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Carbaryl Susceptibility and Detoxication Enzymes in Gypsy Moth (Lepidoptera: Lymantriidae): Influence of Host Plant

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ABSTRACT Toxicity of carbaryl and levels of detoxifying enzymes were determined in larvae of gypsy moth, *Lymantria dispar* (L.), reared from hatching on either white alder, *Alnus rhombifolia* Nutt., or Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. Foliar chemistry was also examined. We treated larvae topically with carbaryl 2 d into the third or the fifth instar and established LD_{50}s based on survival 48 h after treatment. We fed surviving third instars their original diet until death or pupation and assessed sublethal effects. Levels of detoxifying enzymes were determined in larvae of the same age and rearing group. Larvae reared on Douglas-fir were significantly more tolerant of carbaryl. Sublethal effects were found only in larvae fed Douglas-fir, in which mortality after 48 h and time to pupation were significantly greater in treated larvae than in controls. Levels of detoxication enzymes were generally higher in tissues of larvae raised on Douglas-fir, but significantly higher in only a few instances. Nitrogen and total phenolic contents were higher in alder than in Douglas-fir. Terpenes were abundant in Douglas-fir foliage but were not detected in alder.

KEY WORDS allelochemicals, carbaryl, detoxication enzymes

IN INSECT HERBIVORES, the same enzyme systems detoxify plant allelochemicals and insecticides. The best studied of these systems are the polysubstrate monooxygenases (PSMOs), the glutathione transferases, and the esterases (Ahmad et al. 1986, Brattsten 1988a). Detoxifying enzymes are readily inducible by both xenobiotics (e.g., insecticides) and plant allelochemicals, and susceptibility to insecticides often is altered by the host plant of treated insects (Terriere 1984, Brattsten 1988b).

The gypsy moth, *Lymantria dispar* (L.), is highly polyphagous, feeding on >400 plant species. In the Pacific Northwest, gypsy moth can develop successfully on nearly 150 coniferous and deciduous species, including Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, and alder, *Alnus* spp. (Joseph 1989; Miller & Hanson 1989a,b; Miller et al. 1991a,b; Joseph et al. 1991). As broadleaf and coniferous species, respectively, alder and Douglas-fir differ in their allelochemicals and antiherbivory defenses. Although little has been reported on the allelochemistry of alder, the leaves of woody deciduous plants are noted for the presence of a variety of phenolics. We have found no report of monoterpenes in alder foliage in the literature. In contrast, Douglas-fir foliage characteristically produces both complex and simple phenolics and a wide and variable array of monoterpenes (von Rudloff 1973, 1980; Radwan 1975; Perry & Pitman 1983; Redak & Cates 1984).

Many monoterpenes, including several that are dominant in Douglas-fir, induce detoxication enzymes; some monoterpenes are metabolized by insect cytochrome P-450-dependent monooxygenases (Brattsten 1986, Yu 1986, Harwood et al. 1990). Phenolics induce and inhibit detoxication enzymes in insects (Yu 1983, 1984; Cohen et al. 1989; Lindroth 1989a,b), although the influence of phenolics is less well studied.

Here we describe differences in toxicity of carbaryl, a pesticide commonly used to control gypsy moth, to gypsy moth larvae reared on either white alder, *Alnus rhombifolia* Nutt., or Douglas-fir. We relate toxicity to levels of cytochrome P-450, aldrin epoxidase, and glutathione *S*-transferase and to foliar chemistry. This study complements previous studies of insecticide toxicity and detoxication enzymes in gypsy moth (Ahmad & Forgash 1973, 1976, 1978; Forgash & Ahmad 1974; Ahmad 1982, 1983, 1986; Kapin & Ahmad 1980; Sheppard & Friedman 1989; Lindroth et al. 1990). To our knowledge, directly measured levels of microsomal cytochrome P-450 and aldrin epoxidase have not been reported for this insect.

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Materials and Methods

Collection and Rearing of Insects. Eggs were collected from an oak woodland in Maryland in January 1988 and held at 5°C until needed. Experiments were timed to coincide with the phenology of alder and Douglas-fir in the field. Larvae were reared in 148-ml cups (1–10 larvae per cup, depending on size) at 24°C, 50% RH, and a photoperiod of 16:8 (L:D). Larvae were fed ad libitum with fresh field-collected foliage of either alder (fully expanded leaves) or Douglas-fir (1-yr-old needles). All foliage was surface-sterilized in 0.25% sodium hypochlorite, rinsed with distilled water, drip-dried to remove excess surface moisture, and covered with damp paper towels. Foliage was either used immediately or stored sealed in plastic bags at 10°C for up to 3 d; foliage showed no sign of deterioration under these conditions. Before foliage was fed to larvae, stems generally were placed in small test tubes of water plugged with cotton; late instars consumed alder so rapidly that this procedure was unnecessary. Food was changed at least every other day.

