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EFFECTS OF ASPEN PHENOLIC GLYCOSIDES ON GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) SUSCEPTIBILITY TO *BACILLUS THURINGIENSIS*

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ABSTRACT

Performance of the gypsy moth, *Lymantria dispar*, on quaking aspen, *Populus tremuloides*, is strongly affected by foliar concentrations of phenolic glycosides. Because the microbial insecticide *Bacillus thuringiensis* is widely used against gypsy moths and has a mode of action similar to that of phenolic glycosides, we investigated the combined effects of the two toxins on gypsy moth larvae. The experimental design was a 2 x 2 factorial: two levels (0, +) of phenolic glycosides for each of two levels (0, +) of *B. thuringiensis*. The toxins were incorporated into artificial diets and bioassayed against first and fourth instars. *Bacillus thuringiensis* and phenolic glycosides negatively and additively affected larval survival, growth and development times. Both agents slightly reduced consumption rates. In addition, *B. thuringiensis* reduced diet digestibility whereas phenolic glycosides decreased the efficiency with which food was converted to biomass. These results suggest that the efficacy of *B. thuringiensis* applications in aspen forests is likely to be affected by the allelochemical composition of foliage.

The western leading edge of the range of the gypsy moth, *Lymantria dispar* L., is now passing through the Great Lakes states. In Michigan, defoliation increased nearly 10-fold from 1988 to 1991, with over 600,000 acres affected in 1991 (F. Sapio, Michigan Dept. Natural Resources, pers. comm.). Widespread defoliation has not yet occurred in Wisconsin, but gypsy moth populations are now established in the eastern part of the state and control programs are underway.

Establishment of the gypsy moth in the Great Lakes region is of concern for many reasons, notable among them being its impact on the aspen resource. Quaking aspen (*Populus tremuloides*) and bigtooth aspen (*P. grandidentata*) are preferred food plants of the gypsy moth (Lechowicz and Maufette 1986). Unlike northeastern forests, aspens are a dominant component of forests in the Great Lakes area; Wisconsin alone has over 3 million acres of the aspen forest type (Spencer et al. 1990). Moreover, aspen is harvested extensively for production of a variety of pulpwood and plywood commodities (Maass et al. 1990). Extensive defoliation of aspen forests is also of concern because of potential impact on outdoor recreational activities in this area.

Although considered a preferred host, quaking aspen is not uniformly susceptible to defoliation by the gypsy moth. Clonal differences in foliar defensive chemistry are great, and have been implicated as one cause of this

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variation (Lindroth and Hemming 1990, Hemming and Lindroth, unpubl. data). The defensive chemistry of aspen consists almost entirely of phenolic compounds, particularly phenolic glycosides and condensed tannins (Palo 1984, Bryant et al. 1987, Lindroth et al. 1987a). Phenolic glycosides exhibit biological activity toward a variety of Lepidoptera (Bryant et al. 1987, Lindroth et al. 1988, Lindroth and Peterson 1988) and are toxic to gypsy moth larvae at moderate to high doses (> 2% dietary concentration; Lindroth and Hemming 1990, Lindroth and Weisbrod 1991, Hemming and Lindroth, unpubl. data). The putative mode of action of phenolic glycosides involves formation of degenerative lesions in the midgut (Lindroth et al. 1988, Lindroth and Peterson 1988).

Bacillus thuringiensis subspecies *kurstaki* (strain HD-1) is widely used in commercial formulations for control of the gypsy moth. The mode of action of *B. thuringiensis* also involves formation of gut lesions. Endotoxins produced by *B. thuringiensis* are activated in insect guts by proteases, whereupon they bind to brush-border membrane vesicles. Resulting lesions cause lysis of the gut epithelium and death due to septicemia and starvation (Li et al. 1991).

The purpose of this study was to evaluate the combined effects of aspen phenolic glycosides and *B. thuringiensis* on gypsy moth performance. Because the two toxins have similar modes of action, we predicted they would exert additive or synergistic effects when administered together. If such results are found, then differential effects of *B. thuringiensis* are likely to occur for gypsy moths feeding on aspen clones of widely varying defensive chemistry.

MATERIALS AND METHODS

Our feeding experiments were designed as 2 x 2 factorials, with two levels (0, +) of phenolic glycosides for each of two levels (0, +) of *B. thuringiensis*. Gypsy moth larvae used in the experiments were obtained from egg masses provided by the Otis Methods Development Center, Otis Air National Guard Base, MA.

Artificial diets. The four artificial diets used in this study were adapted from the standard high wheat germ formulation of ODell et al. (1985). Methyl paraben, a diet preservative, was not used so diets were autoclaved to inhibit growth of mold. Vitamins, *B. thuringiensis* and phenolic glycosides were incorporated after diets had cooled to 45–50° C. The *B. thuringiensis* product used was Foray 48B (Novo Nordisk Bioindustrials, Danbury, CT). Phenolic glycosides were semi-purified from quaking aspen foliage by the extraction and fractionation procedure of Lindroth et al. (1986). Analysis by HPLC indicated that 90% of the crude preparation consisted of the phenolic glycosides salicortin and tremulacin.

