Oecologia (1989) 80:507–512

Oecologia

Differential effect of tannic acid on two tree-feeding Lepidoptera: implications for theories of plant anti-herbivore chemistry

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Summary. Feeding efficiencies of ultimate instar larvae of two polyphagous tree-feeding Lepidoptera, *Malacosoma disstria* (Lasiocampidae) and *Orgyia leucostigma* (Liparidae), were measured on artificial diets containing from 0% to 8% tannic acid. Relative growth rate (RGR) of *O. leucostigma* was not affected by up to 8% tannic acid, suggesting that *O. leucostigma* has evolved an effective counteradaptation to hydrolyzable tannins. In contrast, as little as 0.5% tannic acid caused a significant reduction in RGR of *M. disstria,* due both to reduced efficiency of conversion of digested food (ECD) and reduced relative consumption rate (RCR), and caused a significant increase in mortality during the pupal stage. Moreover, when reared from hatching on tannin-containing diets, no *M. disstria* larvae survived past the fourth instar.

Although tannins are commonly referred to as "digestibility-reducing substances", tannic acid did not reduce the ability of *M. disstria* or *O. leucostigma* larvae to digest either the whole diet or nitrogen contained in the diet. For *M. disstria,* tannic acid acts as a toxin and a feeding deterrent, but not as a digestibility-reducing substance. Growing evidence that tannins commonly act as toxins warrants a reassessment of their role in anti-herbivore chemistry.

Key words: Tannic acid – Nutritional ecology – *Malacosoma disstria* – *Orgyia leucostigma* – Toxin

Tannins are naturally occurring water-soluble polyphenolic compounds capable of binding to proteins in solution (Haslam 1979; Swain 1979). Tannins have been accorded a major role in plant-herbivore interactions (Feeny 1976; Rhoades and Cates 1976), and are hypothesized to affect insect herbivores in two ways: 1) by forming insoluble complexes with plant proteins in the insect's gut, thereby reducing the availability of plant nitrogen to the herbivore, and 2) by binding to the insect's digestive enzymes, thereby reducing the insect's ability to digest non-protein polymeric components of the diet as well. Two predictions follow from these hypothesized modes of action, and form the subject of this investigation. First, tannins are predicted to affect insect herbivores adversely by reducing their digestive efficiency. Second, by virtue of their ability to bind non-specifically to a wide variety of proteins, tannins are predicted to present an unusually strong barrier to counteradaptation by insect herbivores (Feeny 1976).

This study was initiated in the context of growing evidence that neither of these predictions is correct. Past studies of insect herbivores indicate that, although tannins can reduce growth, they do so by mechanisms other than reducing the insect's ability to digest its food (Bernays 1981; Klocke and Chan 1982; Reese et al. 1982; Berenbaum 1983; Manuwoto and Scriber 1986). Moreover, several insect herbivores appear to have evolved effective counteradaptations that allow them to feed on tannin-rich foods (Bernays et al. 1980; Berenbaum 1983; Manuwoto and Scriber 1986). To date, adverse effects of tannins have been demonstrated primarily in species that do not encounter tannins in their natural diets (Bernays et al. 1980; Berenbaum 1983; Manuwoto et al. 1985; but see Klocke and Chan 1982; Reese et al. 1982).

The purpose of this study was to explore the effect of tannic acid, a hydrolyzable tannin, on the growth and efficiency of food utilization by insects known to ingest tannins in their normal diets. The study was designed to answer two specific questions:

1. Are insects that normally feed on tannin-containing plants adversely affected by tannic acid and, if so,

2. Does tannic acid act as a digestibility-reducing substance, feeding deterrent, and/or toxin?

To answer these questions, I studied the effect of tannic acid on larval growth of two forest Lepidoptera known to feed in nature on foliage containing both hydrolyzable and condensed tannins. Ultimate instar larvae of the forest tent caterpillar, *Malacosoma disstria* (Lasiocampidae) and the white-marked tussock moth, *Orgyia leucostigma* (Liparidae), were fed artificial diets containing from 0% to 8% (dry weight) tannic acid; tree foliage generally contains between 0.5% and 10% tannin (Swain 1979). *M. disstria* has been reported from woody members of at least seven plant families, including Salicaceae, Rosaceae, Fagaceae, Hamamelidaceae, Nyssaceae, Aceraceae, and Tiliaceae (Stehr and Cook 1968). *O. leucostigma* feeds commonly on both deciduous and coniferous trees in at least 8 plant families, including Salicaceae, Rosaceae, Betulaceae, Aceraceae, Tiliaceae, Ulmaceae, Platanaceae, and Pinaceae (Baker 1972).