Carbaryl Bioassay. We treated third and fifth instars 2 d after the molt. Each larva was weighed and treated topically on the thoracic region with carbaryl (technical grade; 99% AI) dissolved in acetone; controls were treated only with acetone. Third instars reared on alder were treated with five doses (range, 2–10 μg of carbaryl/g body weight); fifth instars were treated with seven doses (2–30 μg/g body weight). Third instars reared on Douglas-fir were treated with six doses (24–44 μg/g body weight); fifth instars were treated with doses of 24–600 μg/g body weight). Using an microapplicator (Shardlow Micrometers, Ltd., Sheffield, England), we applied 1 μl solution/10 mg body weight to third instars and 1 μl/100 mg body weight to fifth instars. Each dose was replicated three times for a total of 38 larvae fed Douglas-fir and 45 larvae fed alder. All doses were corrected for the actual amount of carbaryl in the technical grade.

Treated larvae were kept in individual 29.5-ml cups. Mortality was recorded 24 and 48 h after treatment. Because mortality after 24 h was <5% in larvae reared on Douglas-fir, we estimated LD_{50}s on mortality after 48 h. Larvae surviving treatment in the third instar were transferred to individual 148-ml cups, fed fresh foliage of the type they had received before treatment, and observed until adult eclosion to detect subacute effects of treatment.

Enzyme Preparation and Assay. We dissected larvae from each host plant at the same developmental stage as that of larvae treated with carbaryl (n = 25–30 third instars, sexes combined; 8–10 per sex, fifth instars). Dissected tissues were rinsed in 1.15% KCL containing 400 μ*M* phenylmethylsulfonyl fluoride (PMSF) to inhibit protease activity. Midguts were removed, stripped of gut contents, rinsed, and pooled in ice-cold homogenization buffer (0.1 *M* potassium phosphate [pH 7.5, 25°C], 10% glycerol, 100 μ*M* dithiothreitol, 1.0 m*M* ethylenediamine tetraacetic acid, 400 μ*M* PMSF) until all dissections were complete. Body wall, fat body, and associated tissues (designated "carcass") were similarly rinsed and pooled. Microsomes were prepared as described previously by Vincent et al. (1983) and Moldenke et al. (1984), except that preliminary centrifugation at 1,000 × *g* was omitted. Supernatant was stored frozen (−5°C) for later use to determine glutathione S-transferase activity. Cytochrome P-450 content and aldrin epoxidase activity were determined with fresh microsomes. Protein content was determined by the Bradford (1976) assay (BioRad; Richmond, Calif.). Globulins (Sigma Chemical Co., St. Louis, Mo.) were used as the standard proteins. Cytochrome P-450 was determined at 20°C with an Aminco DW-2 spectrophotometer (Baxter Scientific, McGaw Park, IL) as described by Omura & Sato (1964).

Aldrin epoxidase activity of the microsomes was assayed in triplicate at 30°C by the procedures of Moldenke & Terriere (1981) and Feyereisen (1983). Dieldrin production was determined on a Hewlett-Packard model 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with an electron capture detector and a HP-1 10-m capillary column. Temperatures of the injector and detector were 250°C and 300°C, respectively. Oven temperature was programmed from 180 to 230°C at 15°/min, with a 0.5-min. pause at 230°C.

Glutathione s-transferase of the supernatants was assayed with 1,2-dichloro-4-nitrobenzene as substrate. Rate of change of absorbance at 344 nm was determined at 30°C in 0.1 *M* potassium phosphate buffer (pH 7.7), 5 m*M* glutathione, 1 m*M* substrate; reactions were started with the addition of enzyme. An extinction coefficient of 10 mM^{-1} cm^{-1} was used to determine concentration of product. Assays were done in duplicate.

Foliar Chemistry. Each week, subsamples of the fresh field-collected foliage of alder and Douglas-fir fed to the larvae were combined. Foliar terpene, phenolic, and nitrogen content were determined in representative samples as described by Joseph et al. (1991). Brief descriptions of the procedures follow.

Terpene Analysis. Needles that had flushed in the previous year were detached from 5 to 12 branch tips of Douglas-fir, pooled, and frozen in doubled air-tight plastic bags until they were analyzed. Before analysis, samples were warmed to room temperature inside the bags. A subsample was withdrawn, frozen with liquid N_2, and ground with a mortar and pestle. Ground tissue

was transferred to a capped scintillation vial. Moisture content was determined on triplicate samples, which were dried at 105°C overnight.

Terpenes were extracted from 0.5-g subsamples in 1 ml MeOH:H_2O (2:1) and 2 ml of pentane containing fenchone (0.1 mg/ml) as an internal standard (Brooks et al. 1987). Samples were shaken mechanically for 60 min, then centrifuged at room temperature in a IEC HN-SII centrifuge (International Equipment Co., Needham Heights, MA) at ≈200 × *g* for 3–4 min. The pentane, containing the terpenes, was removed and stored at −16°C.

To test for the presence of monoterpenes, we steam-distilled fresh alder leaves for 4 h. In addition, a fresh sample of fully expanded leaves was collected in June and analyzed as described for Douglas-fir.

