Potentiation of *Bacillus thuringiensis* var. *kurstaki* with Thuringiensin on Beet Armyworm (Lepidoptera: Noctuidae)

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ABSTRACT Toxicity of several formulations of *Bacillus thuringiensis* var. *kurstaki* (Berliner) (BTK) to beet armyworm, *Spodoptera exigua* (Hübner), was determined using neonate larvae in a diet incorporation bioassay. LC_{50}'s for formulations of the spore/crystal complex of two BTK isolates, Dipel 2X and Javelin, were 299 μg/ml and 81.2 μg/ml of diet, respectively. The LC_{50} of ABG-6162, an experimental formulation of thuringiensin, was 37.5 μg (AI)/ml of diet. Time of death varied with treatment. With Dipel 2X or Javelin, most mortality (52%) occurred by day 3, whereas with ABG-6162, mortality was greatest (52%) between days 5 and 6. Combinations of thuringiensin and BTK resulted in potentiation, whereas combinations of the two BTK formulations produced an additive effect. Combinations of BTK and thuringiensin increased rate of mortality, with 90% of the total mortality occurring within 3 days.

KEY WORDS *Bacillus thuringiensis* var. *kurstaki*, thuringiensin, exotoxin, potentiation, *Spodoptera exigua*

BEET ARMYWORM (BAW), *Spodoptera exigua* (Hübner), is a polyphagous noctuid that is a primary pest on many agriculturally important crops in the United States (Metcalf et al. 1962). Low economic thresholds and apparent resistance to chemical pesticides (Poe et al. 1973, Meinke & Ware 1978) have increased pesticide use, leading to higher control costs and harmful effects on beneficial insects of other pest species (Johnson et al. 1980, Trumble 1985).

Microbial insecticides containing *Bacillus thuringiensis* var. *kurstaki* (Berliner) (BTK) have been registered since 1961, but recommended field rates in many vegetable crops result in unsatisfactory BAW control (Wyman & Oatman 1977, Schuster 1982). An approach to resolving this problem has been the recent release of the NRD-12 isolate, marketed as Javelin, which has improved activity against *Spodoptera* species. Another approach has been to investigate the potential uses of microbial by-products such as the beta-exotoxin (thuringiensin) produced by some varieties of *B. thuringiensis* (BT). Thuringiensin has a broader insect range than does the delta-endotoxin of BTK (Krieg & Langenbruch 1981).

Potentiation has been documented for interactions using the delta-endotoxin of BT and several chemical insecticides (Herfs 1965, Benz 1971, Salama et al. 1984), although relatively little information is available on interactions between thuringiensin and BTK. Potentiation describes the joint action of insecticides that is more toxic than would be predicted from their separate effects from models of similar action (Busvine 1971). Benz (1975) determined that combinations of BTK and thuringiensin resulted in potentiation against larch bud moth, *Zeiraphera diniana* (Gueneé). Bitoxibacillin, a pesticide containing both BTK and thuringiensin, has been used in the USSR at rates lower than the delta-endotoxin alone against larvae such as BAW, *Agrotis segetum* (Schiffermüller), and *Heliothis zea* (Boddie) (reported as *H. obsoleta* (F.)) (Sebesta et al. 1981).

The purpose of our study was to determine the toxicity of thuringiensin and commercial formulations of BTK containing the HD-1 and NRD-12 isolates to BAW. Subsequently, combinations of BTK and thuringiensin were evaluated.

Materials and Methods

Test materials included Dipel 2X wettable powder containing the HD-1 isolate with 32,000 international units (IU)/mg (Abbott Laboratories), Javelin wettable powder containing the NRD-12 isolate with 16,000 *Spodoptera* units/mg (terminology after Zoecon Corp.), and the liquid ABG-6162 (Abbott Laboratories) containing 1.5% thuringiensin. Neonate BAW larvae used in all tests were obtained from a laboratory colony established in 1982 from insects collected in Orange County, Calif., and maintained on artificial diet (Patana 1969) at 27 ± 1°C and a photoperiod of 16:8 (L:D). Artificial diet was used in all bioassays. Potential inbreeding was reduced by introduction of field-collected insects.