Materials and methods

Experimental organisms

Eggs of both *M. disstria* and *O. leucostigma* were obtained from the Forest Pest Management Institute in Sault Ste.

Marie, Ontario, Canada, from colonies established in 1979 and infused with approximately 10% wild-caught individuals twice each year since then (D. Grisdale, pers. comm.).

The effect of tannic acid on ultimate instar performance of M. disstria and O. leucostigma

From hatching until the onset of the ultimate instar, larvae of each species were reared on Bio Serv Forest Tent Caterpillar Diet or Bio Serv Douglas Fir Tussock Moth Diet containing 0% tannic acid (control diet). Upon molting to the ultimate (fifth for *M. disstria* males and females and *O. leucostigma* males, sixth for *O. leucostigma* females) instar, larvae were removed from control diet, starved for 2 h to clear the gut, and transferred to individual cups containing preweighed amounts of one 0%, 0.5%, 1%, 2%, or 8% tannic acid (Sigma Chemical Co.). Sixteen *M. disstria* and 13–18 *O. leucostigma* larvae were tested on each diet. Standard gravimetric techniques (Waldbauer 1968) were used to measure relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested food (ECD), and relative growth rate (RGR) defined as:

$$\text{RCR} = \frac{\text{weight of food ingested}}{\text{average larval weight} \times \text{days}}$$

$$\text{AD} = \frac{\text{weight of frass}}{\text{weight of food ingested}}$$

$$\text{ECD} = \frac{\text{larval weight gained}}{\text{weight of food ingested} - \text{weight of frass}}$$

$$\text{RGR} = \frac{\text{larval weight gained}}{\text{average larval weight} \times \text{days}}$$

where average larval weight = (initial weight + final weight + weight of silk produced during the instar)/2.

All rearing and testing of both *M. disstria* and *O. leucostigma* was conducted at 22° C under a 16:8 L:D cycle. Fresh diet was provided every 48 h. Immediately after pupation, pupae were sexed (Muggli 1974) and pupae, uneaten food, and frass and silk produced during the instar were dried at 70° C to constant weight. To estimate the initial dry weight of larvae and of food provided, 15 freshly molted ultimate instar larvae and 15 samples of each diet were weighed, dried at 70° C to constant weight, and reweighed.

To determine whether tannic acid reduces the ability of *M. disstria* or *O. leucostigma* to digest dietary protein, the approximate digestibility of nitrogen was measured as:

$$\text{AD(N)} = \frac{\text{nitrogen ingested} - \text{nitrogen in frass}}{\text{nitrogen ingested}}$$

Nitrogen content of food and frass was determined by Kjeldahl analysis with a Tecator Kjeltech 1030 Auto Analyzer and Digestion System. On each diet, frass from 2–3 larvae was pooled to give enough material for two replicate analyses. Food and frass samples were dried, ground through a 40-mesh screen, and digested at 410° C for one hour in 3.0 ml of concentrated sulfuric acid containing 1.5 g potassium sulfate and 0.75 g selenium (Kjeltab TC1527). After cooling, samples were diluted with 25 ml of deionized distilled water, and 25 ml of 35–40% sodium hydroxide was added to convert all digested nitrogen to ammonia. Ammonia was then distilled into 25 ml of 10% boric acid containing 0.001% bromocresol green, 0.0007% methyl red, and 0.002% sodium hydroxide. Original nitrogen content was determined by titration of the resulting ammonia with 0.05 N hydrochloric acid.

During this experiment, *M. disstria* proved susceptible to tannic acid (see Results), but casual observation indicated that adverse effects did not appear for some time after tannic acid was first ingested. To determine more rigorously the degree of exposure necessary to produce adverse effects in *M. disstria*, the following two additional experiments were initiated.

