

CONTROLLING FALL ARMYWORM
(LEPIDOPTERA:NOCTUIDAE) INFESTATIONS IN WHORL
STAGE CORN WITH GENETICALLY MODIFIED *BACILLUS*
THURINGIENSIS FORMULATIONS

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ABSTRACT

Development of more potent strains of *Bacillus thuringiensis* (Berliner) (*Bt*) using recombinant DNA technology may lead to improved insecticidal products for controlling pests like the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), which have been difficult to manage with conventional *Bt*-based products. EG1999, a variant *Bt* strain developed by recombinant DNA technology from EG2348 (the active ingredient of the bioinsecticide Condor[®]), showed improved control of FAW infestations in whorl stage field corn in 1994 (oil flowable and granular formulations) and sweetcorn in 1995 [wetable granule (ECX9526) (WG)] and granular [ECX9526 (G) formulations] as compared with Javelin[®] (WG), an insecticide derived from a naturally occurring *Bt* strain, and methomyl (Lannate[®] LV). In 1994, leaf samples taken 1 h, 1, 2, 3, 5, and 7 d after spraying and assayed in the laboratory with fall armyworm showed that the *Bt* products (recombinant and natural) had less than 48 h residual toxicity to 1st instar larvae. In 1994 freshly hatched FAW were placed on plants in A-frame cages to evaluate this as a method to establish artificial field infestations for use when natural

FAW populations are low. The plant damage and insecticide control in the cages were similar to the heavy natural infestations outside the cages.

Key Words: *Spodoptera frugiperda*, *Bt* formulations, sweetcorn

RESUMEN

El desarrollo de cepas más potentes de *Bacillus thuringiensis* (Berliner) (*Bt*) mediante el uso de la tecnología del DNA recombinante puede contribuir a la mejora de los productos insecticidas para el control de plagas tales como *Spodoptera frugiperda* (J. E. Smith), que han sido difíciles de manejar con productos comerciales a base de *Bt*. EG1999, una variante de una cepa de *Bt* desorrollada mediante la tecnología del DNA recombinante a partir de EG2348 (el ingrediente activo del insecticida Condor®) mostró un mejor control de *S. frugiperda* en maíz en estado de cogollo en 1994 (formulaciones de aceite flowable y granulado), en maíz dulce en 1995 [gránulos humedecibles (ECX9526 (WG)) y formulación granular (ECX9526 (G))] al compararse con Javelin® (WG), un insecticida derivado de una cepa natural de *Bt*, y con methomyl (Lannate® LV). En 1994 las muestras tomadas a 1 hora, y a los 1, 2, 3, 5, y 7 días después de la aplicación y ensayadas en el laboratorio con *S. frugiperda* mostraron que los productos de *Bt* (recombinante y natural) tenían una toxicidad residual al primer instar menor de 24 horas. En 1994 larvas recién eclosionadas fueron puestas sobre plantas en jaulas para evaluar un método para establecer infestaciones de campo cuando las poblaciones naturales de *S. frugiperda* eran bajas. El daño a las plantas y el control con insecticida dentro de las jaulas fueron similares a las fuertes infestaciones naturales que hubo fuera de las jaulas.

Naturally occurring *Bacillus thuringiensis* (Berliner) (*Bt*) based insecticides have had low to moderate effectiveness against the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Gardner & Fuxa 1980; Krieg & Langenbruch 1981; Teague 1993). Biopesticide products containing *Bt* continue to be improved with the use of genetic technologies. ECX9399 (Ecogen, Inc., Langhorne, PA) showed improved toxicity as compared with conventional *Bt* products to FAW in corn field tests in three southeastern states (All et al. 1994). ECX9399 was an oil flowable (OF) formulation of a recombinant DNA variant (EG1999) of EG2348, the active strain in the *Bt* product Condor®. The present research evaluates field efficacy and stability of EG1999 in an (OF), wettable granule (WG), and granular (G) formulation for FAW control in whorl stage field corn (1994) and sweetcorn (1995).

MATERIALS AND METHODS

The field tests were conducted near Athens at the University of Georgia Plant Sciences Farm using Dekalb 689 variety field corn in August 1994 and Silverqueen variety sweetcorn in August 1995. Plots were 4 rows × 6 m long with 95 cm row width and 1 m alleys and were arranged in a randomized complete block experimental design with 4 replications.