Because we were interested in potential interactive effects between *B. thuringiensis* and phenolic glycosides, each toxin was incorporated at a level less than that causing substantial mortality. Based on preliminary experiments, we selected 100 and 500 IU/ml diet as appropriate concentrations for first and fourth instar gypsy moths, respectively. Phenolic glycosides were incorporated at 2% diet wet weight.

Bioassays. We conducted two types of bioassays, first instar survival trials and fourth instar feeding trials, to assess effects of *B. thuringiensis* and phenolic glycosides on gypsy moth performance. For each bioassay, larvae from individual egg masses were distributed across treatments to equalize potential maternal or genetic effects (Rossiter et al. 1990). Larvae were maintained at 25° C, with a 15:9 light-dark cycle.

For survival trials, each of ten replicates per treatment consisted of 15

newly hatched larvae placed into 28 ml plastic rearing cups containing a cube of experimental diet. Insects were provided with diets containing *B. thuringiensis* for only 48 hours, in order to mimic the short-lived viability of the pathogen under field conditions. Afterwards these larvae were switched to corresponding diets without *B. thuringiensis* (i.e., the control and phenolic glycoside diets). All food was replaced at 2–3 day intervals to maintain freshness. We checked rearing containers three times a day for dead larvae or new second instars. We calculated survival rates as the proportion of larvae that survived the first larval stadium, and development times as the time elapsed from onset of a trial to the time at which half of the living larvae molted into the second stadium.

We conducted fourth instar feeding trials in order to measure standard nutritional indices of larval performance. Larvae were reared on control diet through the third stadium. As newly molted fourth instars, larvae were assigned to one of the four experimental diets (10 replicates per treatment). Each replicate consisted of a single larva in a 28 ml plastic cup containing an experimental diet. As for the survival trials, diets containing *B. thuringiensis* were provided for only 48 hours. All diets were replaced at 1–2 day intervals as required. At the end of each trial, we froze the larva, then dried (50° C) and weighed the larva, frass and uneaten food. Dry weights of larvae at the onset of the trials were estimated based on proportional dry weights of a sample of 10 newly molted fourth instars. We calculated nutritional indices on the basis of dry weights, using standard formulas (Waldbauer 1968, Scriber 1977) for relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI).

We analyzed results from the bioassays by two-way analysis of variance (ANOVA), using SAS statistical software. Nutritional indices varied little between male and female larvae, so data from both sexes were pooled prior to analysis.

RESULTS AND DISCUSSION

First instar performance was reduced by both *B. thuringiensis* and phenolic glycosides (Fig. 1). Survival rates decreased 11% in the presence of *B. thuringiensis*, 24% in the presence of phenolic glycosides, and 34% in the presence of both, indicating a simple additive effect. First instar development times were prolonged 40% by *B. thuringiensis* and 27% by phenolic glycosides. The combined effect of both toxins on development time was somewhat less than additive, as indicated by the nearly significant *B. thuringiensis* x phenolic glycoside interaction term.

Fourth instar development times and growth rates were also significantly affected by the dietary toxins (Table 1). In both cases the effects of *B. thuringiensis* and phenolic glycosides were additive. For example, relative growth rates of larvae were reduced 33, 24 and 52% on the *B. thuringiensis*, phenolic glycoside and *B. thuringiensis* plus phenolic glycoside diets, respectively.

Effects of the experimental diets on larval growth can be attributed to changes in consumption rates and digestion and conversion efficiencies (Table 1). That both toxins independently reduced food consumption by larvae is indicated by the fact that the main effects of *B. thuringiensis* and phenolic glycosides were significant, whereas their interaction was not. *Bacillus thuringiensis* decreased the ability of larvae to digest diets (ADs) but phenolic glycosides did not; moreover, the negative effect of *B. thuringiensis* on AD values was ameliorated in the presence of phenolic glycosides. In contrast, phenolic