Terpenes were analyzed on a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector and a SupelcoWax 10 fused silica capillary column (30 m × 0.32 mm i.d., 1:50 split (Supelco Inc., Bellefonte, PA). Injector and detector were at 250°C. Oven temperature was programmed from 60 to 220°C at 5°/min, with a 15-min pause at 220°C. Peak areas were obtained with a Hewlett-Packard 3390A integrator (Hewlett-Packard Co., Palo Alto, CA). Compounds were identified by a combination of gas chromatography–mass spectrometry and peak enrichment with standards. The gas chromatograph–mass spectrometer was a Finnigan 4023 (Finnigan MAT, San Jose, CA) with a 4,500 source set at 140°C, 70 ev, and equipped with the same capillary column described above. Separate response factors for hydrocarbons and monooxygenated and dioxygenated compounds were determined with standards relative to fenchone.

Phenol Analysis. Subsamples of Douglas-fir needles that had flushed the previous year were fresh-frozen. Alder leaves were air-dried after collection and stored in plastic bags at room temperature. Before analysis was begun, alder and Douglas-fir foliage were oven-dried for 24 h at 60°C, ground to pass through a 40-mesh screen, and redried overnight. Phenols were extracted from a 100-mg subsample in 8 ml (Douglas-fir) or 10 ml (alder) MeOH:H_2O (7:3) on a shaker for 1 h. The mixture was centrifuged at ≈200 × *g* in an IEC HN-SII centrifuge at room temperature for 5 min and analyzed as described by Julkunin-Tiitto (1985). An aliquot of extract (50 μl Douglas-fir, 40 μl alder) was brought to 2 ml with distilled deionized water. Folin-Ciocalteu phenol reagent (1 ml) was added and mixed, followed by 5 ml of 20% Na_2CO_3 and 2 ml of distilled water. After 20 min, percentage transmittance was measured at 700 nm with a Bausch & Lomb Spectronic 21 (Bausch & Lomb Inc., Rochester, NY) set to zero with distilled H_2O. A standard curve was prepared with catechin containing an MeOH concentration equivalent to the samples.

Table 1. Toxicity of carbaryl 48 h after treatment to gypsy moth larvae fed alder or Douglas-fir foliage

Host and Instar	*n*	Slope ± SE	LD_{50} (95% CI), μg/g	χ^2
Alder				
Third	45	1.5 ± 0.4	6.9 (4.8– 8.9)	5.07
Fifth	45	0.9 ± 0.2	8.7 (5.8–13.2)	1.29
Douglas-fir				
Third	38	5.8 ± 1.0	34.8 (32.4–37.5)	3.99
Fifth	—	>600	—	—

Nitrogen Determination. Foliage samples were washed in dilute soap solution, rinsed three times in distilled H_2O, oven-dried at 60°C for 48 h, and ground in a Wiley mill to pass a 20-mesh screen. Nitrogen content of foliage was determined by a microKjeldahl technique on an automated Technicon Autoanalyzer II (Anonymous 1975).

Statistical Analyses. Mortality data were subjected to probit analysis (POLO-PC) (LeOra Software 1987). Significance of differences in developmental parameters and enzyme levels was tested by two-sample *t* tests (STATGRAPHICS) (Statistical Graphics 1987).

Results

Toxicity of Carbaryl. The LD_{50} for third instars was nearly five times higher for larvae reared on Douglas-fir (Table 1). Because no dose tested produced mortality within 48 h in fifth instars reared on Douglas-fir, we could not establish an LD_{50} for this treatment. The LD_{50} of fifth instars reared on alder was only 1.5 times higher than that of third instars, but fifth instars reared on Douglas-fir tolerated a dose 17.2 times higher than the LD_{50} for third instars for 48 h.

Most mortality during the first 48 h occurred within 24 h of treatment in third instars reared on alder, but most third instars reared on Douglas-fir died 24–48 h after treatment (Table 2). From 48 h after treatment to pupation, treated larvae on both host plants continued to die at all doses tested (Table 2). Larvae died most frequently when they attempted to molt; some treated larvae failed to pupate or eclose. Although mortality in the 24 h immediately following treatment was higher in larvae reared on alder, overall survival of treated third instars to pupation was higher in this group than in those reared on Douglas-fir (Table 2).

