Host Plant Influence on Activity of *Bacillus thuringiensis* Berliner Against Lepidopterous Pests of Crops[1]

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Abstract Mortality of second-instar *Helicoverpa zea* (Boddie), *Spodoptera exigua* (Hübner) and *Pseudoplusia includens* (Walker) fed *Bacillus thuringiensis* Berliner (Bt), Dipel ES®-sprayed leaves of field grown cotton, soybean and tomato were compared in laboratory bioassays. The median lethal concentration (LC_{50}) for larvae of all species fed Bt-treated leaf tissue was higher for cotton than for soybean or tomato. The LC_{50} for larvae fed Bt-treated soybean and tomato leaves did not differ significantly for any species of insects. When the mean number of days until death was plotted against percent survival at that rate, the mean number of days until death increased with an increase in percent survival. Survival time of the three insect species exposed to Bt increased most rapidly when fed cotton leaves. Results show that foliarly-applied Bt on field-grown cotton leaves is less effective against larvae of these pest species in terms of mortality and speed of kill.

Key Words *Bacillus thuringiensis*, host plant, *Pseudoplusia includens, Helicoverpa zea, Spodoptera exigua,* median lethal concentration

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Bacillus thuringiensis (Bt) is the most commercially successful and widely used biological insecticide in the world (Federici 1999, Lacey et al. 2001). Efficacy is influenced by the host plant for some lepidopterous pests of forest (Appel and Schultz 1994, Moldenke et al. 1994, Farrar et al. 1996, Beveridge and Elek 2001, Kouassi et al. 2001) and field crops (Smith and Hostetter 1982, Meade and Hare 1993, 1994, Faruki and Khan 1996, Luttrell et al. 1998). The mechanistic role of host plant-mediated effects against Bt is related to the quantitative and qualitative variation in allelochemicals in different plant species (Appel and Schultz 1994, Moldenke et al. 1994) and different varieties of some species (Meade and Hare 1993, 1994, Faruki and Khan 1996).

Several examples of host-plant-mediated differences in Bt susceptibility of lepidopterous pests have been reported with deciduous tree species. Efficacy of Bt against *Lymantria dispar* L. was reduced up to five fold on oak, *Quercus* spp., compared to aspen, *Populus tremuloides* Michaux (Appel and Schultz 1994), and showed a >10-fold variation among different host plants (Farrar et al. 1996). Bt-activity was negatively correlated with the concentration of total phenolics, gallotannins, and protein activity in oak leaves (Appel and Schultz 1994). The median lethal concentration

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(LC_{50}) for Bt to the forest tent caterpillar, *Malacosoma disstria* Hübner, was 100-fold greater on aspen than on sugar maple, *Acer saccharum* Marsh (Kouassi et al. 2001). Susceptibility of eucalyptus leaf beetle, *Chrysophtharta bimaculata* (Oliver), to Bt was 104% higher on *Eucalyptus regnans* than *E. nitens* (Beveridge and Elek 2001).

The host plant-mediated alteration of Bt efficacy in field crops is not as well understood. Among field crops, allelochemicals have been shown to significantly reduce growth and survival of lepidopterous larvae on cotton (Chan et al. 1978, Zummo et al. 1984, Hedin et al. 1991) and tomato (Duffey and Isman 1981, Isman and Duffey 1982). Meade and Hare (1994) reported that Bt efficacy against *Spodoptera exigua* (Hübner) and *Trichoplusia ni* (Hübner) was greatest on insects fed resistant lines of celery, *Apium graveolens* var. *rapaceum* (L.). Mortality caused by Bt in *Helicoverpa zea* (Boddie), *Heliothis virescens* (F.), *S. exigua* and *Pseudoplusia includens* (Walker) differed in some instances on cotton and soybean (Luttrell et al. 1998). The survival time of larvae exposed to Bt has been shown to be inversely related to the concentration of Bt (Maczuga and Mierzejewski 1995, Robertson et al. 1996). However, the effect of host plant on survival time of larvae killed by Bt has not been documented. In this study we examined the host plant-mediated variation in susceptibility and time until death of *H. zea, S. exigua* and *P. includens* to Bt on cotton, soybean and tomato.