Toxicity of BTK and Thuringiensin to BAW. Bioassays with BTK were conducted with seven concentrations plus a control for individual treatments. Concentrations (μg/ml of diet) tested were

as follows: Dipel 2X, 50, 100, 200, 400, 800, 1,600, and 3,200; Javelin, 20, 40, 80, 120, 160, 320, and 640. Suspensions were made by adding materials to an aqueous 0.1% Tween 80 solution to produce 50 ml. Controls consisted of 50 ml of an aqueous 0.1% Tween 80 solution. Suspensions were chilled to 10°C and suspended for 30 s on a sonic dismembrator (Fisher) (70% power). ABG-6162 was tested with and without Tween 80. In replicates without Tween 80, a control plus five concentrations of 17.7, 27.9, 33.9, 41.2, and 65.0 μg (AI)/ml of diet were evaluated. Volume was initially brought to 50 ml by the addition of distilled water before incorporation into the diet. In replicates including Tween 80, a control plus seven concentrations (μg [AI]/ml of diet) of 10, 20, 30, 40, 50, 60, and 70 were tested, with initial volumes increased to 50 ml by the addition of 0.1% Tween 80 solution.

Each concentration was added to 1,300 ml of artificial diet after the diet was cooled to 43.5°C in an ice bath. The mixture then was blended for 2 min and ca. 15 ml of diet was poured into clear plastic cups (30 ml). Diet was allowed to dry for 1–4 h before a single neonate BAW (0–4 h old) was placed in each cup. Cups were covered with opaque plastic lids and placed in a growth chamber at 27 ± 1°C and a photoperiod of 16:8. At least 30 insects were tested with each concentration; tests with each concentration were replicated five times. Larval mortality was assessed at 6 days in all treatments.

Data were initially analyzed using the Proc Probit procedure (SAS Institute 1985) after correction for control mortality with Abbott's (1925) formula, and then judged for suitability as described by Vandekar & Dulmage (1982). Remaining values were pooled. Control mortality was ≤10%.

Potentiation. Bioassays with the LC_{25} of each treatment plus control were prepared as described previously, then concurrently evaluated both independently and in combination. Suspensions that contained combinations of BTK and thuringiensin were sonicated before the addition of thuringiensin. Each treatment was replicated six times with 30 larvae per replicate. Mortality was checked at 24-h intervals for 6 days. Determination of potentiation with a χ^2 test was made as described by Finney (1971) and Salama et al. (1984). Comparisons of resulting LC_{25}'s with LC_{25}'s estimated from probit analyses were based on criteria of Vandekar & Dulmage (1982). Control mortality was ≤10%.

Results and Discussion

Toxicity of BTK and Thuringiensin to BAW. The LC_{50} for Javelin (81.2 μg/ml of diet) was lower than the LC_{50} for Dipel 2X (299μg/ml of diet) (Table 1). LC_{50}'s for individual replicates of Javelin were 3.09- to 4.63-fold lower than values from concurrent Dipel 2X replicates. The LC_{50} of ABG-6162 was 37.5 μg (AI)/ml of diet (Table 1). No significant differences were found between tests with or without Tween 80 (by the criteria of Vandekar & Dulmage [1982]).

Table 1. Toxicity of BTK isolates and thuringiensin on *S. exigua*

Treatment	*n*	Slope ± SEM	LC_{50} (95% FL)[a]
Dipel 2X	150	1.64 ± 0.0886	299 (262–338)
Javelin	150	2.51 ± 0.1544	81.2 (73.0–89.0)
ABG-6162	150	4.76 ± 0.3293	37.5 (35.4–39.8)

[a] Dipel 2X and Javelin in μg/ml of diet; ABG-6162 in μg (AI)/ml of diet.

Potentiation. Combinations of BTK and thuringiensin resulted in greater potentiation than has been previously reported for BTK plus organophosphates, carbamates, pyrethroids, or insect growth regulators (Table 2) (Salama et al. 1984). Combining the two BTK formulations produced an additive effect on mortality. Similar results were reported by Salama et al. (1983) for mixtures of two BTK isolates on *Spodoptera littoralis* (Boisduval).