Treated third instars reared on Douglas-fir required significantly longer to pupate than did controls reared on the same diet (males, $t = 4.84$, df = 31, $P = 0.001$; females, $t = 7.04$, df = 32, $P = 0.001$) or treated larvae reared on alder (males, $t = 3.95$, df = 45, $P = 0.001$; females, $t = 3.19$,

Table 2. Carbaryl-induced mortality of gypsy moth larvae fed alder or Douglas-fir foliage and treated 48 h after molt into the third instar

Dose, μg/g	% Cumulative mortality		
	24 h	48 h	Total[a]
	Alder		
2	26.7	26.7	51.1
4	31.1	31.1	62.2
6	40.0	40.0	55.6
8	46.7	46.7	62.2
10	68.9	68.9	80.0
Control	0	0	4.4
	Douglas-fir		
24	2.2	10.5	71.1
28	0	52.6	81.6
32	2.2	71.1	89.5
36	2.2	68.4	89.5
40	2.2	79.0	92.1
44	4.4	89.5	92.1
Control	0	0	7.9

[a] Total mortality includes mortality from treatment through pupation. For larvae reared on alder, initial $n = 45$; for those reared on Douglas-fir, initial $n = 38$.

df = 65, $P = 0.01$) (Table 3). Control females, however, took longer to pupate when fed alder than when fed Douglas-fir ($t = 4.00$, df = 35, $P = 0.001$).

Pupal weights of survivors fed alder and treated in the third instar did not differ from controls (Table 3). Pupal weights of treated (all doses pooled) and control survivors fed Douglas-fir also did not differ. However, females treated with 32 μg/g were significantly heavier than controls (= 1.488 g compared with 0.909 g for controls; $t = 2.28$, df = 18, $P = 0.05$), as were those treated at 36 μg/g (1.556 g; $t = 2.62$, df = 18, $P = 0.01$). Treated females reared on Douglas-fir were significantly heavier than those reared on alder ($t = 2.74$, df = 66, $P = 0.01$), although the controls on the two diets did not differ.

Activities of Detoxication Enzymes. Content and activity of detoxication enzymes were generally higher in tissues from larvae fed Douglas-fir, although differences were not always significant. Recovery of microsomal protein, as a proportion of original larval weight, was about twice as high in larvae fed alder (0.75 versus 0.45%), indicating greater synthesis of non-PSMO protein in larvae fed alder.

Cytochrome P-450 content (Table 4) was significantly higher in the carcass of fifth-instar females fed on Douglas-fir than in the same tissue from larvae reared on alder ($t = 8.57$, df = 3, $P = 0.01$). Cytochrome P-450 was significantly higher in midgut than in carcass in third instars ($t = 4.17$, df = 4, $P = 0.01$) reared on Douglas-fir, in fifth-instar females ($t = 3.35$, df = 4, $P = 0.05$) and males ($t = 5.93$, df = 4, $P = 0.01$) reared on Douglas-fir, and in fifth-instar females reared on alder ($t = 40.95$, df = 2, $P = 0.001$). Midguts of third instars reared on Douglas-fir were higher in P-450 content than were those from fifth instars ($t = 2.34$, df = 7, $P = 0.05$), as were third-instar carcasses of larvae reared on alder ($t = 2.81$, df = 3, $P = 0.05$).

Aldrin epoxidase activity (Table 4) was significantly higher in the carcass of both third instars ($t = 2.83$, df = 3, $P = 0.05$) and fifth-instar females ($t = 3.43$, df = 3, $P = 0.05$) fed Douglas-fir than in the same time from larvae fed alder. Aldrin epoxidase activity did not differ between instars in either midgut or carcass, with one exception: activity was significantly higher in third-instar midgut than in fifth-instar midgut from larvae reared on Douglas-fir ($t = 1.99$, df = 7, $P = 0.05$). Activity was significantly higher in fifth-instar midgut than in carcass in alder-reared females ($t = 11.10$, df = 2, $P = .01$) and in Douglas-fir-reared females ($t = 3.82$, df = 4, $P = 0.01$) and males ($t = 3.08$, df = 4, $P = 0.05$); tissues from the third instar did not differ significantly, regardless of diet.

Glutathione S-transferase activity (Table 4) was significantly higher in the midgut of third instars ($t = 3.31$, df = 3, $P = 0.05$) and in both midgut ($t = 5.27$, df = 3, $P = 0.01$) and carcass ($t = 4.4$, df = 3, $P = 0.05$) of female fifth instars reared on Douglas-fir than in the same tissues of larvae reared on alder. Activity of these enzymes was higher in midgut than in carcass on both diets in the third instars (alder: $t = 31.05$, df = 2, $P = 0.001$; Douglas-fir: $t = 9.45$, df = 4, $P = 0.001$), in fifth-instar females reared on alder ($t = 5.03$, df = 2, $P = 0.05$) and in both females ($t = 9.40$, df = 4, $P = 0.001$) and males ($t = 2.82$, df = 4, $P = 0.05$) reared on Douglas-fir. Activities were significantly higher in midguts of third instars than in those of fifth instars in larvae fed alder ($t = 2.36$, df = 3, $P = 0.05$) or Douglas-fir ($t = 2.35$, df = 7, $P = 0.05$).