Materials and Methods

Cotton, *Gossypium hirsutum* L. (cv. DPL 51), soybean, *Glycine max* L. Merrill (cv. DK 5668), and tomato, *Lycopersicon esculentum* L. (cv. Beefsteak) were grown in research plots at the Agricultural Experiment Station, University of Arkansas, Fayetteville, using standard agronomic practices. Neonates of *H. zea* and *P. includens* were obtained from the Insect Rearing Facility in the Entomology Department where they are maintained on artificial diet (Burton 1969). *Spodoptera exigua* eggs were obtained from the USDA Southern Insect Management Research Unit at Stoneville, MS.

Neonates of the test insects were reared to the second stadium on artificial diet in 30-ml clear plastic cups (Solo Cup Company, Urbana, IL). Aqueous suspensions of Dipel® ES (*B. thuringiensis* subsp. *kurstaki;* 17,600 IU potency per mg, Abbott Laboratories, Chemical and Agricultural Product Division, North Chicago, IL) were applied using a spray table on fully expanded terminal leaves of test plants. The spray apparatus was a CO_2-pressurized boom-type sprayer (Devries Manufacturing, Hollandale, MN) equipped with a Tee Jet 800 2E® hollow-cone nozzle placed 30 cm above the surface of leaves. The apparatus was calibrated to deliver the equivalent of 224 liters/ha. Leaves for the control were sprayed with water. The sprayed leaves were allowed to air dry for approximately 30 min. *Helicoverpa zea, P. includens* or *S. exigua* larvae that had been reared to the second stadium on artificial diet were placed individually in 30-ml plastic cups containing one leaf per cup. At least five Bt-rates were tested for each treatment and each treatment was replicated four times. Each group of 20 to 25 larvae in 30-ml cups was placed in a plastic bag containing moist paper towels to assure adequate humidity and held at 28°C. After 2 d, the leaves were searched daily for surviving larvae. Mortality was recorded through pupation. Leaves in the test were replaced every other day with untreated leaves of the same host.

Total mortality in each treatment was analyzed using probit analysis (Proc Probit, SAS Institute 1998). For each insect/host plant combination, the survival time (mean number of days from Bt-exposure until death) at each Bt dose was determined. Only the larvae that died were considered (Farrar and Ridgeway 1998). The percent survival at each Bt dose was then plotted against the mean time until death (days) of larvae that were killed at that concentration and analyzed to determine their linear relationship using Proc GLM (SAS Institute 1998). Slopes and intercepts for each host plant and insect were compared using Proc GLM Contrast analysis (SAS Institute 1998).

Results

Susceptibility of larvae. *Pseudoplusia includens* was the most susceptible of the 3 species to Bt on all host plants. The LC_{50} for *S. exigua* was greater than that of *H. zea* on cotton and soybean, but did not differ significantly on tomato. When host plants were compared, the LC_{50}s for Bt against second instars of all 3 insect species on cotton were at least 6 and 10 fold greater than those on leaves of soybean or tomato plants, respectively. The LC_{50} did not differ significantly between soybean and tomato for any species tested (Table 1).

Larval survival time. Percent larval survival and mean number of days until death for all 3 species fed Bt-treated leaves were positively correlated for all host plants. The slopes of these equations for larval response to Bt-treated cotton were significantly less than for larvae on Bt-treated soybean or tomato, for all 3 insect species. The slope for the larval response to Bt-treated soybean and tomato differed only with *P. includens*, where the slope for soybean was significantly greater (Table 2, Fig. 1).

