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TANNIN SENSITIVITY IN LARVAE OF *Malacosoma disstria* (LEPIDOPTERA): ROLES OF THE PERITROPHIC ENVELOPE AND MIDGUT OXIDATION

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Abstract—Final-instar *Malacosoma disstria* fed artificial diets containing tannic acid develop lethal pupal deformities. We examined some of the factors potentially underlying tannin sensitivity in this species, including the permeability of the peritrophic envelope to tannic acid and the chemical fate of tannic acid in the gut. Tannic acid does not penetrate the peritrophic envelope of *M. disstria*, demonstrating that the containment of tannic acid within the endoperitrophic space is not sufficient to protect an insect herbivore from the adverse effects of ingested tannins. Ingested tannic acid undergoes extensive chemical modification in the midgut. Only 19–21% of the high molecular weight components of the tannic acid ingested was recovered in the frass. Of two possible chemical fates of ingested tannic acid, oxidation is the predominant chemical transformation, whereas little hydrolysis occurs. Measurements of gut redox parameters showed that conditions in the midgut favor the oxidation of phenols. However, similar conditions occur in the midguts of *Orgyia leucostigma*, in which no oxidation occurs. Therefore, oxidizing gut redox conditions do not necessarily lead to polyphenol oxidation in lepidopteran larvae. We conclude that the sensitivity of *M. disstria* to ingested tannins is a consequence of their oxidation in the midgut.

Key Words—Lepidoptera, Lasiocampidae, *Malacosoma disstria*, *Orgyia leucostigma*, larva, tannin, tannic acid, peritrophic membrane, oxidation.

INTRODUCTION

Insect herbivores vary greatly in their sensitivity to ingested tannins. In tannin-sensitive species, low levels of dietary tannins can have a variety of adverse

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consequences, ranging from reduced growth and abnormal development to death. Whereas at one time it was believed that the adverse effects of tannins resulted from their proclivity to bind with proteins in the gut and inhibit protein digestion (Feeny, 1976; Rhoades and Cates, 1976), it is now recognized that tannins and other polyphenols can act by a variety of different mechanisms in insects, including inhibition of feeding (Schultz, 1989; Hagerman and Butler, 1991), reduction in the efficiency of utilization of nutrients (Felton et al., 1989), and formation of lesions in the epithelial layer of the midgut (Bernays et al., 1980; Steinly and Berenbaum, 1985). It seems likely that in many cases the agent responsible for these effects is an oxidation product (e.g., a quinone) or a by-product of oxidation (e.g., superoxide anion radicals, hydroxyl radicals, or hydrogen peroxide) rather than the polyphenol itself (Appel and Martin, 1990; Appel, 1993).

Two factors are likely to be important in determining whether an insect will be adversely affected by ingested tannins: (1) the permeability of the peritrophic envelope (multiple layers of peritrophic membranes) to tannins or cytotoxic transformation products of tannins, and (2) the chemical conditions in the midgut that determine the chemical fate of ingested tannins.

We have recently reported a study of the permeability of the peritrophic envelope to tannic acid in tannin-tolerant larvae of the white-marked tussock moth, *Orgyia leucostigma* (Lymantriidae), and of the chemical fate of ingested tannic acid in this species (Barbehenn and Martin, 1992). We determined that in these larvae the peritrophic envelope is impermeable to tannic acid and that virtually all (90–100%) ingested tannic acid is egested unchanged in the frass. Thus, tannin tolerance was associated with the effective containment of tannic acid within the endoperitrophic space and a lack of oxidation of tannic acid in the gut. In his seminal study of tannins and the oak moth, *Operophtera brumata*, Feeny (1970) also presented evidence suggesting that the peritrophic envelopes of *O. brumata* larvae are impermeable to tannins.

In the present study we have undertaken a similar exploration of the fate of ingested tannic acid in the tannin-sensitive larvae of the forest tent caterpillar, *Malacosoma disstria* (Lasiocampidae), a polyphagous tree-feeder (Stehr and Cook, 1968). Despite a wide host range that includes many tannin-producing trees (e.g., *Quercus* spp.), *M. disstria* larvae are highly sensitive to dietary tannins. When as little as 0.5% (dry weight) tannic acid is included in an artificial diet, early-instar larvae are unable to complete development, and larvae fed such a diet during the final instar develop lethal pupal deformities (Karowe, 1989). Like many oak-feeding Lepidoptera, this species feeds on its hosts during the time of leaf expansion, when tannins are present at their lowest levels (Feeny, 1968).

In this study, our experiments address the following questions: (1) Is the peritrophic envelope of *M. disstria* larvae permeable to tannic acid? (2) Is tannic acid chemically transformed during its passage through the gut? (3) If chemical

transformation occurs, in what region(s) of the gut does this process take place? (4) By what process(es) are tannins chemically transformed? and (5) Are there differences in the redox conditions in the midguts of the tannin-sensitive larvae of *M. disstria* and the tannin-tolerant larvae of *O. leucostigma*?