Foliar Chemistry. Average content of total phenolics over the experimental period (late April through early June) was ≈75% higher in alder (mean = 175.6 mg/g dry foliage, SD = 21.9) than in Douglas-fir (mean = 101.3 mg/g, SD =

Table 3. Sublethal effects of carbaryl on gypsy moth larvae fed alder or Douglas-fir foliage and treated 48 h after molting into the third instar

Diet	Days to pupation		Pupal weight, g	
	Treated[a]	Control	Treated[a]	Control
Alder				
Males	25.7	26.2	0.432	0.430
Females	34.1	33.7	0.903	0.875
Douglas-fir				
Males	30.1	26.2[b]	0.452	0.439
Females	38.0	29.9[c]	1.136	0.909

[a] Data pooled from survivors at all treatment doses.
[b] Significantly different from treated ($t = 4.83$, df = 31, $P = 0.001$).
[c] Significantly different from treated ($t = 7.02$, df = 32, $P = 0.001$).

Table 4. Levels of detoxifying enzymes in tissues of gypsy moth larvae reared on alder or Douglas-fir[a]

Instar	Midgut		Carcass	
	Alder	Douglas-fir	Alder	Douglas-fir
	Cytochrome P-450 (pmol/mg protein)			
III	101.5 (48.8)	175.7 (49.5)	36.5 (10.1)	52.7 (12.5)
V[b]	81.0	126.3 (20.8)	8.0	39.7 (14.7)
V[c]	104.0 (2.8)	107.0 (35.5)	19.3 (1.1)	42.0 (3.6)**[d]
	Aldrin epoxidase (pmol/min/mg protein)			
III	555.0 (555.8)	1,037.0 (637.8)	30.5 (10.6)	254.3 (105.8)*
V[b]	344.0	587.7 (211.1)	12.5	186.9 (78.0)
V[c]	481.0 (55.1)	443.3 (101.4)	36.8 (10.3)	185.7 (57.8)*
	(Glutathione transferase (nmol/min/mg protein)			
III	22.7 (0.2)	36.8 (5.7)*	5.0 (0.8)	5.4 (0.6)
V[b]	19.2	26.2 (10.4)	4.5	7.7 (5.3)
V[c]	10.6 (2.0)	22.9 (2.8)**	2.9 (0.8)	6.8 (1.1)*

Within enzyme type and instar, values marked with asterisks differ significantly between diets by t test. *, $P = 0.05$; **, $P = 0.01$; see text for other statistics.

[a] Mean (SD) of two or three experiments, except male fifth instars reared on alder ($n = 1$).

[b] Males.

[c] Females.

11.8). In alder, phenolic content rose from 184.5 mg/g dry foliage in late April to 204.7 mg/g dry foliage in early May, then declined to a minimum of 139.3 mg/g in late May. Phenolic content of Douglas-fir foliage during the study declined slightly, from 124.2 mg/g dry foliage in late April to a plateau of 95–100 mg/g during May and a minimum of 76.4 mg/g in early June.

We found no monoterpenes in alder. As expected, terpenes were abundant in the Douglas-fir, however. Total terpene content during the period of the study fluctuated within the range of 5.4–9.4 mg/g foliage (dry weight), averaging 6.8 mg/g foliage over the season. About 90% of the terpenes were monoterpenes, of which the most abundant (α-pinene, β-pinene, and sabinene) averaged 14.2, 54.6, and 12.3%, respectively.

Foliar nitrogen content in both alder and Douglas-fir remained nearly constant through the spring, averaging 2.6% (SD = 0.2) for alder and 1.2% (SD = 0.1) for Douglas-fir over the feeding season.

Discussion

LD_{50}s for larvae fed alder were similar to those reported by Ahmad & Forgash (1975) for *L. dispar* raised on artificial diet, but the tolerance of larvae reared on Douglas-fir was significantly higher. Although detoxifying enzymes were not elevated in all tissues and stages of larvae fed Douglas-fir, the increases that we found may have been sufficient to enhance tolerance. Enzymes not measured by our assay methods also may have been involved in detoxication; carbaryl is detoxified by several enzymatic pathways, including hydrolysis, oxidation, and conjugation (Kuhr & Dorough 1976, Ahmad et al. 1980, Agosin 1985, Brattsten et al. 1986, McCord & Yu 1987). Other mechanisms (e.g., reduced penetration caused by diet-related changes in cuticular lipids) also may have increased insecticide tolerance.

High levels of PSMO activity have been associated with polyphagy (Krieger et al. 1971, Brattsten et al. 1977, Ahmad 1983; but see Krieger et al. 1976, Rose 1985) and with insects that feed on terpene-rich host plants (Rose 1985). On this basis, PSMO levels in gypsy moth should be high relative to other insects and higher in larvae fed Douglas-fir than in those fed alder. In fact, aldrin epoxidase activity was low relative to other insects and even other Lepidoptera, and differences between diet treatments were significant in only a few instances. Our results are thus consistent with those of Ahmad (1986) and Sheppard & Friedman (1989), who also reported relatively low PSMO-related activities in gypsy moth, as well as differential induction of these activities by host plant and by α-pinene in artificial diet. Because of the many differences in techniques used, however, comparisons among studies must be done cautiously.