Prior to spraying, a FAW baited pheromone trap was monitored weekly for moth flights and the field was sampled for larvae and damage by examining 50 plants at random at 3 to 5 day intervals. Spraying was initiated when 50% of the plants showed damage by larvae (scarification of leaves). In both years plants were in the two leaf stage, about 25 cm tall, when insecticide application was initiated. Sprays were ap-

plied with a CO₂ backpack sprayer in 190 liter/ha spray volume, at 3.2 kg/cm² pressure, with a TG3 nozzle about 20 cm over the plant whorl. Granular formulations were applied in an 18 cm wide band over the row using a Noble® granular spreader and small plot procedures described in All & Hammes (1977). Four applications of the recombinant DNA *Bt* insecticides were made at 7-day intervals. In 1994 (OF) and (G) formulations of ECX9426 were tested and in 1995 (WG) and (G) formulations of ECX9526 were evaluated (see Table 1). Methomyl (Lannate® LV) was used as a conventional insecticide standard in 1994 and 1995, and a *Bt* (subspecies *kurstaki*) standard, Javelin® (WG), was used in 1994 at recommended rates for a conventional *Bt* standard.

In 1994 artificial infestations using neonate FAW were conducted by placing an A-frame cage 120 cm long × 60 cm wide × 105 cm high covered with lumite insect screen (32 × 32 mesh) on top of a treated 5 × 10 cm wood base that had been buried 2.5 cm deep in the middle of one row of each plot. The cage covered 6 to 8 plants and was removed at the time of spraying. After the sprays dried, 5 freshly hatched FAW larvae (obtained from the Insect Biology and Population Management Research Laboratory, USDA-ARS, Tifton GA) were placed in the whorl of each plant; the plants were infested again 3 days later. Infestations were continued after each spray application.

Efficacy was determined at selected intervals by examining all plants and rating damage as 1 = no damage or very slight (<5%) defoliation, no feeding or excrement in the whorl ("no whorl damage", NWD); 2 = light (5-10% defoliation), NWD; 3 = moderate defoliation (up to 20%), NWD; 4 = light to moderate defoliation (up to 20%) and light whorl damage (WD); 5 = moderate to heavy defoliation (20-40%) and moderate WD; 6 = heavy (>40%) defoliation and severe WD; and 7 = only midribs of leaves left, whorl destroyed. Five (1994) or 10 (1995) plants in each plot were examined for dead or live larvae and their size was classified as 1 (instar 1 or 2); 2 (instar 3 or 4), or 3 (instar 5 or 6).

One month after the final rating, the remaining plants within each plot and in the cages (1994) were harvested and weighed. Samples of the plants were then dried for 10 days at 88°C to obtain biomass estimates of the plants, and the weight data were converted to dry weight.

In 1994 a study was conducted to determine the toxicity and residual effects of the spray treatments on FAW larvae. Samples were taken from the midsection of leaves in each plot about 1 h after the first spray application, as well as 1, 2, 3, 5, and 7 d post spraying. Leaves (each treatment replicated 8 times) were cut into 5 cm sections and placed in petri dishes containing moistened filter paper. Each leaf sample was infested with 5 neonate larvae and the plates were sealed with parafilm and placed in an incubator (31°C) for 24 h. The plates were then unsealed and larval mortality was determined and defoliation rated on an ascending scale of 1 = no feeding to 6 = heavy feeding. Surviving larvae were then placed in individual diet cups containing pinto bean diet and their status was visually monitored for 7 d. Final mortality counts were made, larval size measured under a dissecting microscope, and the larvae dried for 8 d at 88°C and weighed. The data were analyzed using analysis of variance and Duncan's new multiple range analysis procedures on SAS microcomputer based statistical analysis software (SAS 1985).

RESULTS AND DISCUSSION

Natural infestations were higher in 1994 than 1995, but untreated check plants suffered serious damage (damage rating of 6.3 in natural infestations and 6.7 in cages in 1994 as compared with 4.1 natural infestation in 1995) both years. When plants

TABLE 1. DAMAGE RATINGS OF FAW INFESTATIONS IN WHORL STAGE FIELD CORN (1994) AND SWEETCORN (1995) TREATED WITH RECOMBINANT DNA *Bt* FORMULATIONS AND SELECTED CONVENTIONAL INSECTICIDE STANDARDS.

Treatment and Rate ¹	% Control ²			No. Live Larvae/Plant ²		
	Fields Plants		Cage Plants	Field Plants		Cage Plants
	18 d	28 d	21 d	18 d	28 d	21 d
1994						
ECX9426 OF 1.2	32 a	36 a	52.5 a	3.0 ab	1.7 a	6.0 a
ECX9426 OF 2.4	64 b	44 a	79.1 b	2.3 ab	1.0 b	2.0 ab
ECX9426 G 5.6	32 a	28 a	50.0 a	2.5 ab	1.6 ab	4.8 ab
ECX9426 G 11.3	68 b	72 b	79.9 b	1.6 bc	0.5 bc	2.0 ab
Javelin® WG 1.1	40 a	28 a	31.3 a	3.5 a	1.0 bc	4.0 ab
Methomyl 0.5	80 c	64 b	86.1 b	0.5 c	0.5 c	1.8 b
1995						
ECX9526 WG 1.1	60 a	33 b	—	0.3 a	0.45 a	—
ECX9526 WG 2.4	67 a	52 a	—	0.3 a	0.53 a	—
ECX9526 G 5.6	20 b	3 b	—	0.3 a	0.70 a	—
ECX9526 G 11.3	20 b	13 b	—	0.2 a	0.55 a	—
Methomyl 0.5	72 a	69 a	—	0.1 b	0.38 a	—

