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NOTE

Toxicity of *Bacillus thuringiensis* subsp. *kurstaki* to Gypsy Moth, *Lymantria dispar,* Fed with Alder or Douglas-Fir

Bacillus thuringiensis subsp. *kurstaki* is being applied increasingly to control forest and agricultural pests, either directly or as a component of transgenic plants (Höfte and Whiteley, 1989; McGaughey and Whalon, 1992). Host plant or plant allelochemicals influence efficacy of *B. thuringiensis* in several insects (Brewer and Anderson, 1990; Reichelderfer, 1991). However, host plant effects on control of gypsy moth, *Lymantria dispar* (L.), with *B. thuringiensis* have not been demonstrated.

This study examined the response of gypsy moth larvae reared on either white alder, *Alnus rhombifolia,* or Douglas-fir, *Pseudotsuga menziesii,* to treatment with *B. thuringiensis* subsp. *kurstaki.* These potential hosts for gypsy moth in the Pacific Northwest (Miller and Hanson, 1989) differ in their allelochemistry, nutrients, and capacity to induce detoxifying enzymes in gypsy moth larvae (Joseph *et al.,* 1991; Moldenke *et al.,* 1992).

Egg masses were collected in January, 1990, from oak woodlands in Seneca State Park, Maryland. The egg masses were stored at 10°C for 2–3 months. As needed, eggs were pooled from several masses, warmed to 24°C, washed for 10 min in 15% formalin to kill virus, and rinsed twice in distilled water (5 min/rinse, with stirring). After hatching, larvae were reared on field-collected foliage of Douglas-fir or white alder, which was replaced at least every other day. Rearing was at 24°C under a 16:8 hr L:D photoperiod.

The *B. thuringiensis* used was a preparation of Javelin (5% a.i. technical concentrate; 26.1 BSU) provided by Sandoz Crop Protection Corp. (Wasco, CA). Javelin is derived from the NRD-12 *kurstaki* strain (serovar H:3a,3b), which is effective against certain lepidopterans that are relatively unsusceptible to HD-1 and other *kurstaki* strains (DuBois, 1985). Like products derived from HD-1, the P1 crystals (δ-endotoxins) produced by the NRD-12 strain characteristically contain CryIA(a), CryIA(b), and CryIA(c) protoxins (Moar *et al.,* 1990); Javelin also contains cryII proteins.

The Javelin was mixed into a slurry and diluted in distilled water to a stock concentration of approximately 7 mg (dry weight)/ml. The stock was diluted as necessary to obtain the final concentrations and incorporated into a diet of ground Douglas-fir or alder foliage bound with agar. This type of diet, rather than fresh foliage, was used because it more closely approximates standard assay techniques (Beegle, 1990) and allows for more uniform administration of dose than would the use of fresh foliage. Douglas-fir needles were ground fresh in liquid nitrogen in order to preserve terpenes and passed through a 10-mesh screen; alder foliage, which does not contain detectable terpenes (Moldenke *et al.,* 1992), was air-dried and ground to pass through a 20-mesh screen. Foliage and *B. thuringiensis* were added after the agar had been autoclaved and cooled to 50°C.

Two days after molting, third instars were placed on treated diet corresponding to the foliage on which they had been feeding; control larvae were given diet with no *B. thuringiensis.* The experiment was repeated three times with alder-reared larvae (30 larvae/dose), and the data were pooled (total n/dose = 90); only 30 larvae reared on Douglas-fir were available per treatment. After 48 hr, larvae were placed on fresh foliage of the original host and observed daily until death or pupation. Monoterpene, phenolic, and nitrogen contents of foliage used to rear larvae were determined from weekly foliage samples (Joseph *et al.,* 1991).

Significance of differences in mortality was determined by χ^2. Significance of effects on time to pupation and pupal, adult, and ovary weights was evaluated by t test (Statgraphics, STSC, Inc., Rockville, MD, 1987).

Mortality was independent of dose in both host plant treatments (Table 1). Lack of dose response, which has been reported by other workers (van Frankenhuyzen *et al.,* 1991) may have resulted from reduced ingestion caused by either unpalatability of the treated diet (Gould *et al.,* 1991) or by recovery of larvae at higher doses after replacement on fresh foliage.

At each time interval up to 96 hr, mortality was significantly higher in larvae fed treated Douglas-fir diet than in those fed treated alder diet (Table 1). Up to one-third of total mortality from treatment to pupation in treated larvae on both diets occurred after the initial 96-hr observation period. Latent mortality after treatment with *B. thuringiensis* has been reported previously in gypsy moth (Ahmad *et al.,* 1978) and in *Trichoplusia ni* (Gharib and Wyman, 1991).