Obtaining data on enzyme activities in these insects was difficult because of their small size (especially third instars) and the limited number of larvae available in the appropriate developmental stage. These experimental difficulties may have contributed to the high variability in some of the data and in one instance prohibited a second experiment. Nevertheless, most of our data are within the range of variability common in the literature, and we were able to obtain data with 20–25 larvae, rather than several hundred as required by Ahmad (1986). Similar experiments in subsequent years (A.F.M., unpublished data) yielded comparable values for these enzyme activities.

Larvae in this study fed on only one plant, which may have influenced detoxication levels; sequential feeding on two or more host plants affects levels of these enzymes, PSMOs in particular (Ahmad 1983, Brattsten et al. 1984, Siegfried & Mullin 1989). Low levels of N or other nutrients in Douglas-fir may have inhibited induction of both the PSMOs and the glutathione transferases, although Lindroth et al. (1991) found no response of these enzymes to nutrient limitation. Both food plants may have had an inducing action, with isozymes with higher affinity for the substrates assayed being preferentially induced in Douglas-fir. PSMO levels in larvae reared on artificial diet are lower than those reared on either foodplant we tested (A.F.M., unpublished data).

On the basis of past research (Ahmad & Forgash 1975, Ahmad 1986), we expected higher specific activity for PSMOs in fifth instars compared with third instars. However, we found no significant differences between the instars. This disagreement may result from differences in physiological stage of the insects and in the different techniques used for assaying enzymes and determining protein content.

Subacute effects of exposure to insecticide, such as reduced fecundity and delayed development, may affect the dynamics of insect populations (Raffa 1986). Chronic toxicity and compensatory behavior (such as prolonged, diminished, or enhanced food consumption) in insects surviving insecticide application can affect not only the insects, but the stress to which they subject their hosts. Delayed development, such as that which we observed in treated larvae relative to untreated larvae reared on Douglas-fir, could affect the suitability of foliage for diet, prolong exposure to parasites and predators, affect mating chances, and increase exposure to adverse (or more favorable) weather conditions. The higher N levels in alder probably contributed to the superior ability of larvae surviving insecticide treatment to recover and develop on a schedule comparable to controls.

Because pupal weights of treated and untreated females fed alder did not differ, carbaryl treatment probably did not affect fecundity. Fecundity of Douglas-fir-reared females that survived treatment at 32 and 36 μg/g may even have been increased over that of untreated insects, according to the relationship between pupal weight and fecundity derived by Miller et al. (1991 a,b).

The terpene profile of the Douglas-fir foliage fed to the larvae was consistent with that reported by von Rudloff (1973) for the species in west-central Oregon. The dominant monoterpenes, α- and β-pinene, have frequently been reported to be inducers of detoxication activity, but Sheppard & Friedman (1989) reported that α-pinene administered in artificial diet increased activities above those in controls in only one of the two PSMO activities they assayed. The diet they used may have contained inducers, so that control levels were also higher than constitutive (Ahmad & Forgash 1978). Terpenes present in lesser amounts also may have contributed to or been responsible for induction. Brattsten et al. (1984), for example, reported that PSMO activities in southern armyworm, *Spodoptera eridania* (Cramer), were induced more by limonene, which was present in our Douglas-fir foliage at low levels, than by equivalent doses of α- or β-pinene.

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References Cited

Agosin, M. 1985. Role of microsomal oxidations in insecticide degradation, pp. 647–712. *In* G. A. Kerkut & L. I. Gilbert [eds.], Comprehensive insect physiology, biochemistry, and pharmacology, vol. 12. Pergamon, New York.

Ahmad, S. 1982. Roles of mixed-function oxidases in insect herbivory, pp. 41–47. *In* J. H. Visser & A. K. Minks [eds.], Proceedings of the 5th international symposium on insect–plant relationships (Wageningen 1982). Centre for Agricultural Publishing and Documentation, Wageningen.

1983. Mixed-function oxidase activity in a generalist herbivore in relation to its biology, food plants and feeding history. Ecology 64: 235–243.

1986. Enzymatic adaptations of herbivorous insects and mites to phytochemicals. J. Chem. Ecology 12: 533–560.

Ahmad, S. & A. J. Forgash. 1973. NADPH oxidation by microsomal preparations of gypsy moth larval tissues. Insect Biochem. 3: 263–273.

1975. Toxicity of carbaryl and diazinon to gypsy moth larvae: changes in relation to larval growth. J. Econ. Entomol. 68: 803–806.

1976. Toxicity of carbaryl to gypsy moth larvae parasitized by *Apanteles melanoscelus*. Environ. Entomol. 5: 1183–1186.

1978. Gypsy moth mixed function oxidases: gut enzyme levels increased by rearing on wheat germ diet. Ann. Entomol. Soc. Am. 71: 449–452.

Ahmad, S., A. J. Forgash & Y. T. Das. 1980. Penetration and metabolism of [^{14}C] carbaryl in larvae of the gypsy moth, *Lymantria dispar* (L.). Pestic. Biochem. Physiol. 14: 236–248.