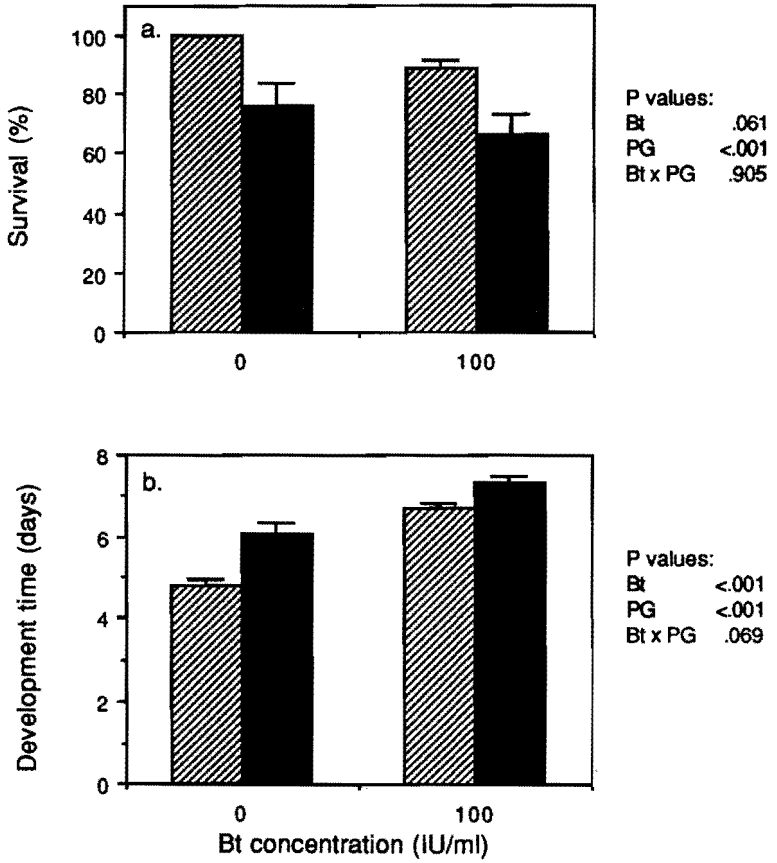

Figure 1. Effects of *B. thuringiensis* and phenolic glycosides on survival (a) and development time (b) of first instar gypsy moth larvae. Error bars indicate ± 1 SE. Hatched and shaded bars indicate diets lacking and containing phenolic glycosides, respectively.

glycosides reduced the efficiency with which larvae converted digested food into biomass (ECD values) but *B. thuringiensis* did not. Finally, because ECI is the mathematical product of AD and ECD, it follows that this parameter was significantly reduced by both *B. thuringiensis* and phenolic glycosides, and that the reduction due to *B. thuringiensis* was less pronounced in the presence of phenolic glycosides.

Results from this study illustrate that both aspen phenolic glycosides and dietary *B. thuringiensis* negatively affect the performance of gypsy moth larvae. The effects of these toxins are, however, primarily additive rather than synergistic in nature. This additive effect is consistent with the mode of action of both toxins, which appears to involve the formation of degenerative lesions in larval gut epithelia. The modes of action of the compounds are not

Table 1.—Effects of *B. thuringiensis* and phenolic glycosides on nutritional indices of fourth instar gypsy moths (X ± 1 SE).

Diet	Development time (days)	RGR ($mg \cdot mg \cdot day^{-1}$)	RCR ($mg \cdot mg \cdot day^{-1}$)	AD (%)	ECD (%)	ECI (%)
Control	5.4±0.1	0.21±.01	0.56±0.2	52.7±0.1	71.9±3.3	37.6±1.2
B. thuringiensis	7.2±0.4	0.14±.01	0.52±.02	39.9±2.2	70.5±4.9	27.8±2.1
Phenolic glycoside	7.1±0.6	0.16±.01	0.54±.02	47.4±0.8	59.7±2.2	28.3±1.0
B. thuringiensis ± phenolic glycoside	10.0±0.5	0.10±.01	0.42±.03	44.4±3.0	55.9±5.5	23.8±1.2
P values:						
B. thuringiensis	<.001	<.001	.002	<.001	.520	<.001
Phenolic glycoside	<.001	<.001	.022	.868	.003	<.001
Interaction	.200	.747	.120	.029	.763	.051

entirely similar, however, because *B. thuringiensis* suppressed ADs whereas phenolic glycosides reduced ECDs.

Few studies have investigated the effects of specific plant allelochemicals on the efficacy of *B. thuringiensis* as an insecticidal agent, and results have differed for various compounds. Trumble et al. (1991) found that psoralens and *B. thuringiensis* had an additive effect in prolonging development time in *Spodoptera exigua*. Krischik et al. (1988) showed that the alkaloid nicotine inhibited the toxicity of *B. thuringiensis* against *Manduca sexta*, whereas the flavonoid rutin had no effect. Felton and Dahlman (1984) found that effects of *B. thuringiensis* on *M. sexta* were enhanced by the nonprotein amino acid L-canavanine. The authors suggest that these results may be due to the combined effects of both *B. thuringiensis* and L-canavanine on gut permeability and function, biological activity similar to that which we have proposed for the action of *B. thuringiensis* and phenolic glycosides on the gypsy moth.

Gypsy moth performance on aspen is strongly and inversely correlated with phenolic glycoside concentrations (Hemming and Lindroth, unpubl. data) and these concentrations vary by over an order of magnitude among aspen clones (Lindroth et al. 1987b, Hemming and Lindroth, unpubl. data). Given that phenolic glycosides and *B. thuringiensis* have additive effects on gypsy moths, it follows that the efficacy of *B. thuringiensis* applications in aspen forest types may be influenced by the chemical composition of foliage. Future research will address that possibility.

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