The effect of tannic acid on performance of M. disstria during the first 70 h of the ultimate instar

To determine the degree of exposure to tannic acid necessary to produce adverse effects in fifth instar larvae, a second group of 75 *M. disstria* larvae was reared and tested in identical fashion, except that 1) the test was concluded after the first 70 h (approximately the first third) of the ultimate instar, and 2) at the conclusion of the test, the larva was starved for 2 hours to clear the gut. Standard nutritional indices and AD(N) were calculated as described above.

The effect of tannic acid on early instar growth and survivorship of M. disstria

To determine the degree of exposure necessary to produce adverse effects in early instar larvae, 132–176 newly hatched larvae were placed into 25-ml polystyrene cups containing Bio Serv Forest Tent Caterpillar Diet with 0%, 1%, 2%, or 8% tannic acid. Initially, each cup contained approximately 25 larvae, five from each of five females. Larval density was reduced to 10/cup upon reaching the third instar, and to 5/cup upon reaching the fourth instar. Diet was replaced every 48 hours. Survivorship and molting were monitored daily through the fourth instar only, since no larvae on any tannic acid diet survived past this stage.

Gut pH

Since pH affects the ability of tannins to bind to proteins in solution (Hagerman and Butler 1978), pH in the foregut and middle midgut was measured using a microelectrode fitted to a Metrohm/Brinkmann pH-103 meter. Larvae were reared on control diet until the onset of the ultimate instar and then allowed to feed for 72 h on 0%, 0.5%, 1%, 2%, or 8% tannic acid diet before determination of gut pH. Gut pH was measured for 17 to 20 *M. disstria* larvae and seven *O. leucostigma* larvae per diet.

Statistical analysis

Mean values of RCR, AD, ECD, and RGR were compared by ANOVA. When ANOVA revealed significant differences among treatments (diets), pairwise comparisons were performed by calculating the least significant difference (LSD; Sokal and Rohlf 1981). Homogeneity of variances between treatments (diets) was satisfied in all cases except ECD for *O. leucostigma;* in this case, equality of means was tested using Games and Howell's Approximate Test for Equality When Variances are Heterogeneous (Games and Howell 1976).

Results

The effect of tannic acid on ultimate instar performance of M. disstria and O. leucostigma

M. disstria and *O. leucostigma* were affected very differently by dietary tannic acid. While *O. leucostigma* was virtually unaffected, the growth and survivorship of *M. disstria* were dramatically reduced when tannic acid was ingested during the ultimate instar.

Relative growth rate (RGR) and efficiency of conversion of digested food (ECD) of ultimate instar *M. disstria* larvae were significantly reduced on diets containing 0.5%, 1%, or 8% tannic acid (Table 1). The presence of 0.5% or 8%, but not of 1%, tannic acid also caused a significant reduction in relative consumption rate (RCR), but not of total food consumed during the ultimate instar. Decreases in RGR and ECD were significantly greater on 8% tannic acid than on all other diets. In addition, both male and female *M. disstria* fed 8% tannic acid formed significantly smaller pupae than those fed the control diet (Table 1). For unknown reasons, no significant reductions in any nutritional index were evident for larvae fed diet containing 2% tannic acid.

In contradiction to its proposed role as a digestibility-reducing substance, tannic acid did not reduce the ability of *M. disstria* larvae to digest either whole food or dietary nitrogen; no tannin-containing diet caused a significant reduction in either AD or AD(N) (Table 1). On all diets, larvae digested dietary nitrogen considerably more efficiently than whole food.

Ingestion of any level of tannic acid during the ultimate instar significantly increased the frequency of lethal pupal malformations (Fig. 1). The frequency of malformed pupae was 75%, 50%, 56%, and 44% on 0.5%, 1%, 2%, and 8% tannic acid diets, respectively, but only 6% on the control diet (tannin-containing vs. tannin-free diets: $\chi^2 = 13.2$, df = 1, $p = 0.0003$; among tannin-containing diets: $\chi^2 = 3.35$, df = 3, $p > 0.3$). A separate rearing confirmed that malformed pupae never eclosed successfully.