Ahmad, S., L. B. Brattsten, C. A. Mullin & S. J. Yu. 1986. Enzymes involved in the metabolism of plant allelochemicals, pp. 73–152. *In* L. B. Brattsten

& S. Ahmad [eds.], Molecular aspects of insect–plant associations. Plenum, New York.

Anonymous. 1975. Technicon autoanalyzer II methodology: individual simultaneous determination of nitrogen and phosphorus in BD acid digests. Technicon Corporation Industrial Method No. 334-74A. Technicon Corp., Tarrytown, NY.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248–254.

Brattsten, L. B. 1986. Fate of ingested plant allelochemicals in herbivorous insects, pp. 211–256. *In* L. B. Brattsten & S. Ahmad [eds.], Molecular aspects of insect–plant associations. Plenum, New York.

1988a. Enzymic adaptations in leaf-feeding insects to host-plant allelochemicals. J. Chem. Ecol. 14: 1919–1939.

1988b. Potential role of plant allelochemicals in the development of insecticide resistance, pp. 331–348. *In* P. Barbosa & D. K. LeTourneau [eds.], Novel aspects of insect–plant interactions. Wiley, New York.

Brattsten, L. B., C. F. Wilkinson & T. Eisner. 1977. Herbivore–plant interactions: mixed function oxidases and secondary plant substances. Science 196: 1349–1352.

Brattsten, L. B., C. K. Evans, S. Bonetti & L. H. Zalkow. 1984. Induction by carrot allelochemicals of insecticide-metabolizing enzymes in the southern armyworm (*Spodoptera eridania*). Comp. Biochem. Physiol. 77C: 29–37.

Brattsten, L. B., C. A. Gunderson, J. T. Fleming & K. N. Nikbahkt. 1986. Temperature and diet modulate cytochrome P-450 activities in southern armyworm, *Spodoptera eridania* (Cramer), caterpillars. Pestic. Biochem. Physiol. 25: 346–357.

Brooks, J. E., J. H. Borden, H. D. Pierce, Jr. & G. R. Lister. 1987. Seasonal variation in foliar and bud monoterpenes in sitka spruce. Can. J. Bot. 65: 1249–1252.

Cohen, M. B., M. R. Berenbaum & M. A. Schuler. 1989. Induction of cytochrome P450-mediated detoxification of xanthotoxin in the black swallowtail. J. Chem. Ecol. 15: 2347–2355.

Feyereisen, R. 1983. Polysubstrate monooxygenases (cytochrome P-450) in larvae of susceptible and resistant strains of house flies. Pestic. Biochem. Physiol. 19: 262–269.

Forgash, A. J. & S. Ahmad. 1974. Hydroxylation and demethylation by gut microsomes of gypsy moth larvae. Int. J. Biochem. 5: 11–15.

Harwood, S. H., A. F. Moldenke & R. E. Berry. 1990. Toxicity of peppermint monoterpenes to the variegated cutworm, *Peridroma saucia* Hübner (Lepidoptera: Noctuidae). J. Econ. Entomol. 83: 1761–1767.

Joseph, G. 1989. Host suitability studies of Douglas-fir and white alder to the gypsy moth. M.S. thesis, Oregon State University, Corvallis.

Joseph, G., J. C. Miller, R. E. Berry, J. Wernz, A. F. Moldenke & R. G. Kelsey. 1991. White alder and Douglas-fir quality and interegg-mass influences on larvae development of gypsy moth, *Lymantria dispar*. J. Chem. Ecol. 17: 1783–1799.

Julkunen-Tiitto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. J. Agric. Food Chem. 33: 213–217.

Kapin, M. A. & S. Ahmad. 1980. Esterases in larval tissues of gypsy moth, *Lymantria dispar* (L.): optimum assay conditions, quantification and characterization. Insect Biochem. 10: 331–337.

Krieger, R. I., P. Feeny & C. F. Wilkinson. 1971. Detoxification enzymes in the guts of caterpillars: an evolutionary answer to plant defense? Science 172: 579–580.

Krieger, R. I., C. F. Wilkinson, L. J. Hicks & E. F. Taschenberg. 1976. Aldrin epoxidation, dihydroisodrin hydroxylation, and P-chloro-N-methylalanine demethylation in six species of saturniid larvae. J. Econ. Entomol. 69: 1–5.

Kuhr, R. J. & H. W. Dorough. 1976. Carbamate insecticides: chemistry, biochemistry, and toxicology. CRC, Cleveland, OH.

LeOra Software. 1987. Polo-PC: a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA.

Lindroth, R. L. 1989a. Biochemical detoxication: mechanism of differential tiger swallowtail tolerance to phenolic glycosides. Oecologia 91: 219–224.

1989b. Differential esterase activity in *Papilio glaucus* subspecies: absence of cross-resistance between allelochemicals and insecticides. Pestic. Biochem. Physiol. 35: 185–191.

