.2)	92 (35)
Bt3	ND	1.57	38.2 (9.4)	65 (16)
GL20-43[2]	ND	1.14	248.5 (-)	10 (-)
GL20-42[2]	ND	1.00	1242.5 (-)	2 (-)
HD-73	3a3b	0.00	- (-)	0 (0)

[a] Each isolate was tested in 5 concentrations (5, 10, 20, 40, and 60 µg/ml diet). [1] = New isolates obtained from Kenyan soils and [2] = from Israeli soils. ND = not determined.
[b] SE = Standard Eror.

Table 8. Insecticidal activities of powders prepared by lyophilization and spray drying of isolates K26-21, MF4B-2 and Bt_{24} of Bacillus thuringiensis, on second instar larvae of Spodoptera littoralis[a].

Isolate number	Slope	LC_{50} (µg/ml)[b]	% of Bt_{24}
Bt24 (spray dry)	4.15	35.2 a	100
MF4B-2 (spray dry)	1.87	22.6 b	155
K26-21 (spray dry)	3.02	14.3 c	246
K26-21 (lyophilized)	4.15	17.2 c	204

[a] Each isolate was tested in 5 concentrations (5, 10, 20, 40, and 60 µg/ml diet).
[b] Numbers followed by the same letter are not significantly different (P=0.05, ANOVA).

Powdered preparations of isolate K26-21, MF4B-2 and Bt_{24} were prepared by spray drying for the field experiments and a sample of isolate K26-21 was also prepared by lyophilization. The insecticidal activity of the powders of isolate K26-21 was 2.04 fold (lyophilized), and 2.46 fold (spray dried), MF4B-2 (pray dried) was 1.55 of that of spray dried Bt_{24} on a w/w per ml diet basis. In addition, isolate K26-21 produces 2-3 fold dry matter/ml fermentation broth. Therefore this isolate may be more efficient and less expensive to produce than Bt_{24} or other isolates of B. thuringiensis var. aizawai used against the Egyptian cotton leafworm, S. littoralis (Table 8).

Screening for isolates with high insecticidal activity against the African armyworm, Spodoptera exempta.

Preliminary screening of B. thuringiensis isolates (at a dilution of 1:45 of the fermentation broth) for their insecticidal activity against second instar larvae of S. exempta have indicated that 17 isolates caused 100% mortality, 36 isolates caused 50-98% mortality, 18 caused 20-49% mortality and 9 caused less than 20% mortality (Table 9).

According to LC_{50} values of selected isolates of Bacillus thuringiensis, 9 of them were more insecticidal than Bt_{24} on the armyworm larvae. The best ones were 7f (10.9 fold), K26-8 (8.2 fold), MF4B-2 (7.7 fold), and L1-6 (4.2 fold) (Table 10). In laboratory bioassay experiments, 4th and 5th instar larvae of the armyworm were also killed (100% mortality) by Bt isolates at a 1:45 dilution of the original culture broth (Table 11).

Second instar larvae of S. exempta, are more susceptible than those of S. littoralis to different Bt isolates: HD-133 (28 fold) Bt_{24} (30 fold) and 7f (40 fold). In addition, the isolates causing the highest insecticidal activity against S. littoralis, are not the best ones against S exempta, and vise versa. The best isolates against S littoralis (in a descending order) were: K26-21, MF4B-2, MR1-37, K26-8 and Bt24. The best isolates against S. exempta were: 7f, HD-593 K26-8, MF4B-2, K30-3, HD-593, MR1-37, Bt57, K26-21 and Bt24 (Table 12).

Table 9. Preliminary screening of the various isolates of *Bacillus thuringiensis* (compared to Bt[illegible]), for their insecticidal activity against second instar larvae of *Spodoptera exempta*[a].

Isolate No.[b]	Mortality (%)	Isolate No.[b]	Mortality (%)	Isolate No.[b]	Mortality (%)
7f	100	A3[k]	100	Bt3	100
Bt15	100	Bt15	100	Bt17	100
Bt18	100	Bt24	100	Bt40	100
Bt53	100	Bt54	100	HD-133	100
HD-146	100	HD-151	100	HD-593	100
K26-8[k]	100	k26-21[k]	100	L1-6	100
MF4B-2[k]	100	MR1-37[I]	100	Bt55	98
Bt 57	98	NRD-12	98	IH-A (7BMP)	98
6105	97	HD-263	95	K30-3[k]	95
Ber 1715	93	Bt30	92	HD-498	92
HD-601	92	Bt56	90	HD-120	88
HD-695	88	Bt23	83	Ao18	80
HD-155	80	HD-220	75	GL13-[I]	75
Ko4[k]	75	Bt20	73	HD-129	72
Bt29	70	Bt16	67	Bt25	65
HD-73	65	Bt4	62	Bt49	62
HD-305	60	HD-229	60	Koe[k]	60
Bt13	57	Bt26	57	Bt5	55
HD-1	52	M14[I]	52	M16[I]	42
M2[I]	40	S53-1	40	Bt28	37
Bt14	32	HD-23	32	M4[I]	30
Bt35	25	Bt1	25	S32-1[I]	25
HA-3	23	Bt27	22	M13[I]	22
Bt52	20	GL13-2	20	M9[I]	20
B18	20	S39-2	20	Bt6	15
S39-1	15	B17	13	B39	12
S31-3	10	Bt45	8	Bt8	5
S42-2[I]	5	GL20-4	0		

[a] Each isolate was mixed with the diet at a 1:45 dilution of the original culture broth (ca. 5x10[illegible] cfu/ml diet). Forty second instar larvae were used for each isolate, and repeated 3 times.

[b] [k] = New isolates from Kenyan soils and [I] = from Israeli soils.

Table 10. Insecticidal activity (LC_{50} values compared to Bt_{24}) of selected isolates of *Bacillus thuringiensis* on third instar larvae of *Spodoptera exempta*[a].