In contrast to *M. disstria, O. leucostigma* larvae were not adversely affected by ingested tannic acid during the ultimate instar. *O. leucostigma* larvae fed the control diet did not differ significantly from larvae fed any tannic acid diet in RGR, ECD, AD, AD(N), or male or female pupal weight (Table 2). Moreover, RCR was significantly increased on diets containing 0.5% and 8% tannic acid, indicating that tannic acid acts as a feeding stimulant for *O. leucostigma*.

On all diets, the midgut fluids of ultimate instar *M. disstria* and *O. leucostigma* larvae were highly alkaline (Table 3). Although the midgut fluid of *O. leucostigma* is slightly more alkaline that that of *M. disstria,* in both species the alkalinity is sufficient to prevent the precipitation of most proteins by tannins (Hagerman and Butler 1978; Martin and Martin 1983).

The effect of tannic acid on performance of M. disstria during the first 70 h of the ultimate instar

Although ingested tannic acid clearly exerts adverse effects on *M. disstria* larvae when measurements were made over

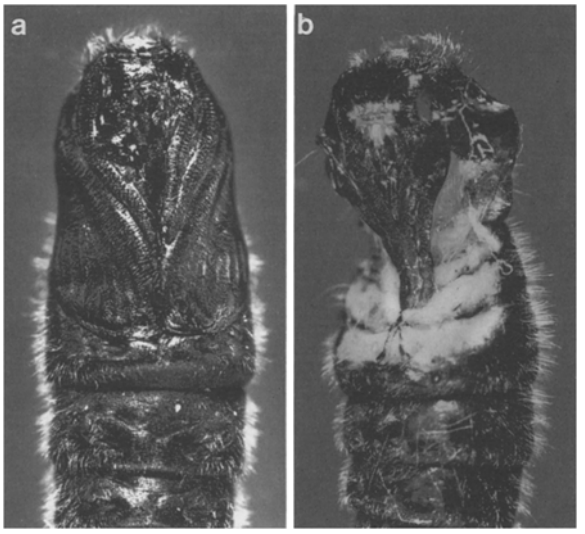

Fig.1 a, b. Typical appearance of *M. disstria* pupae from the control diet (**a**) and 0.5%, 1%, 2%, and 8% tannic acid diets (**b**). All malformed pupae failed to produce adults

Table 1. Nutritional indices for *Malacosoma disstria* fed one of five test diets during the entire ultimate instar. Means are given, with standard deviations in parentheses. Means followed by different letters are significantly different at $P < 0.05$ by LSD

Diet	n	RGR	RCR	ECD	AD	AD(N)
0%	16	1.25a (0.25)	0.696a (0.131)	0.395a (0.093)	0.538a (0.063)	0.741a (0.044)
0.5%	16	0.83b (0.38)	0.533b (0.150)	0.291b (0.101)	0.569a (0.035)	0.715a (0.046)
1%	16	0.93b (0.34)	0.603abc (0.135)	0.305b (0.109)	0.585a (0.070)	0.722a (0.055)
2%	16	1.09ab (0.36)	0.691ac (0.199)	0.331a (0.104)	0.555a (0.068)	0.726a (0.069)
8%	16	0.56c (0.34)	0.566bc (0.252)	0.199c (0.121)	0.574a (0.109)	0.753a (0.046)
$LSD_{0.05(75)}$		0.24	0.127	0.077	0.051	0.629*

* Sample size = 6 for each diet; $LSD_{(0.05,25)}$ is given

Table 2. Nutritional indices for larvae of *O. leucostigma* fed for the entire ultimate instar on one of five test diets. Means are given, with standard deviations in parentheses. Means followed different letters are significantly different at $P < 0.05$ by LSD

