Relative Activity of Commercial Formulations of *Bacillus thuringiensis* Against Selected Noctuid Larvae (Lepidoptera: Noctuidae)[1]

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Abstract Diet incorporation and spray chamber assays compared the activity of three commercial formulations of *Bacillus thuringiensis* Berliner against *Heliothis virescens* (F.), *Helicoverpa zea* (Boddie), *Pseudoplusia includens* (Walker), and *Spodoptera exigua* (Hübner). The commercial products evaluated were Condor OF® (Ecogen Inc., Langhorne, PA), Dipel ES® (Abbott Laboratories, North Chicago, IL), and Javelin WG® (Sandoz Crop Protection Corp., Des Plaines, IL). Variation in results of the diet incorporation studies illustrated the importance of including a standard reference formulation in the design of the studies and suggested that some standardization of insect strains used in such assays may be important also. Overall results of the diet incorporation studies were similar to those obtained in the spray chamber studies where insects were assayed on sprayed cotton and soybean. Dose-mortality regressions were developed for each *B. thuringiensis* formulation applied against each insect species on cotton and soybean over a 28-day observation period. These regressions may be helpful in the development of recommendations for the use of *B. thuringiensis* on cotton and soybean. They may also have value in establishing base-line levels of susceptibility for future resistance monitoring efforts. Median lethal concentrations (LC50s) tended to be slightly higher on cotton than on soybean. The most susceptible insect species was *H. virescens*, and the least susceptible was *S. exigua*. LC50s for *H. zea* and *P. includens* were similar and intermediate between those of *H. virescens* and *S. exigua*. Javelin WG tended to exhibit the highest activity per unit weight of commercial product, but the relative differences among the commercial formulations were influenced by insect species.

Key Words *Bacillus thuringiensis*, *Heliothis virescens*, *Helicoverpa zea*, *Pseudoplusia includens*, *Spodoptera exigua*, cotton, soybean

Commercial formulations of *Bacillus thuringiensis* Berliner have been available for use on a variety of field crops for more than three decades (Hall 1963). Field tests of commercial *B. thuringiensis* products on cotton were made as early as the 1960's (Cowan et al. 1960, Pfrimmer and Merkl 1962). Many field experiments have re-examined the efficacy of *B. thuringiensis* products for control of cotton pests since

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these initial tests. Results have been variable (see references in Luttrell et al. 1982, Ali and Young 1993), and the market for this microbial insecticide on cotton has been small in comparison to that for traditional chemical insecticides. In recent years, there has been small but increased use of *B. thuringiensis* products on soybean crops. Field efficacy tests of *B. thuringiensis* against soybean pests are less common than those for cotton, although some field testing of commercial formulations was done as early as 1973 (Yearian et al. 1973). Several laboratory studies (Rogoff et al. 1969, Ignoffo et al. 1977, MacIntosh et al. 1990) have compared the relative activity of *B. thuringiensis* against a range of lepidopterans typically associated with the soybean system. Most lepidopteran species attacking soybean are susceptible to *B. thuringiensis*.

Interest in expanding the role of *B. thuringiensis* and other microbial insecticides in cotton and soybean IPM programs tends to increase when pest populations develop resistance to conventional insecticides, and alternative control measures are needed for effective crop protection. The largest historical markets for *B. thuringiensis* products on cotton and soybean were those that developed over the past decade. Most Cooperative Extension Service recommendations for control of cotton and soybean pests across the Mid-South now include options for the use of *B. thuringiensis* products. This was not true a decade ago. The increased reliance on *B. thuringiensis* in the Mid-South is due partially to the declining efficacy of traditional insecticides for control of *Heliothis virescens* (F.) populations on cotton (Luttrell et al. 1987, Elzen et al. 1992) and *Pseudoplusia includens* (Walker) populations in soybean (Felland et al. 1990, Leonard et al. 1990). Interest in *B. thuringiensis* also has been stimulated by the availability of a number of different commercial products developed by expanding biotechnology. These new *B. thuringiensis* products are supported by aggressive market development strategies, and competition among industry groups for a share of this small but visible market is keen. As a result, farmers and agricultural consultants inquire about expected product performance and comparative activity of different commercial products. Although differences among the products and the dose applied may be small in terms of expected insect mortality (Luttrell et al. 1982, Ali and Young 1993), differences in cost of these products per unit area at the farm level can be significant.