Isolate No.[b]	Serotype[c]	slope	LC_{50} (mg/ml)	% of Bt_{24}	LC_{50} ($x10^6$ cfu/ml)	% of Bt_{24}
7f	7 aizawai	1.61	0.43	1088	0.43	1284
K26-8[k]	ND	1.14	0.57	821	1.07	516
MF4B-2[k]	ND	0.92	0.61	767	1.08	511
L1-6[k]	ND	0.86	1.11	422	1.43	386
A3[k]	ND	1.03	2.08	225	1.86	297
MR1-37[I]	ND	1.52	2.70	173	2.04	271
K26-21[k]	ND	0.99	3.89	120	2.68	206
Bt17	ND	1.94	9.42	50	3.36	164
K30-3[k]	ND	1.32	1.68	279	3.91	141
HD-593	7 aizawai	1.05	2.66	176	4.81	115
Bt57	ND	1.33	3.59	130	4.92	112
Bt24	6 entomoc.	2.70	4.68	100	5.52	100
HD-73	3a3b kurs.	1.07	12.38	38	29.82	19

[a] the washed pellet of each isolate was diluted (20, 40, 80, 160, 320, and 640 mg/ml diet, 60 larvae/dilution.
[b] [k] = New isolates obtained from Kenyan soils and [I] - from Israeli soils.
[c] ND = not determined.

Table 11. Insecticidal activity of several isolates of *Bacillus thuringiensis* on 4th-5th instar larvae of *Spodoptera exempta*[a].

Isolate No.	Serotype[b]	Mortality, (%)
Bt 15	ND	100
Bt54	ND	100
M16[I]	ND	100
HD-695	1	100
7f	7	100
Bt35	ND	80
HD-498	10	80

[a] Pellets of the washed isolates were diluted to give 1:45 dilution of the original culture medium (ca. $5x10^6$ cfu/ml diet), 40 larvae /isolate.
[b] ND = not determined.

Table 12. Comparison of the insecticidal activities of selected isolates of *Bacillus thuringiensis* on second instar larvae of *Spodoptera littoralis* and *S. exempta*[a].

Isolate No.[b]	Serotype[c]	*S. littoralis* slope	*S. littoralis* % of Bt[24]	*S. exempta* slope	*S. exempta* % of Bt[24]
1 K26-21[k]	ND	1.55	244	0.99	120
2 MF4B-2[k]	ND	1.56	180	0.92	767
3 MR1-37[j]	ND	1.46	163	1.52	173
4 K26-8[k]	ND	1.57	145	1.14	821
5 HD-593	7 aizawai	1.76	102	1.05	176
6 Bt24	6 entomocidus	1.55	100	2.70	100
7 Bt17	ND	3.11	87	1.94	50
8 K30-3[k]	ND	1.72	61	1.32	279
9 7f	7 aizawai	0.81	60	1.61	1088
10 Bt57	ND	2.53	52	1.33	130
11 HD-73	3a3b kurstaki	0.00	0	1.07	38

[a] The data for this Table were taken from Tables 5 and 10. The LC_{50} values were calculated from the data recorded for culture broth pellet weight/ml diet.

[b] [k] = New isolates obtained from Kenyan soils and [j] = from Israeli soils.

[c] ND = not determined.

Field applications of Bacillus thuringiensis strain preparations against the African armyworm, Spodoptera exempta.

The purpose of the field experiments in 1988 was to learn the problems of Bt applications against S. exempta. The information on field infestation with the pest were irregular and late. This resulted in application on 4th and 5th instar larvae, which are considerably more tolerant to Bt. In the first experiment (Table 13), numbers of surviving larvae were not reduced in the Bt-treated plots. However, the granular preparation did reduce it (52% survival). 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 might have been as a result of direct larval mortality following ingestion of the treated grass, as observed (but not counted), in the numbers of cadavers. 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. These 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.

Table 13. Effect of preparations of *Bacillus thuringiensis* on 4th-5th instar larvae of the African armyworm (*Spodoptera exempta*) in a cinadon grass field in Lambwe valley Kenya.

Treatment	No. living larvae[a] Pre	Post	%[b]	% of control[c]
Short grass				
Non-treated	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				
Non-treated	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) treatment form pre-treatment 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 main reasons for failing to get significant numeric reductions in surviving larvae in the preliminary field trial were: the small plots without protective borders and the relatively well developed larvae. 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.

In the second experiment (Table 14), a great number of dead and moribund larvae were observed 24 h. after spray application of Bt aizawai and very little larval movement on the ground, 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. After 2 and three days, the treated plots were much greener than 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. While many moribund and dead larvae were observed in The treated plot, 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.

Table 14. Effect of application of Bacillus thuringiensis var aizawai (IH-A) powder preparation, on cinadon grass in the field, Son larval (4th-5th instars) populations on Spodoptera exempta.

Time after Treatment (Days)	Non-treated control		Bt aizawai		
	Number Larvae[a]	% of initial count	Number Larvae[a]	% 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 untreated control.

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 evident in the vegetation. There was also a significant decrease in the larval population in the treated plots. 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 treated and untreated plots are actually much greater than indicated in Table 14. The treated plots were the only green areas in an otherwise totally stripped grassless plain. (Fig. 5)