Developmental time from treatment to pupation of treated alder-fed larvae increased significantly ($P \leq 0.001$) relative to untreated larvae, but was unaffected

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TABLE 1

Percentage of Mortality of Third-Instar Gypsy Moth Fed White Alder or Douglas-fir and Treated with *Bacillus thuringiensis*, subsp. *kurstaki*[a]

Dose (ppm)	48 hr		72 hr		96 hr		Total	
	Alder	Douglas-fir	Alder	Douglas-fir	Alder	Douglas-fir	Alder	Douglas-fir
0	0	0	0	0	0	0	0	0
25	2.2	—	21.1	—	30.0	—	38.9	—
50	6.7	10.0	27.8	63.3	40.0	83.3	48.9	83.3
100	4.4	20.0	36.7	46.6	51.1	56.6	58.8	76.6
200	2.2	20.0	27.8	53.3	36.7	60.0	51.1	83.3
300	3.3	30.0	30.0	60.0	35.6	70.0	51.1	93.3
350	6.7	23.3	31.1	63.3	36.7	73.3	47.8	76.6

[a] Diets differed significantly for each post-treatment interval (χ^2, $P \leq 0.05$).

in larvae fed Douglas-fir (Table 2). With the exception of male pupae from the Douglas-fir diet, treatment with *B. thuringiensis* did not affect ovarial, pupal, or adult weight. Fecundity of treated surviving females should therefore be about the same as that of untreated insects (Miller *et al.*, 1991a,b).

Foliage of the two food plants differed during the feeding period in levels of nitrogen (mean % dry weight: alder, 2.0%; Douglas-fir, 1.3%), phenolics (alder, 1146 mg/g; Douglas-fir, 368 mg/g), and terpenes (absent in alder; 3973 μg/g in Douglas-fir). Such differences are consistent with earlier studies (Moldenke *et al.*, 1992; Berry *et al.*, 1993). In those studies, however, alder-fed larvae were more, rather than less, susceptible to the chemical insecticides used.

The higher susceptibility of larvae reared on Douglas-fir could be due to any of several mechanisms that affect the mode of action of δ-endotoxins. Terpenes, which are common in Douglas-fir, are well-known inducers of detoxifying enzymes; in this study, however, they provided no protection against *B. thuringiensis* and perhaps potentiated it. Terpenes could induce activating proteases, change the conformation of binding sites, or disrupt membrane structure (Andrews *et al.*, 1980). The higher nitrogen available to alder-fed larvae may have increased their vigor and, thereby, their tolerance to *B. thuringiensis* (Rossiter *et al.*, 1990). Binding with dietary tannins, especially the types common in alder, could inactivate the proteinaceous *B. thuringiensis* toxins (Luthy *et al.*, 1985; Keating *et al.*, 1989); however, since such complexes should dissociate readily in the alkaline larval midgut (Schultz and Lechowicz, 1986; Hagerman and Robbins, 1987), this mechanism seems unlikely to have enhanced tolerance in alder-fed larvae.

This study indicates that host plant may influence toxicity of *B. thuringiensis* to gypsy moth. Applications of *B. thuringiensis* might differ in efficacy because of host-plant-induced conditions in the insect digestive tract. Diet-dependent differences in phenolics or midgut pH, for example, might differentially induce or inhibit the toxin-activating proteases. Alternatively, terpenes, tannins, or other allelochemicals might inhibit microbial growth (Reichelderfer, 1991). The effects of

TABLE 2

Sublethal Effects of Treatment with *Bacillus thuringiensis* subsp. *kurstaki* in Gypsy Moth Reared on White Alder or Douglas-fir[a,b]

Effect	Alder		Douglas-fir	
	Control	Treated	Control	Treated
		Females		
Days to pupation	25.3 (0.3)	28.0 (0.2)***	29.0 (1.0)	28.7 (1.0)
Pupal weight (g)[c]	2.11 (0.05)	2.09 (0.03)	1.42 (0.06)	1.52 (0.11)
Adult weight (g)[c]	1.23 (0.03)	1.22 (0.02)	0.80 (0.04)	0.83 (0.07)
Ovary weight (g)[c]	0.84 (0.02)	0.84 (0.01)	0.57 (0.03)	0.63 (0.05)
		Males		
Days to pupation	21.0 (0.2)	22.3 (0.2)***	23.0 (0.6)	23.3 (0.5)
Pupal weight (g)[c]	0.54 (0.01)	0.54 (0.01)	0.47 (0.01)	0.43 (0.01)*
Adult weight (g)[c]	0.10 (0.00)	0.10 (0.00)		

[a] *B. thuringiensis* was incorporated into artificial diet consisting of ground foliage of either alder or Douglas-fir bound with agar. Larvae were treated 48 hr after molting into the third instar and were kept on the treatment diet for 48 hr.

[b] Expressed as mean (SE). Significance of differences between treated and control insects was determined by *t* test: (*) $P \leq 0.05$; (***) $P \leq 0.001$. Dada from larvae from all dosages were pooled in the "Treated" category.

[c] Wet weight.

different host plants, even at the cultivar level (Meade and Hare, 1993), are important considerations in the use of *B. thuringiensis* to control this pest. Studies combining host-plant switches at the time of treatment, while beyond the scope of this study, would be useful in expanding our knowledge of host-plant influences on toxicity of *B. thuringiensis*.

KEY WORDS: *Bacillus thuringiensis; Lymantria dispar; Alnus rhombifolia; Pseudotsuga menziesii;* Gypsy moth; Alder; Douglas-fir; Allelochemicals; Insect/host-plant interactions.

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