Industrial groups producing *B. thuringiensis* have standard internal procedures for quantifying the potency of their different products. Unfortunately, these procedures vary from company to company, and different companies report potency on product labels in different units (e.g., international units, *Spodoptera* units, amount of active ingredient or lepidopteran active protein, etc.). The standardization procedures described by Dulmage (1973, 1975) and Beegle et al. (1986) are no longer universally used. This is due, in part, to the differential activities of *B. thuringiensis* isolates and proteins against different insect species (Ignoffo et al. 1977, Luttrell et al. 1982, MacIntosh et al. 1990).

Reported herein are results of studies conducted at two universities comparing the relative activity of three commercial formulations of *B. thuringiensis* against four species of Lepidoptera commonly found in cotton and soybean in the Mid-South. These studies were initiated to assist Cooperative Extension Service specialists with recommendations about the use of *B. thuringiensis* on cotton and soybean. They also provide quantification of the variability associated with measuring potency of different *B. thuringiensis* products.

Materials and Methods

The activity of three commercial formulations of *B. thuringiensis* were compared in diet incorporation and spray chamber assays against *H. virescens*, *P. includens*, *Helicoverpa zea* (Boddie), and *S. exigua* (Hübner) larvae. Diet incorporation assays were conducted at Mississippi State University and the University of Arkansas. Spray chamber assays were conducted at Mississippi State University. Insects used in diet incorporation assays were laboratory colonies reared according to the procedures of Jenkins et al. (1995). Separate colonies were used at each location, and insects were not exchanged between locations. Field colonies of *H. virescens*, *H. zea*, and *S. exigua* collected from cotton fields in Leflore Co., MS, were also included in diet incorporation assays at Mississippi State University. Insects from the field colonies had been reared on a wheat-germ casein diet (King et al. 1985) in the laboratory for less than three generations. The laboratory colonies had been maintained for many generations (more than 5 yrs with some colonies) in laboratory culture. Spray chamber assays were conducted with insects obtained from the USDA/ARS research facilities at Stoneville, MS.

The commercial formulations of *B. thuringiensis* used in all studies were: Condor OF® (an oil-flowable formulation of *B. thuringiensis* var. *kurstaki* strain EG 2348 from Ecogen Inc., Langhorne, PA, with 7.5% active ingredient [lepidopteran active toxin]), Dipel ES® (an emulsifiable suspension of *B. thuringiensis* var. *kurstaki* from Abbott Laboratories (North Chicago, IL) with 3.5% active ingredient and a potency of 17,600 IU (international units) per mg of product), and Javelin WG® (a wettable granule formulation of *B. thuringiensis* var. *kurstaki* from Sandoz Crop Protection Corp. (Des Plaines, IL) with 6.4% active ingredient and a potency of 52,863 *Spodoptera* units or 31,718 IU/mg). All diet incorporation assays at a given location were conducted from samples of the same commercial lot of each *B. thuringiensis* product. The product samples used for spray chamber assays were new commercial lots and were not the same as those used in the diet incorporation assays. The diet incorporation assays were conducted over a 2-yr period in advance of the spray chamber assays.

Diet incorporation assays. Procedures for diet incorporation assays were similar at both locations and were modifications of those described by Dulmage et al. (1976). Each bioassay included a range of 5 to 6 concentrations of one of the commercial *B. thuringiensis* products incorporated into the insect diet. At Mississippi State University, wheat-germ casein diet (King et al. 1985) was flash sterilized and obtained from the USDA Gast Rearing Facility at Mississippi State while it was hot (~70°C). At the University of Arkansas, the diet was prepared in the laboratory. The *B. thuringiensis* formulations were added to the liquid diet at both locations when the temperature of the diet was cooled to 45 to 50°C. Smaller volumes were used at Mississippi State University where the diet was poured into 1.25-ml cells on plastic assay plates (NuTrend Container, Corrigan & Co. Inc., Jacksonville, FL). Each plate represented a single replicate of a given concentration and included 24 cells. A total volume of 30 ml of diet was used on each plate. At the University of Arkansas, 15 ml of diet was poured into 30-ml clear plastic cups (Solo Cup Company, Urbana, IL). A replicate of a concentration consisted of 25 cups. The diet was allowed to cool for at least 2 h at both locations before a single neonate was placed individually in cells or cups. The assay plates at Mississippi State University were sealed with a ventilated polyethylene cover (Clear Lam Package, Elk Grove Village, IL), while the plastic cups were covered with paper lids (WLMA Crop-RCV-FAC, Inc., Newark, NJ) at the University of