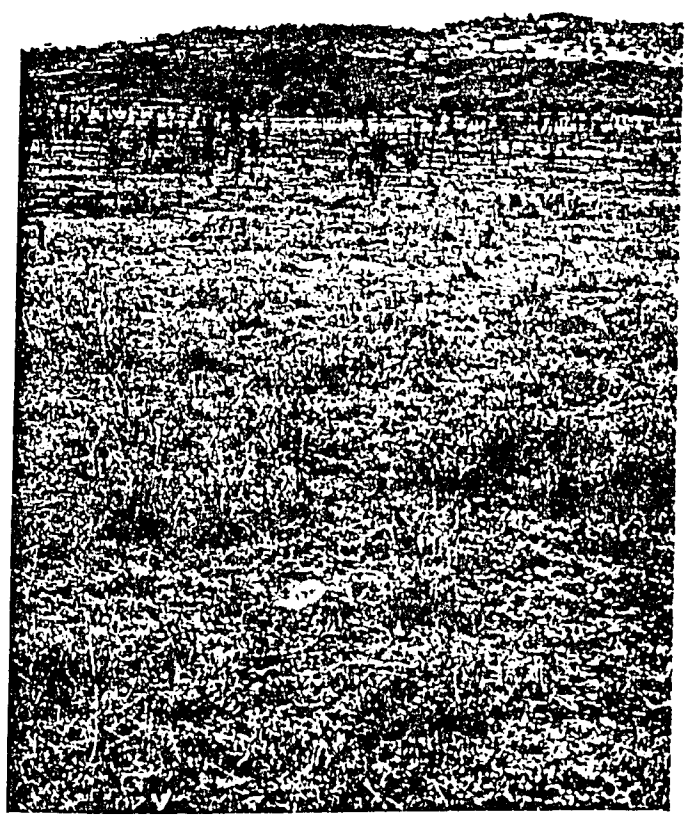


Fig. 5. Effect of Bacillus thuringiensis on prevention of damage to cinadon grass caused by gregarious larvae of the African armyworm Spodoptera exempta. The green plot in the center were sprayed with Bt aizawai strain IH-A, while the front and the back plots were untreated control plots.

The results obtained thus, indicate that the strain (IH-A) of Bt aizawai was very effective in reducing the damage of the armyworm larvae also when they were in their advanced development instars. The population density in the treated plot was constantly lower than that in the untreated control, and reduction in larval population was generally significant.

The third experiment was carried out on 15x15m plots, which reduced the effect of migration from the untreated control plots. Generally, there was a steady decrease in the survival of larval populations in both treated and untreated plots, while the decrease in the Bt treated plots was significantly stronger (Table 15 and Fig. 6). 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 (5th-6th) larvae were observed alive in the area, presumably as a greater dose or longer feeding time are required to kill large larvae. The grass also appeared to be much more healthy than in the control plot, with much new leaf growth observed.

From the third day after Bt application, a sharper decline in larval count was observed in the control as compared to the treated plots. This may be due to partial migration of larvae from the untreated to the treated plots, and the entry of the mature larvae to soil for pupation. Larvae ingesting sub-lethal concentrations of the endotoxin, developed slower and pupate after an extended period of time.

Table 15. 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. 6).

Time after Treatment (days)	Non-treated control. Number Larvae[a]	% of initial count	Bt aizawai 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 each, in 15x15m plots.

[b] Percentage was calculated as % of initial count of treated vs. non-treated control.

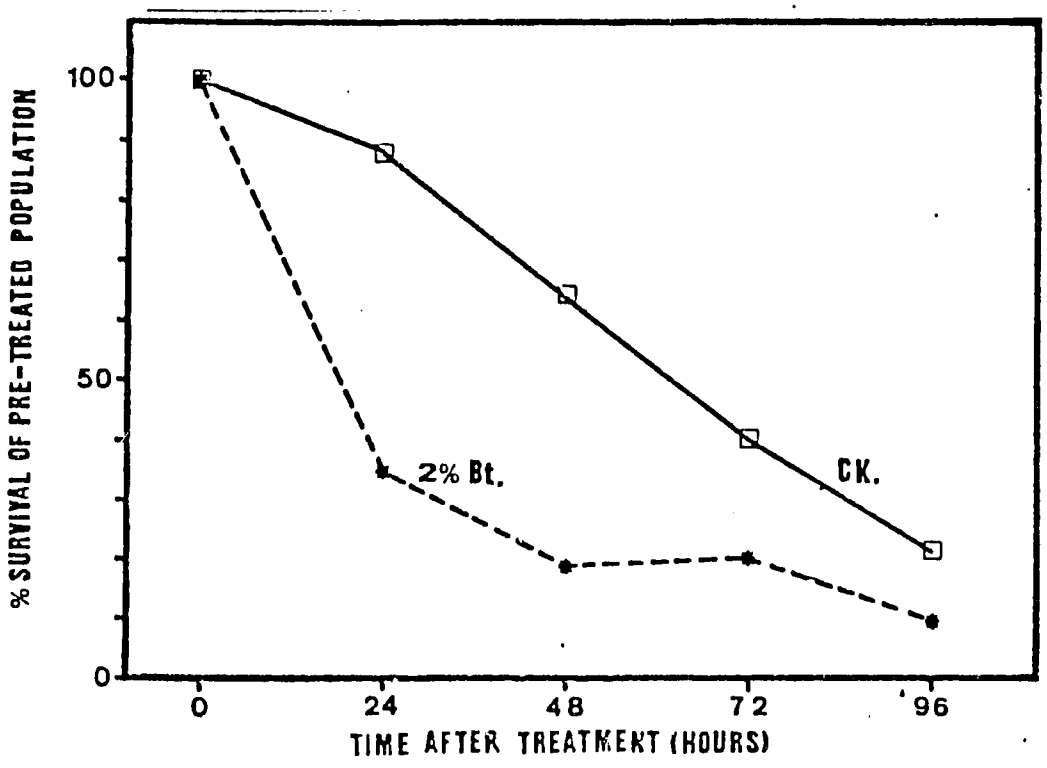


Fig. 6. Effect of spray application of a powdered preparation of *Bacillus thuringiensis* var *aizawai* (IH-A 2% w/v) on survival of 5th instar larvae of *Spodoptera exempta* in 15x 15m plots in Lambwe valley.