Diet	n	RGR	RCR	ECD	AD	AD(N)
0%	18	1.64ab (0.25)	0.844a (0.126)	0.459a (0.054)	0.460a (0.042)	0.650a (0.072)
0.5%	13	1.78a (0.30)	0.988b (0.202)	0.423a (0.060)	0.476ab (0.050)	0.657a (0.070)
1%	13	1.55b (0.29)	0.901ab (0.154)	0.401a (0.032)	0.486a (0.035)	0.664a (0.075)
2%	13	1.53b (0.20)	0.827a (0.143)	0.440a (0.088)	0.474ab (0.059)	0.655a (0.063)
8%	18	1.77a (0.29)	0.987b (0.190)	0.455a (0.110)	0.444b (0.045)	0.689a (0.030)
$LSD_{0.05(70)}$		0.20	0.120	0.064*	0.034	0.764**

* equality of means determined by Games and Howell (1976) Approximate Test for Equality When Variances are Heterogeneous

** Sample size is 6 for each diet; $LSD_{(0.05,25)}$ is given

Table 3. Foregut and mid-midgut pH for *M. disstria* and *O. leucostigma* after feeding on control diet through the penultimate instar and one of five test diets for the first 72 hours of the ultimate instar. Means are given, with standard deviations in parentheses. Means followed by different letters are significantly different at $P<0.05$ by LSD

Diet	*O. leucostigma*			*M. disstria*		
	n	Foregut pH	Midgut pH	n	Foregut pH	Midgut pH
0%	8	7.55a (0.45)	10.18a (0.24)	20	7.74a (0.38)	10.07ab (0.59)
0.5%	6	7.43a (0.32)	10.20a (0.40)	17	7.44bc (0.30)	9.81ab (0.57)
1%	7	7.49a (0.35)	10.29a (0.48)	18	7.45bc (0.29)	9.99ab (0.67)
2%	7	7.50a (0.42)	10.29a (0.29)	17	7.53ab (0.29)	9.70a (0.42)
8%	7	7.37a (0.38)	10.30a (0.44)	20	7.22c (0.61)	10.10b (0.60)
$LSD_{0.05(70)}$		0.43	0.41	$LSD_{0.05(87)}$	0.27	0.38

Table 4. Nutritional indices and final weight for *M. disstria* fed one of five test diets during the first 70 hours of the ultimate instar. Means are given, with standard deviations in parentheses. Means followed by different letters are significantly different at $P<0.05$ by LSD

Diet	n	RGR	RCR	ECD	AD	AD(N)	Final Dry Weight (g)
0%	13	0.327a (0.096)	1.14abc (0.25)	0.548a (0.080)	0.507a (0.052)	0.798a (0.031)	0.0200a (0.0086)
0.5%	15	0.386ab (0.065)	1.11ab (0.15)	0.715b (0.086)	0.486ab (0.041)	0.810a (0.017)	0.0247ab (0.0060)
1%	15	0.330a (0.091)	1.04a (0.17)	0.739b (0.130)	0.450b (0.040)	0.798a (0.020)	0.0216ab (0.0059)
2%	16	0.371ab (0.072)	1.19bc (0.12)	0.678b (0.086)	0.469b (0.057)	0.795a (0.034)	0.0245ab (0.0077)
8%	16	0.397b (0.097)	1.24c (0.19)	0.675b (0.085)	0.472ab (0.063)	0.826a (0.021)	0.0263b (0.0077)
$LSD_{0.05(70)}$		0.062	0.13	0.069	0.038	0.328*	0.0053

* Sample size is 5 for each diet; $LSD_{(0.05,20)}$ is given

the entire ultimate instar, no adverse effects were evident when measurements were restricted to the first 70 hours of the instar (Table 4). Rather, *M. disstria* larvae appear initially to benefit from ingestion of tannic acid. Larvae fed 8% tannic acid diet displayed significantly higher RGR, ECD, and final weight, and consumed significantly more food than larvae fed the control diet, and all tannin-containing diets produced significant increases in ECD. While AD was significantly reduced by diet containing 1% tannic acid, it was not affected by diet containing 0.5%, 2%, or 8% tannic acid. Moreover, no tannin-containing diet produced a significant decrease in AD(N) relative to the control diet (Table 4), indicating again that tannic acid did not reduce the ability of *M. disstria* to digest dietary protein.