Arkansas. At both locations, larvae were held at a temperature of 28 to 30°C until mortality was assessed 7 d later. Each concentration was repeated for a minimum of four times. Twenty or more replicates were completed with some formulations and species. Data were analyzed at Mississippi State University using the probit analysis procedures of POLO-PC (LeOra Software 1987) and at the University of Arkansas using the Proc Probit procedures in SAS (SAS Institute 1988). All dosages (concentrations) were expressed as µg of formulated product per ml of diet.

Spray chamber assays. Procedures described by Luttrell et al. (1987) were used to develop dose-mortality regressions for each of the four species of Lepidoptera exposed to each of the three commercial formulations of *B. thuringiensis* on cotton and soybean. In addition to providing dose-mortality models from sprayed plant tissue for comparison to the diet incorporation assays, these data provided direct comparisons between insect mortality on cotton and soybean. Cotton terminals and terminal trifoliates of soybean were harvested from untreated plots at the Plant Science Research Farm, Mississippi State University. The plant stems were trimmed and placed in water in test tubes with the terminal bud and surrounding foliage remaining exposed in an upright position (i.e., plant tissue in water pics). Holding boxes designed to position the water pics in a uniform manner under a spray nozzle and to allow caging of larvae with ventilated plastic cups were used to support the water pics. Each holding box contained 10 water pics (10 cotton terminals or 10 soybean trifoliates). Two holding boxes represented a treatment replication (20 cotton terminals or 20 soybean trifoliates and 20 larvae). Each *B. thuringiensis* product was evaluated at five different use rates (doses), usually 0.1, 0.3, 1.0, 3.0, and 10.0 fold the recommended use rate on the product labels. The test was replicated four times.

The plant tissue was sprayed in an automated spray chamber (To et al. 1995) with the two holding boxes positioned approximately 8 to 10 cm to the right and left of the center-line of the spray swath (Smith et al. 1995). Nozzle height was approximately 45 cm above the top of plant terminals. A spray volume of 93.4 liters of total spray per hectare was applied through a single TX-6 nozzle. When spray deposits were dry (15 to 30 min after application), 2-day-old (late first instar) larvae from each of the four species were placed individually on a treated plant terminal or trifoliate and caged with ventilated, clear plastic cups. Holding boxes containing caged larvae and water pics were stored in a temperature controlled room at 28°C. After 2 d, the plant tissues were searched for surviving and dead larvae. Surviving larvae were transferred to fresh wheat-germ diet and held for subsequent mortality observations at 7, 14, 21, and 28 days posttreatment. Data from individual observation times were subjected to probit analysis using the POLO-PC procedure (LeOra Software 1987). Multiple linear regression models (SAS Institute 1988) were developed describing larval mortality as a function of dose and days posttreatment for each combination of insect species, *B. thuringiensis* formulation, and plant species. Dose (application rate) was expressed as kg of formulated product per hectare.

Results

Diet incorporation assay. Differences in median lethal concentrations (LC50s) were associated with variation between laboratories, and among colonies, species, and commercial formulations. Variation in LC50s between the Arkansas and Mississippi State laboratories ranged from a difference of 1.2 fold with *P. includens* exposed to Javelin WG to a 95.5 fold difference with *S. exigua* exposed to Dipel ES (Table 1).

Table 1. Dosage-mortality response of Arkansas and Mississippi colonies of *Heliothis virescens, Helicoverpa zea, Pseudoplusia includens,* and *Spodoptera exigua* exposed to commercial formulations of *Bacillus thuringiensis* in diet incorporation studies.