In summer 1988, The control experiment of the armyworm outbreak was carried out with powdered preparations of isolates: Bt_{24} (one concentration) and IH-A (3 concentrations) applied on the vulnerable larval instar (3rd), although 4th-6th instar larvae were also found in relatively low numbers. Results summarized in Table 16, clearly indicate that larval population density in the 0.5 and the 1.0% w/v treated plots were reduced by 64.5 and 60.1% respectively (these treatments were not significantly different from each other). A significantly (P=0.05) greater reduction of 95.6% was obtained by the 2.0% treatment with Bt aizawai. The Anova test indicated that both experiment and time, were the most important factors influencing larval population levels. This effect is expected as a consequence of concentration effect on field efficacy and the expected reduction in larval population levels with time. The analysis of the transformed data for treatment effect showed that overall, the 2% treatment of Bt aizawai was the most effective in reducing larval populations in the field. The time at which larval populations were counted was also significant. The most significant drop in surviving larval numbers occurred between 0 and 24 hours, followed by 24 to 48 hours. The final count was not significantly different from that of 48 hours. Thus, the greatest reduction in larval population levels for all treatments occurred within the first 48 hours after Bt application.

The Bt entomocidus preparation was applied at a 2% (w/v) concentration, so that toxicity of the treatment was comparable to that of 1% Bt aizawai, in terms of insecticidal activity units (Bt aizawai had 65000 IUs while Bt entomocidus had only 35000 IUs) The treatment also induced significant larval mortality over the 72 h. Larval population was reduced by 87.% as compared to 60% by 1% of Bt aizawai.

The treated areas appeared to be much healthier than the controls. Thus, all treatments appeared to have affected the larvae and prevented feeding on the crop, either as a result of direct and rapid larval mortality, or as a result of reduced feeding by larvae ingesting sub-lethal doses of the toxin.

Table 16. Control of the African armyworm, *Spodoptera exempta* (3rd instar larvae) with powdered preparations of *Bacillus thuringiensis* var. *aizawai* (IH-A) and *B. thuringiensis* var *entomocidus*[a] (Bt_{24}).

Treatment	Number of surviving larvae[b] Time after spraying (hours) 0	24	48	96
Control	331	309	337	325
% of pre-treatment	100	93.2	101.8	98.0
% reduction	-	6.8 a	-1.8 a	2.0 a
IH-A (0.5%)	328	220	175	119
% of pre-treatment	100	64.4	50.3	34.8
% of control	-	69.1	49.4	35.5
% reduction	-	30.9 b	50.6 d	64.5 d
IH-A (1.0%)	306	214	131	115
% of pre-treatment	100	73.6	42.8	39.1
% of control	-	76.6	42.0	39.9
% reduction	-	23.4 b	58.0 d	60.1 d
IH-A (2.0%)	307	109	33	13
% of pre-treatment	100	33.1	11.3	4.1
% of control	-	35.5	11.1	4.2
% reduction	-	64.5 d	89.9 e	95.6 e
Bt_{24} (2%)	332	162	93	42
% of pre-treatment	100	48.7	28.0	12.7
% of control	-	52.3	27.5	13.0
% reduction	-	42.7 c	72.5 d	87.0 e

[a] The preparation of isolate IH-A was more concentrated (65000 IU) than that of Bt_{24} (35000 IU). Therefore, 2% of the Bt24 is comparable to 1% of the IH-A preparation.

[b] Larval counts from 30 squares of 20x20cm (3 replicates 15x15m). Numbers followed by the same letter are not significantly different (P=0.05 ANOVA).

Spring 1989

Monitoring moth populations and subsequent search for egg masses in Lambwe valley.

In previous years, the presence of population outbreaks of the armyworm were noticed usually, only when the larvae have already reached their 4th or 5th instar. At this stage, they have already caused a considerable amount of damage to the crops and they are more resistant to biological or chemical pesticides. This was also the reason for partial success of our use of Bt to control the armyworm in the field in 1988. Rose et al. have accumulated a great deal of information regarding the biology and migration patterns of the armyworm. They have placed a sparse network of traps in several countries in Africa. However, this kind of network may provide a general view on their migration direction. For tracking down the actual farms in which massive landing of moths occurs, a denser network of traps is required. Information on areas where armyworm outbreaks occurred in recent years may be of great benefit to guide the placement of pheromone traps.

Using this valuable information, we have decided to set up more traps in the outbreak season of spring 1989. Our timing of setting up the pheromone traps network (as suggested by Dr. Rose) was just right for
tracking down the time and locations of moth population appearance in this region. This is clearly demonstrated in Table 17. First counts on February 28th were very low (0-1/trap). It increased to a peak in March 21st (5-721/trap) and from there on, it declined in April 9th (0-26/trap). From the 9 locations, spread over an area of 625km², the concentration of moth appearance was mainly in 2 sites in Makende, in which during 8 days 421 and 1190 moths were recorded per trap (Table 17). In Mogaka, a high number of moths/trap (254) was recorded on February 21st, but before and after this date, very few were trapped. The reason for that may be that this site is located on the way of their migration. They did not land there for egg deposition, as very few ovipositions were found there in the fields. In Lambwe west a,

about 97 moths were recorded in the same date. Lower numbers were counted in the traps stationed in the other locations.

Table 17. Trapping of male moths of Spodoptera exempta in Lambwe valley* with a network of pheromone traps.

Location	Number of adult males trapped											
	28/2	3/3	9/3	13/3	16/3	21/3	22/3	23/3	24/3	26/3	28/3	9/4
1 Makende	0	0	1	26	9	721	220	144	96	13	1	0
2 Makende	1	0	4	22	26	114	187	59	35	15	0	0
3 Mogaka F.	0	0	2	15	1	254	19	–	12	15	–	21
4 L. West a	0	0	9	40	–	97	8	–	8	9	0	5
5 Lochol	0	0	0	–	–	44	2	–	1	15	–	15
6 Koguta F.	0	0	1	38	–	38	0	–	4	8	1	26
7 L. West b	0	0	–	–	–	17	0	–	2	0	11	–
8 L.G. Park	0	0	0	–	–	10	0	–	–	–	–	11
9 Ogongo	0	0	0	14	–	5	1	–	–	0	3	2

* Nine traps were placed of different locations covering an area of ca. 625 km^2 (25x25 km). Moth counts were made in each trap 12 times during the period from 28/2/1989 - 9/4/1989.