Table 5. Survivorship during each of the first four instars of *M. disstria* larvae fed artificial diet containing 0%, 1%, 2%, or 8% tannic acid. Sample size at the onset of each instar is given in parentheses

Diet	Instar			
	I	II	III	IV
0%	0.773 (176)	0.912(136)	0.976(124)	0.992(121)
1%	0.818 (131)	0.879 (107)	0.447 (94)	0.000 (42)
2%	0.953 (148)	0.943 (141)	0.669 (133)	0.000 (89)
8%	0.987 (148)	0.932 (146)	0.356 (136)	0.000 (48)
	$\chi^2=45.89$ $P<0.001$	$\chi^2=4.49$ $P>0.2$	$\chi^2=126.01$ $P<0.0001$	$\chi^2=299.46$ $P<0.0001$

Table 6. Number of days from egg hatch to the onset of the n^{th} instar for *M. disstria* larvae fed one of four test diets (mean ± S.D.). Sample sizes are given in parentheses. Means followed by different letters are significantly different at $P<0.05$ by Mann-Whitney U test

Diet	Egg to 2^{nd}	Egg to 3^{rd}	Egg to 4^{th}	Egg to 5^{th}
0%	3.85±0.68a (134)	7.07±0.85a (124)	11.23±1.29a (121)	15.95±1.33 (120)
1%	3.94±0.39a (106)	8.26±0.81c (88)	14.78±1.76c (36)	none survived
2%	4.01±0.85a (141)	8.11±1.32bc (131)	13.56±1.59b (82)	none survived
8%	3.69±0.83b (147)	7.85±0.72c (136)	14.23±1.77bc (48)	none survived

The fact that tannic acid causes reduced growth and efficiency of food utilization over the entire ultimate instar (Table 1) while improving performance in the first part of the instar obviously implies that adverse effects are manifested only during the latter part of the instar.

The effect of tannic acid on early instar growth and survivorship of M. disstria

No *M. disstria* larvae survived past the fourth instar when fed artificial diet containing 1%, 2%, or 8% tannic acid (Table 5). Ingestion of tannic acid resulted in significantly higher survivorship of first instar larvae, but significantly lower survivorship of third and fourth instar larvae. Developmental rate was also significantly reduced by tannic acid after the first instar (Table 6). By the end of the second instar, larvae on all tannic acid diets were conspicuously smaller than those on the control diet.

Discussion

Clearly, not all insects that normally feed on tannin-containing foliage are immune to the potential adverse effects of tannins. Growth, development, and survivorship of *M. disstria* are dramatically reduced by tannic acid in an artificial diet. Indeed, the very high mortality of *M. disstria* larvae fed artificial diet containing 0.5% tannic acid poses the question of how this species avoids the toxicity of tannins present in their normal food plants. Perhaps tannins ingested by *M. disstria* in nature are less toxic than tannic

acid, or perhaps their toxicity is diminished by interactions with other dietary components. In any event, it is clear that not all tannin-feeding insects are tannin-adapted insects. In contrast, *O. leucostigma* is unaffected by ingested tannic acid and does appear to be tannin-adapted.

The hypothesis that tannins possess generalized digestibility-reducing properties that make them particularly resistant to counteradaptation is not supported by the responses of *M. disstria* and *O. leucostigma* to tannic acid. For *M. disstria,* tannic acid does not act as a digestibility-reducing substance but, instead, acts as a toxin and a feeding deterrent. Tannic acid did not reduce the ability of *M. disstria* larvae to digest either whole food or dietary protein, but did significantly reduce relative consumption rate (RCR) and the efficiency with which digested food is converted into larval biomass (ECD). The designation of tannic acid as a "toxin" follows from its demonstrated ability to cause mortality of *M. disstria.* When fed to larvae from the time of hatching, tannic acid caused 100% mortality by the fourth instar and, when fed to larvae during the ultimate instar, tannic acid caused over 60% mortality during the ensuing pupal stage. As would be expected of a toxin, mortality was independent of tannic acid concentration over the range of concentrations used in this experiment.