¹Rates of ECX9426 (OF) are presented as volume (liters) of formulated product per ha, ECX9526 (WG), Javelin® (WG), ECX9426 (G) and ECX9526 (G) as weight (kg) of formulated product per ha and rate of methomyl as weight (kg) of active ingredient per ha.

²Means followed by the same letter within a column and year are not significantly different in Duncan's new multiple range test ($P < 0.05$). Percent control values for treatment are based on damage ratings compared with untreated checks.

were examined for dead and live larvae, insects of all sizes were found in all of the treatments. Table 1 shows that in 1994 the higher rate of both the ECX9426 (OF) and (G) formulations gave superior percent control [$1 - (\text{treatment damage rating/check rating}) \times 100$] as compared to Javelin® (WG) at a rate of 1.1 kg/ha. However, only the (G) formulation was statistically similar to methomyl at 0.5 kg active ingredient per ha in the 28 d sample.

The high 1994 natural infestation rate resulted in levels of plant damage similar to the injury that occurred in the infestations in cages. Relative efficacy of the various treatments in the cages was similar to their performance on the non-caged plants in plots subjected to natural infestations. These results indicate that use of artificial infestations in A-frame cages could be a useful method to simulate high natural infestations in situations where indigenous FAW populations are low.

In 1995 the percent FAW control at the 2.4 kg rate of ECX9526 (WG) was similar to that provided by methomyl, but efficacy of ECX9526 (G) was reduced substantially compared with a similar formulation used in 1994 (Table 1). Overall, weather was drier in 1995 during the test period, and this may have negatively influenced FAW control by the (G) formulation by reducing dispersion of the bacteria within the whorls.

In the 1994 leaf sample assay of the two ECX9426 (OF) rates, Javelin® (WG), and methomyl, all of the treatments were highly toxic to larvae exposed to leaves taken one hour following spraying (Table 2). The granular formulation of ECX9426 was not tested because of poor leaf coverage. Toxicity of all of the *Bt* products dropped dramatically on the leaves collected 24 h after spraying, demonstrating a rapid loss in residual potency under field conditions. FAW mortality on *Bt* sprayed leaves was reduced even further 2 days after spraying and was less than 8% (statistically similar to the check leaves) on days 3, 5, and 7 after spraying (for this reason only data up to day 3 are presented in Table 2). Methomyl residues on sprayed leaves were highly toxic to FAW larvae up to the 2 day sample, but substantially less mortality occurred on leaves collected on day 3. Toxicity of methomyl residues was less than 5% on days 5 and 7 and was not significantly different from mortality on check leaves after day 3.

The growth of larvae surviving from exposure on treated leaves and then placed on diet for 7 d was determined as an indicator of physiological inhibition resulting from insecticide toxicity. Weight and length measurements followed similar trends, therefore only size measurements are presented in Table 3. Survivors from all of the insecticide leaf samples taken on the same day as spraying were significantly smaller than the larvae exposed on check leaves after 7 d on diet. The size of survivors was reduced on the high, but not the low, rate of ECX9426 (OF) on 24 and 48 h leaf samples; whereas the size of survivors from the Javelin® treatment was not reduced in the 24 h leaf sample or thereafter. Reduced FAW growth by survivors on methomyl treated leaves occurred for up to 3 d after spraying, but not from leaves collected at 5 and 7 d after spraying. These results indicate that residues of ECX9426 (OF) sprayed on corn leaves at a rate of 2.4 kg/ha reduce growth of FAW survivors and are significantly more effective than Javelin® (WG) at 1.1 kg/ha. However, the data also demonstrate the weak residual activity (less than 2 d) of *Bt* products on corn leaves for FAW.

TABLE 2. FAW LARVAL MORTALITY IN 1994 FOLLOWING FEEDING ON CORN LEAF SAMPLES COLLECTED AT SELECTED INTERVALS AFTER TREATMENT WITH *Bt*-BASED AND CONVENTIONAL INSECTICIDE PRODUCTS.