This set up, provided us with important information required for tracking down the fields, where the egg masses have been deposited, in order to apply the preparations of B. thuringiensis to the infested fields at the correct timing to meet the hatching larvae at their most susceptible stage. All experiments were carried out during this outbreak period in two sites in Makende (Fig. 7), because in these sites many egg masses were deposited. The third site where many moths have been trapped, only few egg masses have been observed. This was probably due to the fact that most of the moths continued their migration.

The female moths preferred to lay their eggs first in grass fields. However, the egg masses were not deposited low on the green leaves, but on the dry straw about 20cm above ground (Fig 8). The larvae

hatching from these locations spread with the wind in an area of 35cm in diameter on the grass. Thus, they are scattered, each larva has more food and space, and they may have a better chance to escape from predation. Next ovipositions were observed in the young maize field on the lower surface of the lower leaf (of 4-5). This is completely different from that of S. littoralis, which lay their egg masses on the youngest leaves of cotton. Thus they can be easily observed. Later on, the egg masses were found also in the millet field.

Fig 7. A view of Makende farm site 1, from front to back: Young Cinadon grass, tall (ca. 70cm) Maize, Young (2-4 leaves) Maize, and Millet.

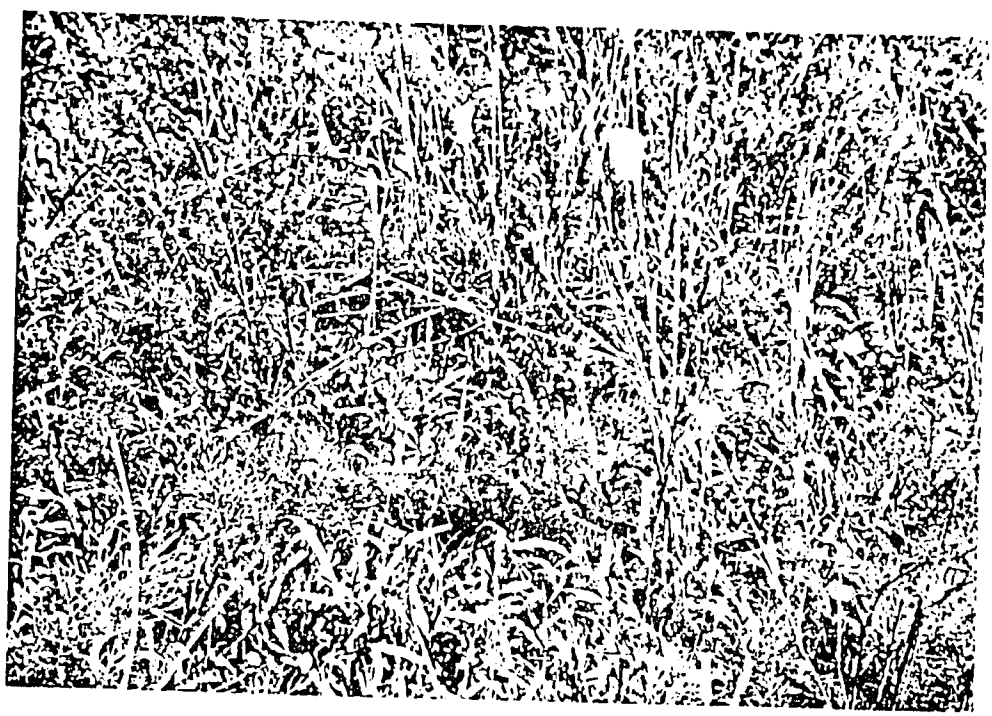
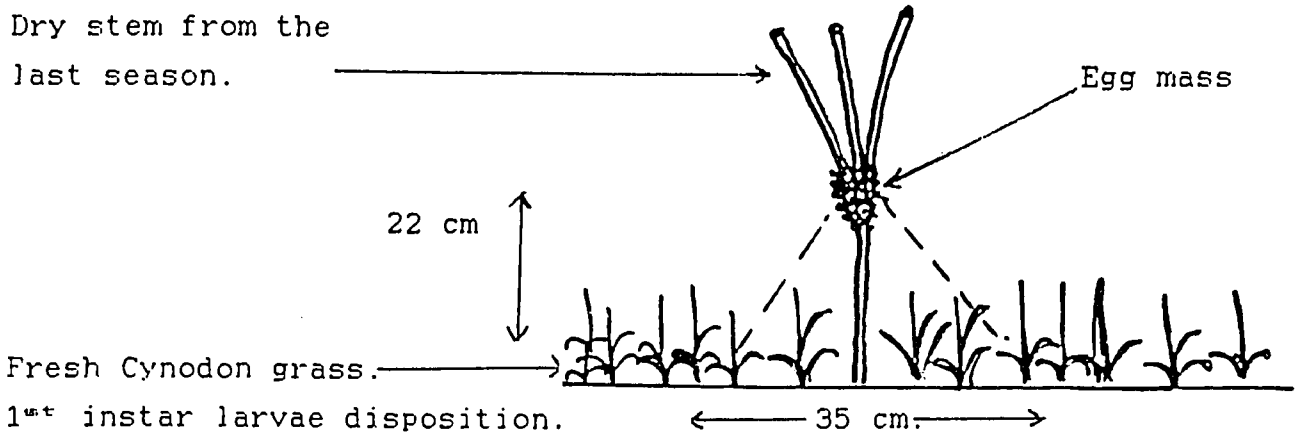
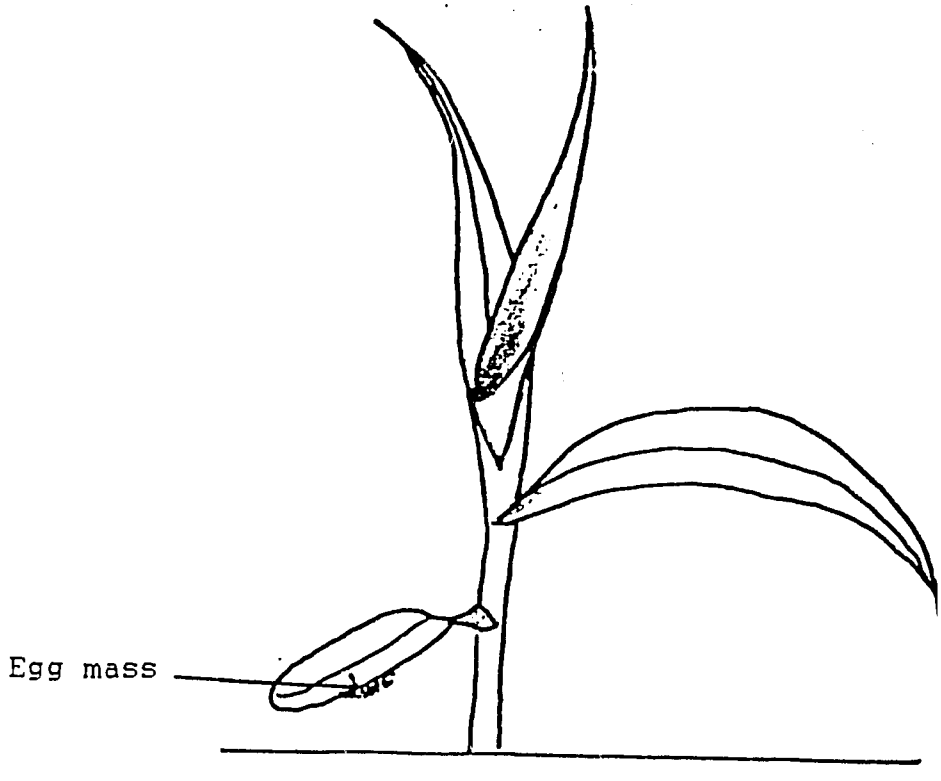