Discussion

The LC_{50} of Bt against three lepidopteran larval pests on leaves of Bt-treated cotton was typically several fold greater than on soybean or tomato leaves. This

Table 1. The LC_{50} at pupation of second-instar lepidopteran pests exposed to Dipel ES on leaves of field plants

Insect/ host plant	No. of larvae	LC_{50}* (mg/ml)	Fiducial limits		Intercept	Slope
			Lower	Upper		
		H. zea				
Cotton	500	29.50	23.73	38.26	−2.08	1.42
Soybean	500	2.43	1.96	2.93	−0.87	2.26
Tomato	480	2.84	2.07	3.98	−0.49	1.08
		P. includens				
Cotton	500	9.25	7.32	12.32	−1.58	1.64
Soybean	500	1.00	0.66	1.38	−0.00	1.24
Tomato	480	0.33	0.00	1.02	0.60	1.24
		S. exigua				
Cotton	550	87.68	70.09	112.81	−2.36	1.21
Soybean	400	14.04	5.19	41.70	−1.34	1.17
Tomato	550	4.75	2.06	9.89	−1.08	1.49

* LC_{50}s determined using the Probit Procedure (SAS 1998). Significance was based on overlapping fiducial limits.

Table 2. Regression equations of percent larval survival and mean number of days until death on Bt-treated leaves of cotton, soybean and tomato

Host plant	Regression equation for larval survival*	Test of significance between slopes of host plants		$P > F$
	H. zea			
Cotton	18.29 + 11.24 X Mean day	Cotton	Soybean	0.0475
Soybean	−80.87 + 35.65 X Mean day	Soybean	Tomato	0.8401
Tomato	−74.29 + 38.43 X Mean day	Tomato	Cotton	0.0334
	P. includens			
Cotton	−31.80 + 26.64 X Mean day	Cotton	Soybean	0.0387
Soybean	−102.15 + 49.63 X Mean day	Soybean	Tomato	0.0065
Tomato	−369.15 + 147.68 X Mean day	Tomato	Cotton	0.0029
	S. exigua			
Cotton	25.45 + 5.11 X Mean day	Cotton	Soybean	0.0019
Soybean	−141.54 + 50.85 X Mean day	Soybean	Tomato	0.1747
Tomato	−90.16 + 31.43 X Mean day	Tomato	Cotton	0.0100

* For each insect-host combination, the mean time until death (Mean day) of larvae was calculated for each Bt-dose. Percent survival of larvae at each dose was plotted against the mean days to death to determine their linear relationship using Proc GLM (SAS Institute 1998).

indicates that Bt is less active against larvae on foliage of cotton than on the other host plants. Luttrell et al. (1998) compared these same species of lepidopterous insects on Bt-treated terminals of cotton and soybean, but found little if any difference in susceptibility between host plants. They did find some variability in activity among the different Bt-products for lepidopteran larval pests, *P. includens* and *S. exigua.* The difference in results between the two studies may be due to the different plant part used in that we used fully-developed leaves, while they used terminals. The concentration of certain secondary chemicals such as, tannins, anthocyanin, flavonoids, etc., in fully-developed leaves of cotton plants is greater than in terminal tissues (Zummo et al. 1984, Hedin et al. 1991). Many of these secondary chemicals are bacteriostatic and bactericidal (Smirnoff and Hutchison 1965), and have been shown to reduce the efficacy of Bt-endotoxin against different insects (Maksymiuk 1970, Reichelderfer 1991, Krischik et al. 1988, Appel and Schultz 1994, Navon et al. 1993). The decrease in effectiveness of Bt-treated cotton foliage against larvae that we observed may be related to a greater concentration of these secondary chemicals. Also, protease inhibitors (MacIntosh et al. 1990) may have enhanced the toxicity of Bt against the larvae; these may have been in higher concentration in cotton leaves. Further, the effect of Bt on tomato leaves may have been related to certain allelochemicals (such as chlorogenic acid and polyphenol oxidase) present there. These have been shown to retard larval growth (Elliger et al. 1980, Duffey and Felton 1991, Frelichowski and Juvik 2001) and enhance the efficacy of Bt against *H. zea* larvae (Ludlum et al. 1991).