The finding that, when present, adverse effects of tannins are due to toxicity or feeding deterrence and not to digestibility-reduction is consistent with the results of other studies. Incorporation of 20% tannic acid in the diet of the grasshoppers *Locusta migratoria* and *Oedaleus senegelensis* significantly reduced ECD and RCR, but did not affect AD (Bernays 1978; Bernays et al. 1981). Both tannic acid and the condensed tannin quebracho significantly reduced RCR and ECD of the southern armyworm, *Spodoptera eridania,* but did not affect AD (Manuwoto and Scriber 1986). *Liriodendron* tannins increased mortality of the black swallowtail, *Papilio polyxenes* (Berenbaum 1983), presumably due to bacterial septicemia resulting from rupture of the gut wall (Steinly and Berenbaum 1985). Cotton condensed tannin acted only as a feeding deterrent for the cotton budworm, *Heliothis zea* (Klocke and Chan 1982; Reese et al. 1982). In the only in vitro study of unambiguous biological relevance, even extremely high levels of tannic acid failed to inhibit digestion of protein by enzymatically active gut fluid of the tobacco hornworm, *Manduca sexta* (Martin et al. 1987).

The response of *O. leucostigma* to dietary tannic acid suggests that counteradaptations to tannins are not particularly difficult to evolve. Growth, digestive efficiency, and conversion efficiency of ultimate instar *O. leucostigma* larvae were not influenced by ingestion of up to 8% tannic acid, and relative consumption rate was significantly increased on diets containing 0.5% and 8% tannic acid. Indeed, *O. leucostigma* resembles an "adapted specialist" that is immune to, and stimulated to feed by, a secondary compound that is toxic to "nonadapted" insect herbivores.

A growing number of studies of both Orthoptera and Lepidoptera indicates that counteradaptation to tannins may be common among tree-feeding herbivores. All five species of tree-feeding acridid grasshoppers tested by Bernays et al. (1980) were unaffected by up to 20% tannic acid. Similarly, the silkmoth, *Callosamia promethea,* was unaffected by up to 3% tannic acid or quebracho condensed tannin (Manuwoto and Scriber 1986), and the tiger swallowtail, *Papilio glaucus,* was unaffected by natural concentrations of tannin extracted from its host *Liriodendron* (Berenbaum 1983).

Moreover, adapted herbivores may benefit from the presence of tannins in their food plants. Both AD and ECD increased significantly when tannic acid was added to the diet of the tree locust, *Anacridium melanorhodon* (Bernays et al. 1980). In addition, tannic acid has been shown to stimulate feeding of a variety of tree-feeding Lepidoptera, including *Orygia leucostigma* (this study), *O. antiqua, Lymantria dispar, Euproctis chrysorrhoea, Malacosoma neustria, Operophtera brumata, Amphidasis beularia, Acronycta aceras,* and *Phalera bucephala* (Gornitz 1954 in Bernays 1981).

To date, four mechanisms have been proposed to confer a general immunity to tannins: alkaline guts (Feeny 1970; Berenbaum 1980), gut surfactants (Martin and Martin 1984), digestive *B*-glucosidases (Bernays and Woodhead 1982), and adsorptive peritrophic membranes (Bernays 1978). Of these, only adsorptive peritrophic membranes necessarily result in the inactivation of tannins. Gut alkalinity, surfactancy, and digestive B-glucosidases are proposed to confer immunity by preventing the formation of insoluble tannin-protein complexes. In doing so, however, these three widespread characteristics of insect gut fluid may increase the probability that solublized tannins or their component phenolic acids pass into the body cavity, and therefore may actually potentiate the ability of tannins to exert toxic effects on insect herbivores. This may in part account for the vulnerability of *M. disstria* to tannic acid despite midgut pH in excess of 9.7.

Given that tannins are toxins or feeding deterrents and not digestibility-reducing substances, the assumption that apparent and unapparent plants are defended by chemicals with fundamentally different modes of action must be questioned. This study and others make it increasingly clear that, like small ephemeral plants, large long-lived plants are defended by toxic secondary chemicals.

Acknowledgements. I am grateful to J. Cresswell, J. Lehman, B. Rathcke, H. Van't Hof, and particularly M.M. Martin for constructive criticisms on an earlier draft of this manuscript. This work was supported by NSF Grant BSR-8421268 to M.M. Martin and by a Block Grant from the University of Michigan Rackham School of Graduate Studies.

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Received August 18, 1988