	Arkansas Laboratory Colonies			Mississippi Laboratory Colonies			Mississippi Field Colonies		
Commercial Formulation	n	Slope ± SEM	LC50 (95% C.I.) µg of formulated product/ml of diet	n	Slope ± SEM	LC50 (95% C.I.) µg of formulated product/ml of diet	n	Slope ± SEM	LC50 (95% C.I.) µg of formulated product/ml of diet
					H. virescens				
Condor OF	712	1.69 ± 0.12	7.5 (5.2–10.2)	1752	0.95 ± 0.07	2.9 (0.6–6.6)	2918	1.19 ± 0.06	3.2 (0.8–7.1)
Dipel ES	740	1.49 ± 0.13	10.4 (9.0–12.2)	3083	1.24 ± 0.05	3.1 (2.4–3.9)	2800	1.19 ± 0.07	2.4 (0.6–4.9)
Javelin WG	645	2.66 ± 0.20	3.5 (3.2–3.8)	2760	2.15 ± 0.14	2.9 (2.1–3.8)	3046	1.56 ± 0.08	1.3 (0.7–1.8)
					H. zea				
Condor OF	736	1.30 ± 0.16	55.3 (46.2–68.7)	847	0.48 ± 0.06	7.9 (1.9–39.3)	937	1.03 ± 0.11	***
Dipel ES	920	1.91 ± 0.17	142.8 (125.2–163.1)	842	0.75 ± 0.08	6.9 (2.6–17.2)	652	0.68 ± 0.10	***
Javelin WG	939	3.84 ± 0.36	34.0 (31.5–36.9)	1255	1.40 ± 0.14	6.3 (2.8–10.2)	1040	1.48 ± 0.16	21.9 (10.2–39.9)
					P. includens				
Condor OF	744	2.80 ± 0.28	34.6 (30.6–39.3)	1837	3.22 ± 0.43	29.0 (13.8–38.8)	—	—	—
Dipel ES	891	2.19 ± 0.19	69.5 (62.3–79.1)	1579	1.20 ± 0.08	5.8 (2.9–9.5)	—	—	—
Javelin WG	926	4.69 ± 0.33	21.3 (20.1–22.6)	1761	3.51 ± 0.32	17.3 (12.5–21.7)	—	—	—
					S. exigua				
Condor OF	970	1.07 ± 0.10	297.3 (253.2–356.5)	537	0.81 ± 0.13	10.8 (0.4–34.70)	1812	***	***
Dipel ES	740	2.32 ± 0.15	343.9 (311.5–381.4)	478	0.69 ± 0.13	3.6 (0.6–9.0)	1458	0.91 ± 0.11	37.1 (11.4–129.3)
Javelin WG	1378	1.76 ± 0.12	58.6 (53.1–65.7)	671	1.18 ± 0.10	34.1 (11.0–73.0)	1576	0.67 ± 0.08	23.7 (8.6–67.1)

* Dose expressed as µg of formulated product per ml of diet. Original equations have the form y = a + bx; where y = probit mortality, a = intercept, and b = slope, and x = log dose.

** All assays conducted by placing neonate larvae on artificial diet containing varying concentrations of the commercial *B. thuringiensis* products. Mortality data were recorded after 7 days of exposure.

*** Statistically significant regression models were not obtained.

In general, LC50s from the two laboratories varied more with Dipel ES than with Condor OF and Javelin WG. Assays with Javelin WG resulted in similar results at both research locations with all four insect species, although the laboratory colony of *H. zea* at Mississippi State was more susceptible than the *H. zea* colony at Arkansas and the field colony of *H. zea* at Mississippi State. This colony has been in laboratory culture for more than 15 yrs and has a history of being extremely susceptible to many insecticides (Luttrell et al. 1991), including *B. thuringiensis* (Mascarenhas 1994, Wan 1994). Larger differences in LC50s were observed in the Arkansas data than in the Mississippi State data. Dipel ES was comparatively more active in the Mississippi State studies than in the Arkansas studies.