Spodoptera exigua was less affected by Bt than were *H. zea* or *P. includens.* In previous studies on dietary bioassays on artificial diet (Luttrell et al. 1998, 1999), and

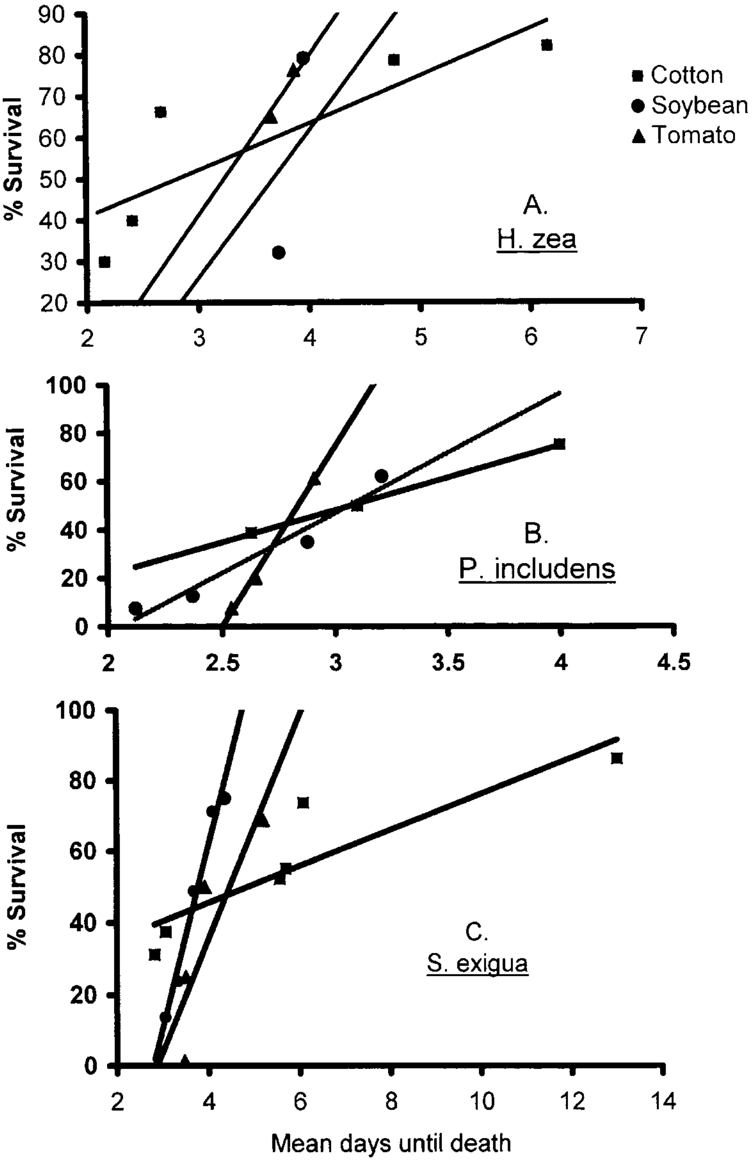

Fig. 1. Survival of *H. zea* (A), *P. includens* (B) and *S. exigua* larvae (C) in response to concentration of Bt-applied to foliage of cotton (■), soybean (●) and tomato (▲). For each insect-host combination, the mean time until death (mean day) of larvae was calculated for each concentration. Percent survival of larvae at each dose was plotted against the mean days to determine their linear relationship.

cotton and soybean (Luttrell et al. 1998), the activity of Bt for *S. exigua* was less than for *H. zea* or *P. includens.*

In all tested insect species, percent survival of larvae on Bt-treated leaves was correlated with the mean number of days until larval death. However, this relationship differed somewhat with insect species and with host plants. Larvae fed Bt-treated leaves died faster on tomato and soybean than on cotton. For all three species, the time until death of Bt-treated larvae on field-grown cotton increased more with an increase in percent survival than did that of those on tomato or soybean. This may, however, have been related to secondary chemicals in plants that alter Bt activity or rate of larval development. The phenolics of tomato (Ludlum et al. 1991, Duffey and Felton 1991) or soybean (MacIntosh et al. 1990) enhance efficacy of Bt and may increase the speed of kill. Similarly, leaves of cotton plants contain high concentration of certain phenolics, such as tannins (Chan et al. 1978, Zummo et al. 1984, Hedin et al. 1991) as well as other chemical groups (Chan et al. 1978, Zummo et al. 1984, Hedin et al. 1991) that slow insect development, and may alter Bt activity.

In conclusion, host plant effects must be considered when Bt is applied against pest species on crops. These results suggest that, especially on cotton, Bt may be less active than on some crops and that the speed of killing may differ as well.

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