Fig. 8. Egg masses of Spodoptera exempta laid on dry straw (marked with white plastic bands) left from the last season, in a young Cinadon grass field (3 weeks after the start of the rainy season.

SITES IN WHICH EGG MASSES OF SPODOPTERA EXEMPTA HAVE BEEN FOUND IN THE PRESENT STUDY. SPECIFIC LOCATIONS OF ADULT APPEARANCE WERE TRACKED BY PHEROMONE TRAPS



IN A GRASS FIELD



IN A MAIZE FIELD

Control of Spodoptera exempta with powdered preparations of isolates of Bacillus thuringiensis.

Strain MF4B-2 (one of our best new isolates obtained from a Kenyan soil) controlled 95% of the neonate larvae, when applied on a cinadon grass field at only 0.2% concentration (1/10 of that ordinarily used Table 18).

Table 18. Control of neonate larvae of Spodoptera exempta by application of a Bacillus thuringiensis preparation of isolate MF4B-2[a] on egg masses on a cinadon grass field[b] in Makende, site 1.

Treatment	No. of egg masses/plot	Number of surviving larvae Total[c]	per egg mass[d]	% of control
Control	31	534	17.4 a	100
Bt	41	49	0.9 b	5.2

[a] Concentration of the spray suspension was 0.2% (w/v).

[b] Larval counts were recorded 5 days after spray application.

[c] Total numbers counted around 7-15 marked egg masses ($0.5m^2$/egg mass)/plot (3x3m, 4 replicates). The grass field dimensions were 115x32m.

[d] Numbers followed by the same letter are not significantly different (P=0.05, ANOVA).

Another of our best new isolates (K26-21, also obtained from a Kenyan soil) controlled an average of 92% of 2nd to 3rd instar larvae on young maize (Table 19 and Fig 9). Similar control was obtained by application of three different Bt concentrations, indicating that the concentration of 0.4% of this isolate was suitable to control 3rd instar larvae of the armyworm.

Table 19. Control of 2nd and 3rd instar larvae of *Spodoptera exempta*[a] by application of a *Bacillus thuringiensis* preparation of isolate No. K26-21 on Maize plants in Makende, site 2[b]

B. thuringiensis concentration (%)	Number of plants	Number of surviving larvae Total	per plant[c]	% of control
0	303	367	1.21 a	100
1.6	207	21	0.10 b	8.3
0.8	275	21	0.07 b	5.9
0.4	216	20	0.09 b	7.4

[a] Four replicated plots (3x3m)/treatment of young maize plants (5 leaves) arranged in a Latin square.

[b] Counts of living larvae were recorded 2 days after Bt application.

[c] Numbers followed by the same letter are not significantly different (P=0.05, ANOVA).