Variation in the susceptibility of the different species can be estimated by comparing LC50s for a given *B. thuringiensis* formulation across the different colonies tested (Table 1). Differences in comparisons of LC50s among species varied greatly depending upon which colony of a given species was used in the comparison. However, *H. virescens* was consistently the most susceptible species, *S. exigua* was the least susceptible, and *H. zea* and *P. includens* were usually intermediate in susceptibility. LC50s for *P. includens* ranged from 3.9 to 11.9 fold higher than those for *H. virescens* in Condor OF assays, 2.3 to 28.0 fold higher than those for *H. virescens* in Dipel ES assays, and 4.9 to 17.2 fold higher than those for *H. virescens* in Javelin WG assays. A similar level of variation between colonies was observed in comparison of the other species. The range of susceptibility for *P. includens* and *H. zea* overlapped. For example, differences between LC50s for *P. includens* and *H. zea* exposed to Condor OF ranged from 3.7 fold higher for *P. includens* to 1.9 fold higher for *H. zea.* When LC50s were averaged across all dose-mortality regressions, regardless of laboratory or colony tested, LC50s for *P. includens*, *H. zea*, and *S. exigua* were 7.4, 9.5, and 24.5 higher than those for *H. virescens*, respectively.

The diet incorporation assays (Table 1) included ten separate comparisons of Condor OF, Dipel ES, and Javelin WG. LC50s averaged across all species and colonies in the Arkansas data indicated that LC50s for Condor OF (86.4 µg/ml of diet) were 2.9 fold higher than those for Javelin WG (29.35 µg/ml diet) and 4.8 fold higher than those for Dipel ES (141.7 µg/ml of diet). In similar comparisons in the Mississippi data, LC50s for Javelin (15.3 µg/ml of diet) were 3.5 fold higher than those for Dipel ES (4.3 µg/ml of diet) and those for Condor OF (10.7 µg/ml of diet) were intermediate between Dipel ES and Javelin WG. Average slopes for Condor OF, Dipel ES, and Javelin WG across all species were 2.2, 1.9, and 3.2, respectively, in the Arkansas data and 1.8, 1.4, and 1.9, respectively, in the Mississippi data. Average slopes across all data from Arkansas and Mississippi, were 1.5, 1.5, and 2.6, respectively, for Condor OF, Dipel ES, and Javelin WG.

Spray chamber assays. Dose-mortality regressions describing the mortality of *H. virescens*, *P. includens*, *H. zea*, and *S. exigua* 7 d after initial exposure to cotton and soybean plant tissue treated with Condor OF, Dipel ES, and Javelin WG are listed in Table 2. LC50s for *H. virescens* were always lower than those for the other species and were 1.5 to 2.8 fold higher on cotton than on soybean. The differences in LC50s on cotton and soybean were not statistically significant. LC50s for *P. includens*, *H. zea*, and *S. exigua* were similar for the two-crop plants. Slopes of the regression lines (Table 2) were similar across all commercial formulations, pest species, and crop plants tested. They ranged from 1.08 with *P. includens* exposed to Dipel ES on soybean to 2.06 with *H. zea* exposed to Javelin WG on soybean, but most slopes were between 1.4 and 1.8 and were statistically similar based on overlap of 95%

Table 2. Dosage-mortality response at 7 days posttreatment for *Heliothis virescens, Helicoverpa zea, Pseudoplusia includens,* and *Spodoptera exigua* larvae exposed for 48 hr to cotton and soybean treated with Javelin WG, Dipel ES, and Condor OF in spray chamber assays.