Lindroth, R. L., B. D. Anson & A. V. Weisbrod. 1990. Effects of protein and juglone on gypsy moths: growth performance and detoxification enzyme activity. J. Chem. Ecol. 16: 2533–2547.

Lindroth, R. L., M. A. Barman & A. V. Weisbrod. 1991. Nutrient deficiencies and the gypsy moth, *Lymantria dispar*: effects on larval performance and detoxication enzyme activities. J. Insect. Physiol. 37: 45–52.

McCord, E., Jr. & S. J. Yu. 1987. The mechanisms of carbaryl resistance in the fall armyworm, *Spodoptera frugiperda*. Pestic. Biochem. Physiol. 27: 114–122.

Miller, J. C. & P. E. Hanson. 1989a. Laboratory studies on development of gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), larvae on foliage of gymnosperms. Can. Entomol. 121: 425–429.

1989b. Laboratory feeding tests on the development of gypsy moth larvae with reference to plant taxa and allelochemicals. Oreg. Exp. Stn. Bull. 674.

Miller, J. C., P. H. Hanson & D. N. Kimberling. 1991a. Development of the gypsy moth (Lepidoptera, Lymantriidae) on Douglas-fir foliage. J. Econ. Entomol. 84: 461–465.

1991b. Development of the gypsy moth (Lepidoptera: Lymantriidae) on Garry oak and red alder in western North America. Environ. Entomol. 20: 1097–1101.

Moldenke, A. F. & L. C. Terriere. 1981. Cytochrome P-450 in insects. 3. Increase in substrate binding by microsomes from phenobarbital-induced house flies. Pestic. Biochem. Physiol. 16: 222–230.

Moldenke, A. F., D. R. Vincent, D. E. Farnsworth & L. C. Terriere. 1984. Cytochrome P-450 in insects. 4. Reconstitution of cytochrome P-450-dependent monooxygenase activity in the house fly. Pestic. Biochem. Physiol. 21: 358–367.

Omura, T. & R. Sato. 1964. The carbon-monoxide binding pigment of microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. 239: 2370–2379.

Perry, D. A. & G. B. Pitman. 1983. Genetic and environmental influences in host resistance to herbivory: Douglas-fir and the western spruce budworm. Z. Angew. Entomol. 96: 217–228.

Radwan, M. A. 1975. Genotype and season influence chlorogenic acid content in Douglas-fir foliage. Can. J. For. Res. 5: 281–284.

Raffa, K. R. 1986. Devising pest management tactics based on plant defense mechanisms, theoretical and practical considerations, pp. 301–328. *In* L. B. Brattsten & S. Ahmad [eds.], Molecular aspects of insect–plant associations. Plenum, New York.

Redak, R. A. & R. G. Cates. 1984. Douglas-fir (*Pseudotsuga menziesii*)–spruce budworm (*Choristoneura occidentalis*) interactions: the effect of nutrition, chemical defenses, tissue phenology, and tree physical parameters on budworm success. Oecologia 62: 61–67.

Rose, H. A. 1985. The relationship between feeding specialization and host plants to aldrin epoxidase activities of midgut homogenates in larval Lepidoptera. Ecol. Entomol. 10: 455–467.

Sheppard, C. A. & S. Friedman. 1989. Endogenous and induced monooxygenase activity in gypsy moth larvae feeding on natural and artificial diets. Arch. Insect Biochem. Physiol. 10: 47–56.

Siegfried, S. & C. D. Mullin. 1989. Influence of alternative host plant feeding on aldrin susceptibility and detoxification enzymes in western and northern corn rootworms. Pestic. Biochem. Physiol. 35: 155–164.

Statistical Graphics Corporation. 1987. STATGRAPHICS. Users' guide. STSC Inc., Rockville, MD.

Terriere, L. C. 1984. Induction of detoxication enzymes in insects. Annu. Rev. Entomol. 29: 71–88.

Vincent, D. R., A. F. Moldenke & L. C. Terriere. 1983. NADPH-cytochrome P-450 reductase from the house fly, *Musca domestica*. Insect Biochem. 13: 559–566.

von Rudloff, E. 1973. Geographical variation in the terpene composition of the leaf oil of Douglas-fir. Pure Appl. Chem. 34: 401–410.

1980. Chemosystematic studies in the genus *Pseudotsuga*. IV. Inheritance and geographical variation in the leaf oil terpenes of Douglas-fir from the Pacific Northwest. Can. J. Bot. 58: 546–556.

Yu, S. J. 1983. Induction of detoxifying enzymes by allelochemicals and host plants in the fall armyworm. Pestic. Biochem. Physiol. 19: 330–336.

1984. Interactions of allelochemicals with detoxication enzymes of insecticide-susceptible and resistant fall armyworms. Pestic. Biochem. Physiol. 22: 60–68.

1986. Consequences of induction of foreign compound-metabolizing enzymes in insects, pp. 153–173. *In* L. B. Brattsten & S. Ahmad [eds.], Molecular aspects of insect–plant associations. Plenum, New York.

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