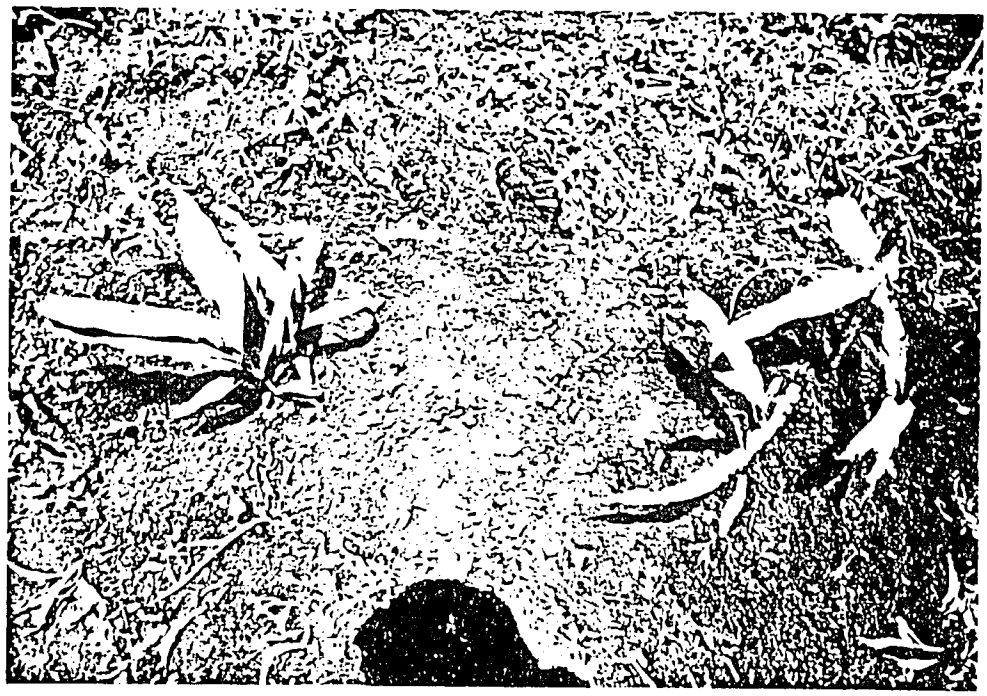


Fig. 9. Control of larvae (2nd and 3rd instars) of *Spodoptera exempta* with a powdered preparation of *Bacillus thuringiensis* (isolate K26-21) on young Maize plants (2-4 leaves) in Makende farm site 2, two days after spray application. The intact plants on the left were treated while the damaged ones on the right were untreated controls.

In order to evaluate to some extent the damage prevented by the use of Bt on young maize plants, the weight of the fourth leaves was recorded. The control leaves had lost 16.6% (fresh weight) or 12.2% (dry weight) as compared to that of Bt-treated ones (Table 20).

Table 20. Effect of application of a *Bacillus thuringiensis* preparation of isolate K26-21 (1.6% w/v) to control 3rd instar larvae of *Spodoptera exempta*, on young maize plants in Makende site 2, on weight of the 4th emerged leaf[a], Makende site 2.

Treatment	Leaf weight (g)[b]		Differential weight (g)[c]	
	Fresh	Dry	Fresh	Dry
Control	115.3	20.19	-22.9 (16.6%)	-2.8 (12.2%)
Bt	138.2	22.99	- -	- -

[a] Before spray application, the third leaves of the maize plants had already been damaged by the larvae, while the 4th leaves were still intact. Therefore, the 4th leaf was used for comparison.

[b] Weights of 12 leaves/treatment (4 replicates, 3 leaves/replicate) were recorded 2 days after Bt application.

[c] Percent of leaf tissue consumed in the control leaves as compared to leaf weight in the Bt-treated leaves. Weight of control leaves are significantly (P=0.05, ANOVA) lower that that of the Bt-treated ones.

On a wider scale experiment (2000m²) on a millet field, a Bt preparation (strain IH-A), controlled 92% third of the armyworm larvae (3rd instar, Table 21).

Table 21. Control of 3rd instar larvae of Spodoptera exempta with a preparation of Bacillus thuringiensis var aizawai isolate IH-A (2% w/v) in a large millet field[a] in Makende site 1.

Counting time	Number surviving larvae/0.5m²[b] Non-treated	Bt-treated	% of control
Pre-treatment	62.5	62.5	-
After treatment	31.8 a	2.3 b	7.9

[a] The whole millet field (2000m²) was sprayed, except for 4 plots (4x4m) left as untreated controls.

[b] Counts of living larvae were recorded from 12 random squares (0.5m²)/treatment, 1 day before, and 2 days after application.

[c] Numbers followed by the same letter are not significantly different (P=0.05, ANOVA).

One of the important problems of the armyworm, is that after the larvae complete to destroy the plants in one field, they "march" to the neighboring fields and continue to destroy the crops there last year the whole crops were destroyed by the armyworm outbreak in Makende site 1. Therefore, a special care should be taken to control the armyworm populations in the wild grass bands around the fields. A grass band area of ca. 3700m² was sprayed with Bt (strain IH-A at 2% w/v) When the larvae were already developed (4th and 5th instars). Even at this stage a considerable control (85%) was obtained (Table 22). Larval counts were made in this experiment by counting the larvae collected from 10 seeps of 180° using the Californian sweep net.

A considerable number of large larvae were collected with the Californian sweep net in the untreated plots. Therefore, it was suggested that in small farms in cases where biological or chemical

control preparations are not available, it might be possible to collect most of the larvae by "Sweeping out", and thus, save the adjacent crops. In such a preliminary treatment, during about 45 min., of sweeping, about 10000 larvae were removed. Some of the larvae fell to the ground during the sweeping, Therefore, the treatment was repeated on the second and on the third day. Very few remained to be swept on the third day. The farmer who had lost his crop last year during the armyworm outbreak, was very grateful, realizing that this year this treatment saved his crop

Table 22. Control of 4th (some at 3rd and some at 5th) instar larvae of Spodoptera exempta[a] on a large cinadon grass band[b] around the maize field in Makende site 1 with a preparation of Bacillus thuringiensis var aizawai (IH-A).

Sampling date (April 1989)	Number of surviving larvae[b] non-treated	Bt-treated	% of control
4	944	944	0 0
6	239	60	74.9
7	340	51	85.0
11[c]	89	19	78.7

[a] Dimensions of the field sprayed with 2% of Bt suspension were 3700m². Four randomized plots (4x4m) were left as untreated controls.

[b] Counts of living larvae were made with a Californian sweep net, ten sweeps of 180°/count

[c] In the last sampling most larvae entered the soil for pupation. pupation of larvae ingesting sub-lethal concentrations of Bt is delayed.

A bait preparation Killed after 24 h. 9 from 25 (4th- 5th instar) larvae of S. exempta. additional 11 were killed after 4 days the other 3 left died before pupation. All of the larvae in the control remained alive. and pupated. It may be used as a barrier around crops, to prevent massive migration of the larvae into the crop.