Commercial Formulation	Assays with Cotton			Assays with Soybean		
	n	Slope (SEM)	LC50 (95% C.I.) kg of formulated product/ha	n	Slope (SEM)	LC50 (95% C.I.) kg of formulated product/ha
			H. virescens			
Condor OF	466	2.01 (0.06)	1.0 (0.6 to 2.2)	388	1.65 (0.064)	0.4 (0.2 to 0.7)
Dipel ES	465	1.97 (0.07)	0.8 (0.5 to 1.3)	378	1.87 (0.070)	0.5 (0.3 to 0.8)
Javelin WG	462	2.01 (0.08)	0.3 (0.3 to 0.5)	402	1.51 (0.059)	0.2 (0.1 to 0.3)
			H. zea			
Condor OF	452	1.17 (0.06)	1.5 (0.7 to 3.0)	378	1.54 (0.06)	1.6 (0.9 to 2.8)
Dipel ES	456	1.31 (0.06)	1.6 (0.9 to 2.6)	360	1.42 (0.06)	1.1 (0.4 to 2.0)
Javelin WG	464	1.61 (0.06)	0.4 (0.3 to 0.7)	383	2.06 (0.08)	0.4 (0.2 to 0.5)
			P. includens			
Condor OF	440	1.19 (0.06)	1.6 (0.6 to 17.2)	444	1.60 (0.06)	0.9 (0.6 to 1.6)
Dipel ES	408	1.71 (0.07)	1.7 (1.0 to 4.0)	433	1.08 (0.05)	3.1 (1.4 to 13.0)
Javelin WG	433	1.68 (0.07)	0.5 (0.3 to 0.9)	446	1.72 (0.07)	0.4 (0.3 to 0.63)
			S. exigua			
Condor OF	422	1.45 (0.061)	3.1 (1.8 to 5.7)	362	1.43 (0.06)	3.22 (1.9 to 5.5)
Dipel ES	431	1.64 (0.065)	1.9 (1.3 to 2.6)	362	1.91 (0.07)	2.56 (1.9 to 3.5)
Javelin WG	431	1.65 (0.064)	1.1 (0.7 to 1.6)	392	1.46 (0.06)	0.82 (0.3 to 1.7)

* LC50 expressed as kg of formulated product/ha. Original equations have the form y = a + bx; where y = probit mortality, a = intercept, and b = slope, and x = log dose.

** All assays conducted by placing 2-day-old larvae on cotton terminals or soybean trifoliates treated with the commercial formulations of *B. thuringiensis* in a spray chamber. Regressions were based on mortality data collected 7 days post initial exposure. Surviving larvae were transferred to artificial diet after 48 h of exposure.

confidence intervals. When comparing LC50s from all commercial *B. thuringiensis* formulations and both crop plants, *H. zea* was 2.1 fold more tolerant of *B. thuringiensis*-treated plant tissue than *H. virescens*. LC50s for *P. includens* and *S. exigua* on the sprayed plant tissue were 2.6 and 4.0 fold higher than that for *H. virescens*.

The LC50s for Javelin WG were lower than Dipel ES for *P. includens* and *S. exigua* on soybean and for *H. virescens* and *P. includens* on cotton (Table 2). Condor OF had a lower LC50 than Dipel ES for *P. includens* on soybean, but similar LC50s were measured for Condor OF and Dipel ES in all other comparisons. Javelin WG had lower LC50s than Condor OF for *H. zea* and *S. exigua* on soybean and for *H. virescens*, *H. zea*, and *S. exigua* on cotton. Average slopes of the regression equations across all species and crop plants were 1.4, 1.6, and 1.7 for Condor OF, Dipel ES and Javelin WG, respectively. The average LC50s expressed as kg of formulated product per ha were 1.67, 1.66, and 0.52 for Condor OF, Dipel ES and Javelin WG across all species and crop plants, respectively.

Consistently lower LC50s were observed with Javelin WG at all posttreatment observation times than with Condor OF and Dipel ES in the spray chamber assays with plant tissue. Statistically significant, dose-mortality regressions were obtained for all four pest species exposed to commercial formulations of *B. thuringiensis* on cotton and soybean at 2 d posttreatment. However, additional mortality was observed between the 2 and 7 d posttreatment observation periods, and LC50s at 7 days posttreatment were less than those at 2 d posttreatment. Some additional mortality was observed at 14, 21, and 28 d posttreatment, but the majority of the total mortality was realized by the 7 d posttreatment observation. Differences between 2 and 7 d posttreatment mortality were greatest for the fruit-feeding species, *H. virescens* and *H. zea*. Differences in mortality between 2 and 7 d were less pronounced for *P. includens* and *S. exigua* on both crop plants.

Multiple linear regression equations were developed that predict larval mortality as a function of dose (kg of *B. thuringiensis* formulated product/ha) and time posttreatment (days) for each pest, crop plant, and *B. thuringiensis* formulation (Table 3). All regression equations had highly significant F values (range from 48.25 to 124.90), and r^2 values indicated that the equations explained 46 to 67% of the variation in larval mortality. The intercepts which were a measure of natural mortality (or unexplained mortality in the untreated controls) ranged from 6.7 to 31.9 across the different pest species and crop plants. Lower intercepts were associated with *H. virescens* and cotton.