Period Between Spray and Collection	Observation Time	% Mortality ¹ Treatment and Rate ²				
		ECX9426 1.2	(OF) 2.4	Javelin® 1.1	Methomyl 0.5	Check
1 h	24 h	47.5 c	55.0 c	72.5 b	97.5 a	0.0 d
	7 d	65.0 c	90.0 ab	82.5 b	100.0 a	0.0 d
1 d	24 h	25.0 b	22.5 b	2.5 c	90.0 a	0.0 c
	7 d	35.0 c	65.0 b	5.0 d	95.0 a	7.5 d
2 d	24 h	12.5 bc	22.5 b	7.5 c	97.5 a	0.0 c
	7 d	15.0 bc	30.0 b	15.0 bc	97.5 a	0.0 c
3 d	24 h	0.0 b	2.5 b	5.0 ab	17.5 a	2.5 b
	7 d	2.5 b	7.5 ab	7.5 ab	20.0 a	2.5 b

¹Means followed by the same letter across a row are not significantly different in Duncan's new multiple range test ($P < 0.05$).

²Rates of ECX9426 (OF) are presented as volume (liters) of formulated product per ha, Javelin® (WG) as weight (kg) of formulated product per ha and methomyl as weight (kg) of active ingredient per ha.

TABLE 3. GROWTH (AFTER 7 DAYS ON DIET) OF FAW LARVAE SURVIVORS FROM CORN LEAF SAMPLES COLLECTED AT SELECTED INTERVALS FOLLOWING SPRAYING WITH *Bt*-BASED AND CONVENTIONAL INSECTICIDE PRODUCTS.

Leaf Collection Following Spray	Mean Size (cm) of Survivors of 7 D ¹ Treatment and Rate ²				
	ECX9426 1.1	(OF) 2.4	Javelin® 1.1	Methomyl 0.5	Check
1 h	1.65 b	1.20 bc	1.33 c	1.00 d	3.00 a
1 d	2.25 ab	1.88 bc	2.95 a	1.10 d	2.83 a
2 d	2.70 ab	2.38 b	2.70 ab	1.05 c	3.00 a
3 d	2.90 ab	2.83 ab	2.78 ab	2.60 b	2.95 a
5 d	2.93 ab	3.00 a	2.93 ab	2.78 b	2.95 ab
7 d	2.75 a	2.75 a	2.90 a	2.73 a	2.95 a

¹Means followed by the same letter across a row are not significantly different in Duncan's new multiple range test ($P < 0.05$).

²Rates of ECX9426 (OF) are presented as volume (liters) of formulated product per ha, Javelin® (WG) as weight (kg) of formulated product per ha and methomyl as weight (kg) of active ingredient per ha.

Silage (dry wt) yield in the treatments paralleled efficacy trends. In 1994, plant production in check and Javelin® plots was 5229 and 6250 kg/ha, respectively, and these were significantly less than yields in the methomyl plots (9408 kg/ha). Yield of the ECX9426 (OF) treatments was 6232 and 6698 for the 1.2 and 2.4 kg/ha rates, respectively. In comparison, the ECX9426 (G) treatments yielded 7619 and 6904 kg/ha, respectively, at the 5.6 and 11.3 kg/ha rates, but these were not statistically different from the other treatments. Yield in 1995 was 6368 kg/ha in check plots, which was significantly less than all of the treatments except the 2.4 kg/ha rate of ECX9526 (G) which averaged 7375 kg/ha. Methomyl at 0.5 kg active ingredient per ha had highest yield with 15,943 kg/ha yield in dry plant matter followed by ECX9526 (WG), 2.4 kg/ha (15,765 kg/ha), > ECX9526 (WG) 1.2 kg/ha (11,606 kg/ha), > ECX9526 (G) (11,445 kg/ha), all of which were statistically similar to each other (Duncan's new multiple range test ($P < 0.05$)), but significantly higher than the yield in check plots.

Overall, the two years of tests with four formulated *Bt* products (ECX9426 (OF), ECX9526 (WG), ECX9426 (G) and ECX9526 (G)) derived from a recombinant DNA strain EG1999 demonstrated that at the rates tested the materials provided improved control of FAW infestations in corn compared with a formulation of an indigenous *Bt* strain (Javelin®). Under certain circumstances, the recombinant DNA *Bt* products produced control of FAW similar to that of a conventional insecticide (methomyl). It appears that the recombinant DNA *Bt* products have low residual potency which has been a problem with most *Bt*-based insecticides (Krieg & Langenbruch 1981). These tests verified 1993 research with EG1999 formulations tested in three southeastern states (All et al. 1994). Ecogen Corp. is currently pursuing registration of an EG1999 product under a tentative trade name of Crystar®.

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