Mortalities for estimated LC_{25}'s of Dipel 2X, Javelin, and ABG-6162 were 21, 20, and 33%, respectively (Fig. 1). These values were not different (Vandekar & Dulmage 1982) from LC_{25}'s estimated by probit analysis.

Temporal differences in the occurrence of mortality was also evident among treatments (Fig. 1). For the BTK formulations, 52–55% of the total mortality occurred by day 3.0. In contrast, 52% of the total mortality occurred between days 5 and 6 for ABG-6162. Similar trends were also evident for all concentrations tested in bioassays done to estimate each probit regression. Although ca. 3 days are normally required to complete development of the first instar at 27°C (Fye & McCada 1972), no neonate BAW exposed to lethal or sublethal concentrations of thuringiensin molted to the second instar after 6 days. Because thuringiensin acts by suppressing RNA transcription (Sebesta et al. 1981), the sudden onset of mortality between days

Table 2. Potentiation of BTK with thuringiensin against neonate *S. exigua*[a]

LC_{25}[b] Dipel 2X	LC_{25} Javelin	LC_{25} ABG-6162	% larval mortality[c]	χ^2 value	Effect
128.5	—	29.6	100	103.9[d]	Potentiation
—	43.8	29.6	99	103.2[d]	Potentiation
128.5	43.8	—	37	0.103	Additive

[a] BTK values in μg/ml of diet; thuringiensin in μg (AI)/ml of diet.

[b] LC_{25} = estimated lethal concentration needed to kill 25% of the test population at day 6.

[c] Pooled data from six replicates with 30 larvae per replicate.

[d] Rejection of the hypothesis that treatment effects are additive at $P < 0.01$.

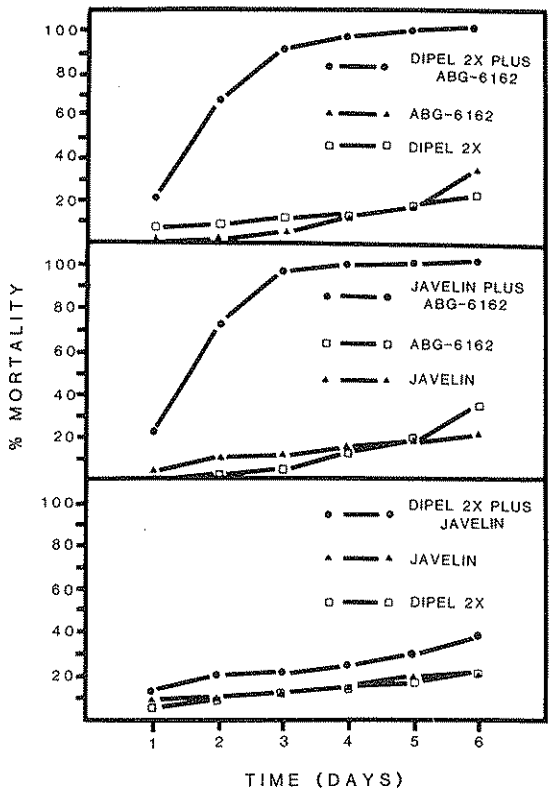

Fig. 1. Percentage of *S. exigua* mortality from LC_{25} values of Dipel 2X, Javelin, and ABG-6162 tested singularly, and potentiation resulting from combinations, in relation to time. Treatments were replicated six times with 30 larvae per replicate.

5 and 6 appears to be in response to a physiologically demanding process such as molting.

Combinations of BTK and thuringiensin substantially increased the mortality rate as compared with any individual compound (Fig. 1). Within 3 days, ≥90% mortality was observed. As in the previous toxicity study, mixing the two BTK formulations produced an additive effect, with only 19% mortality occurring after 48 h.

This study utilized commercially formulated materials that contain compounds potentially capable of affecting the interactions of delta-endotoxin and thuringiensin. However, because both potentiation and accelerated mortality were demonstrated using commercial formulations, the data presented have practical implications for field use in integrated pest management programs. Additional research is necessary to document the effects of combinations of the purified endotoxins and thuringiensin, as well as to determine the most efficacious combinations for BAW control.

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