Discussion

Collectively, the results of these assays support the need for standardizing potency estimates for *B. thuringiensis* commercial products. The variation observed in laboratory assay data illustrate the value of using uniform assay procedures and reference standard techniques described by Dulmage and co-workers two decades ago (Dulmage 1973, 1975, Dulmage et al. 1976). Results of this study also suggest that standardized assay procedures should include a reference strain or colony of the test species involved. Spray chamber assays with cotton terminals and soybean trifoliates were more expensive than diet incorporation assays in terms of labor and supplies, but results provided more direct estimates of the mortality expected in the field.

Comparing the activity of commercial formulations of *B. thuringiensis* at different

Table 3. Coefficients for multiple linear regressions describing mortality of *Heliothis virescens, Helicoverpa zea, Pseudoplusia includens,* and *Spodoptera exigua* larvae exposed for 48 hours to cotton and soybean treated in a spray table assay with Condor OF, Dipel ES, and Javelin WG as a function of dose (kg formulated product per ha) and days posttreatment.

Commercial Formulations	Intercept	Dose Coefficient	Days Coefficient	r^2	F
		H. virescens–Cotton			
Condor OF	7.434	15.720	1.514	0.65	136.1
Dipel ES	12.414	18.382	1.144	0.67	149.5
Javelin WG	6.761	45.491	1.439	0.64	128.5
		H. virescens–Soybean			
Condor OF	17.767	14.559	1.730	0.55	86.6
Dipel ES	22.226	7.497	1.512	0.63	122.8
Javelin WG	15.435	43.209	1.625	0.59	101.6
		H. zea–Cotton			
Condor OF	19.705	3.177	1.841	0.54	85.3
Dipel ES	19.672	2.301	1.875	0.58	100.9
Javelin WG	20.387	9.191	1.775	0.55	89.2
		H. zea–Soybean			
Condor OF	25.397	3.465	1.729	0.58	98.5
Dipel ES	31.978	1.975	1.677	0.46	60.6
Javelin WG	28.284	8.916	1.757	0.59	104.5
		P. includens–Cotton			
Condor OF	20.752	7.811	1.441	0.46	61.1
Dipel ES	25.279	3.225	1.646	0.54	85.5
Javelin WG	26.111	6.720	1.679	0.48	65.6
		P. includens–Soybean			
Condor OF	19.279	8.410	1.388	0.47	62.8
Dipel ES	19.234	3.299	1.413	0.40	48.3
Javelin WG	25.568	7.574	1.344	0.35	39.0
		S. exigua–Cotton			
Condor OF	16.039	3.169	1.187	0.58	99.1
Dipel ES	19.685	3.187	1.279	0.53	81.5
Javelin WG	11.094	5.568	1.693	0.55	88.9
		S. exigua–Soybean			
Condor OF	15.960	2.682	1.687	0.63	122.1
Dipel ES	16.161	2.698	1.833	0.63	124.9
Javelin WG	19.760	5.021	1.730	0.55	86.2

* Equations have the form of % mortality = $a + b_1x_1 + b_2x_2$; a = intercept, b_1 = regression coefficient for dose, x_1 = dose in kg of formulation per hectar, b_2 = regression coefficient for days, and x_2 = days posttreatment.