CONCLUSIONS

Screening of Bt isolates obtained from soil samples collected in Kenya and Israel together with isolates from the stock cultures, for insecticidal activity against larvae of S. exempta, and S. littoralis have indicated the following conclusions:

1. Several new isolates have demonstrated significant higher insecticidal activity than the previous best isolates (Bt[illegible] [entomocidus] and IH-A [aizawai]) against both insect pests, In a descending order:
   Against S. exempta - 7f, K26-8, MF4B-2, K30-3, HD-593, MR1-37.
   Against S. littoralis - K26-21, MF4B-2, MR1-37, K28-30, K26-8.
2. Larvae of S. exempta are considerably more susceptible to Bt than S. littoralis (up to 40 fold for some isolates). Larvae of advanced instars (4th to 6th) of S. exempta, were also controlled by spray application of Bt in the field. Such sprays are considerably less effective against advanced larval instars of S. littoralis.
3. The best of these isolates may be considered for large scale production as a bio-insecticide for the control of these two important pests.

Before 1989, outbreaks of African armyworm larval populations in the fields have been detected only when the larvae have already been considerably developed (4th-6th instar). This was the case in spite of the fact that a great deal of information regarding the weather conditions and its effect on migration patters of this pest in the East African countries (following rain storms) have already been well known (Haggis, 1987; Pedgley et al., 1987). At this larval stage, a great deal of damage had already occurred to the crops and the larvae are more tolerant to all kinds of pesticides. The reasons for that were:

1. Moth traps (based on pheromones, molasses, light etc.) were too scarcely scattered. Only one trap was placed for a large region such as in the whole Lambwe valley. From the results of our field work in 1989, we can conclude that there may be a considerable armyworm population outbreak in several regions of such a valley, but this

could be detected only in 3 out of 9 locations. Therefore, the possibility that only one trap placed in such a vast area, will provide the correct important information on moth appearance in certain fields there, is very slim.

2. The exact locations of the egg masses in the fields was not accurately known before our recent field trials. This gap in early detection was critical for alarming the growers. Therefore, they did not have the crucial information on the necessity to treat their field in the right time. The alarm was in the time when immense masses of large larvae were observed. But at this time, a great deal of damage had already occurred and control, if attempted is less efficient.

In 1989, this knowledge, together with information on fields in which heavy armyworm outbreaks occurred in previous years, and the experience that we have accumulated in scouting for egg masses of S. littoralis in cotton and alfalfa fields in Israel (Broza et al. 1984; 1986), we could clearly demonstrate that the correct use of a network of pheromone traps can serve as the most important tool for scouting for the location of egg masses in the infested fields. This enabled us to learn the sequence of crops on which the eggs are deposited. Namely, first on fresh grass, then on young corn and on millet. Thus, it enabled us to apply the Bt preparations in the right time when larvae were in their most vulnerable stage an excellent control, even with a Bt concentration which was 10 fold lower than that, usually required for the more developed larval instars.

The 1988 outbreak of the African armyworm (Spodoptera exempta) in 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 farms 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 hectares and can be considered wealthy in the general subsistence - type farming which is practiced in the Lambwe valley.

The isolates of Bt entomocidus Bt$_{24}$ and of Bt aizawai (IH-A) which have been produced in the fermentation facilities of Becker Microbial Products for the control of S. littoralis in Israeli fields, have provided a significant good control S. exempta in the fields of Lambwe valley. However, the new isolates may be less costly to produce and more efficient in their insecticidal performance in the fields. we have shown that Bt-treatment may also save the crops from complete destruction, when the larvae in the field are already large and when the efficiency of the Bt is not expressed in larval mortality rates, by reducing larval feeding. The sick larvae may also be more vulnerable to other natural control factors. We have observed that Bt-treated large larvae were carried by ants, while healthy larvae make fast movements to remove the ants away.

It should be emphasized that these fields of small farms are close to the housing and the farm animals are always feeding in these fields. It is therefore impossible to use toxic chemical insecticides. Biological, agro-technological or integrated pest management (IPM) are urgently required to combat his devastating pest.

Grass bands around crops are the main breeding grounds for the armyworm. After the larvae complete to consume the grass, they migrate in large numbers to destroy adjacent crops. The farmers have never considered to control pests on the grass bands around their cultivated crops. The crops of the farm in Makende, which were completely destroyed last year, were saved this year by the careful spraying with Bt of the crops and the grass around around them.

In addition, in small farms, when a bio-insecticide is not available at the required time, a simple agro-technological use of the Californian sweep net for sweeping out the larvae with relatively little work can save the crops from complete destruction. This was clearly shown in our 1989 series of field trials.

Consequent, we would like to suggested a preliminary scheme for an IPM program:

1. Local monitoring by pheromone traps in each of 1-4 adjacent farms. (The method of trapping out using many traps of pheromones or light in locations determined in the early stages of scattered traps have not yet been tested, but may be considered).
2. Scouting for egg masses and first instar larvae.
3. Application of low concentration (0.2%) of Bt (strain effective against the Armyworm) on the neonate larvae.
4. In case that stage 3 was not treated in time, Higher concentrations (1-2%) of Bt and/or baits (based on Bt) will be applied.
   The grass around the field provides a massive reserve for large larvae which will immigrate to the crops. Therefore, treatment of the grass is very important to prevent damage to the crop.
5. A great number of larvae may be removed from the field by a physical means such as sweeping with a net. This can be considered in small farms and save crops.

Further Research Needed:

a) A three years further investigation directed toward:
   1. Accumulating more data on the local nature of a secondary outbreak of the armyworm in South Nyanza.
   2. To improve the control strategy, techniques and the product used.
   3. Scaling up the system to include 30-50 farms.
      To make a cost-benefit analysis.
      To get a Social-Scientist feedback.

   During this stage, the Bt preparations will be produced in Israel.

b) Two to three years of a satellite project to develop a local, inexpensive production line for Bt.

c) To perform a preliminary study on the possible use of Bt to control the African Armyworm in primary outbreak sites in Tanzania and or Eastern Kenya.

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