laboratories without reference standards and uniform samples from the same lot of the products under investigation can lead to different conclusions. The variability in our diet incorporation studies was purposefully reported to emphasize the need for standardized procedures. Beegle et al. (1986) reported a 2-fold difference in potency estimates of the same samples of *B. thuringiensis* assayed at several industrial and governmental laboratories around the U.S. Uniform procedures and reference standards were used in the comparative studies. In our studies, laboratory assay data were potentially influenced by the variability of activity within each formulation (i.e., different samples from different lots of the same commercial product) and the variability in susceptibility of different populations of the test insects to *B. thuringiensis.* Javelin WG had a more pronounced dose effect than the other two formulations. Data from Javelin WG assays were also less variable than those from the Condor OF and Dipel ES assays. In fact, the differences in LC50s between the two laboratories were less than 2 fold if the data for the highly susceptible laboratory colony of *H. zea* (Luttrell et al. 1991, Mascarenhas 1994, Wan 1994) are not included in the Javelin WG data set. Rather large differences in LC50s (10 fold and higher) were observed between the laboratories in assays with Dipel ES. This consistent trend in the data suggests that the Dipel ES samples assayed at the two laboratories had different potencies. These differences were magnified by differences in susceptibility of the test insects. Differences in susceptibility of different populations of the same insect species to *B. thuringiensis* have been reported to be as large as 15 to 20 fold (Stone and Sims 1993, Wan 1994). When the variability in susceptibility of test insects is multiplied by sample variability within *B. thuringiensis* formulation and typical experimental variation, differences in relative estimates of product activity can become quite large. Standardizing the population or strain of test insect could potentially reduce some of this variability in laboratory data.

Dosage-mortality responses from the spray chamber studies indicated that most of the larval mortality from *B. thuringiensis* occurs between 2 and 7 d posttreatment. These data corroborate the findings of Ali and Young (1993) and suggest that the number of larvae recovering from the 2-d exposure to *B. thuringiensis*-treated plant tissue is small (Dulmage et al. 1978). Differences in susceptibility of the different species on cotton and soybean were small, although LC50s were 1.1 to 1.8 fold higher on cotton than on soybean (Table 2). Smith and Hostetter (1982) observed differences this large and larger for *Trichoplusia ni* (Hübner) and *H. zea* larvae exposed to cotton and soybean leaf discs treated with *B. thuringiensis.* Interactions between plant-produced enzymes and activity of *B. thuringiensis* have been reported (Ludlum et al. 1991). Differences in LC50s on cotton and soybean were larger for *H. virescens* than for the other insect species (Table 2).

Differences among the species generally corroborate those reported (Rogoff et al. 1969, Ignoffo et al. 1977, Luttrell et al. 1982, MacIntosh et al. 1990) with *H. virescens* being the most susceptible species tested. The susceptibility of *H. zea* and *P. includens* to the commercial formulations of *B. thuringiensis* were similar and ~2 fold higher than those for *H. virescens* (Table 2). The LC50s for *S. exigua* were 4 fold higher than those for *H. virescens.* The spray chamber data supported the diet incorporation data in that Javelin WG was the most active commercial formulation across all species tested.

The multiple linear regression equations (Table 3) provide comparisons of expected mortality within the range of doses included in this study (generally 0.1 to 10 fold the recommended use rate). Ali and Young (1993) reported mortality for 2-day-

old *H. virescens* and *H. zea* larvae exposed to different rates of Javelin WG on cotton. For *H. virescens* larvae exposed for 4 and 7 days to cotton sprayed with a 0.56 kg per ha rate, they reported mortality levels of 42 and 52%. The regression model for Javelin WG and cotton in Table 3 predicts mortality of 38 and 42% for *H. virescens* larvae exposed to the 0.56 kg per ha rate for 4 and 7 days, respectively. Mortality observed by Ali and Young (1993) at the 1.12 kg per ha rate for 4 and 7 days were 56 and 57%. Those predicted from the regression equation were 63 and 67%. Similarly, observed *H. zea* mortality at different rates and periods posttreatment in the Ali and Young (1993) data were 29, 37, 39, and 41%. Predicted mortality using the *H. zea*–Javelin WG equation for the same rates and periods posttreatment were 32, 38, 38, and 43%, respectively. Although the regression models appear to describe the data of Ali and Young (1993), additional studies are needed to accurately relate the data from spray chamber assays to expected field efficacy. Studies by Ali and Young (1993) were conducted in the same facility as some of the studies reported here. Equipment, laboratory procedures, insect colonies, and *B. thuringiensis* formulations may have been common to both. More variability would be expected in studies conducted at other locations. The spray chamber assays seem to provide quantitative comparative estimates of insect mortality that may be used to refine recommendations for the use of *B. thuringiensis* on cotton and soybean. Further investigation of the relationships between laboratory assay data and field efficacy is needed. The data reported here provide a benchmark for additional studies and base-line references for resistance monitoring efforts.

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