METHODS AND MATERIALS

Insects and Diets. M. disstria and *O. leucostigma* eggs were obtained from the Forest Pest Management Institute (Sault Ste. Marie, Ontario). Larvae were reared at 22°C under a 16-hr light–8-hr dark photoperiod at 70% average relative humidity, unless otherwise noted. *M. disstria* larvae were fed an artificial diet (Addy, 1969), modified by substituting 7.0 g of agar for sodium alginate and adding 4.0 g of methyl paraben (Sigma) per 605 g of dry diet. *O. leucostigma* larvae were reared on *O. leucostigma* diet (Bio-Serv Inc., Frenchtown, New Jersey) unless otherwise indicated.

Permeability of Peritrophic Envelope to Tannic Acid. Mid-fifth-instar (final-instar) larvae ($N = 11$) were fed freshly prepared diet containing 3% dry wt tannic acid (Sigma lot No. 64F-0049) for two days. Intact guts were dissected from chilled (−20°C, 7 min) larvae, the fore- and hindguts were ligated with silk sutures (size 6-0), and a small hole (average area 1.5 $mm^2 \pm 0.1$ SE) was cut through the mid-midgut wall to expose the peritrophic envelope (Barbehenn and Martin, 1992). Dissected gut preparations were rinsed in three saline solutions (227 mM KCl, 3 mM NaCl, 300 mM fructose, pH 6.1) for 30–60 sec and incubated in 2.0 ml of the same solution in a shaker bath (2 hr, 23°C). Aliquots (1.5 ml) of incubating solution were lyophilized, redissolved in 0.75 ml of mobile phase (20% (v/v) aqueous acetonitrile and 1% (v/v) acetic acid) and filtered (0.45 μm, Gelman Acrodisc). Tannic acid in these solutions was quantified using HPLC (10-μl injection; Waters 10-μm C-18 column, 4.6 × 250 mm; flow rate 1.0 ml/min; Shimadzu UV detector, 280 nm, 0.002 AU). Peak areas were integrated with a Shimadzu C-R4A Chromatopac. Control larvae fed a tannin-free diet ($N = 3$) were prepared similarly to determine the presence of interfering peaks in the HPLC chromatograms. The midgut contents of each larva were dried (60°C, two days) and weighed to calculate the amount of tannic acid initially present in each midgut.

In a second test of the permeability of the peritrophic envelope to tannic acid, the above procedure was repeated with the following modifications: final-instar larvae ($N = 11$) were fed artificial diet containing 8% (dry wt) tannic acid or control diet ($N = 2$), holes were cut through the anterior midgut wall, gut preparations were incubated in a pH 7.5 buffered saline solution containing 3 mM sodium chloride, 228 mM potassium chloride, 41 mM magnesium sulfate, 20 mM calcium chloride, 52 mM Tris (hydroxymethyl) aminomethane (Sigma),

7.5 mM Tris(hydroxymethyl)aminomethane hydrochloride, and 440 mM fructose, and 30-μl samples were analyzed using HPLC.

Permeability of Peritrophic Envelope to Brown Pigments. The oxidation of tannins and other polyphenols produces brown pigments that can be quantified by measuring their absorbance at 420 nm (Cilliers and Singleton, 1989). We observed that brown pigments leached out of the fenestrated midguts of *M. disstria* that contained tannic acid. To quantify the amounts of brown pigments that permeated the peritrophic envelope, the incubating solutions from the second experiment described in the previous section (8% tannic acid) were analyzed with HPLC as described above, with the exception that the absorbance of the eluate was measured at 420 nm.

Tannic Acid Budget. Final-instar larvae (N = 13) were placed individually in 30-ml plastic cups with ventilated lids. Larvae were fed artificial diet containing 3% (dry wt) tannic acid for three days, following which they were fed a tannin-free diet until all dark-colored (tannin-containing) frass had been collected. Larvae fed the tannin-free rearing diet (N = 4) served as controls to measure the presence of any substances in the frass that interfere with the measurement of egested tannic acid. Food and frass samples were collected daily, frozen (−20°C), and lyophilized when the collection was completed. The amount of food consumed was calculated from the amount of frass produced and the approximate digestibility of the diet: (mg dry wt frass)/(1 − 0.555), where 0.555 is the value for the approximate digestibility (AD) for final-instar *M. disstria* larvae fed artificial diet containing 2% tannic acid (Karowe, 1989). The percent of ingested tannic acid remaining in the frass was calculated using the formula: (mg frass × % TA in frass)/(mg ingested diet × % TA in diet).

To prepare samples for tannic acid analysis, lyophilized samples (3–7 mg) of control diet (N = 3), frass from larvae fed the control diet (N = 4), 3% tannic acid diet (N = 4), and frass from larvae fed the tannin-containing diet (N = 13) were pulverized with a glass rod in screw-cap centrifuge tubes (1.5 ml) and extracted in 70% acetone (2 × 1.0 ml, 50°C, 45 min) in a shaker. Following centrifugation (13,500g, 15 min), supernatant solutions were pooled within samples, concentrated to 1.0 ml in a stream of nitrogen, lyophilized, redissolved in 1.0 ml of 20% (v/v) aqueous acetonitrile containing 1% (v/v) acetic acid, and filtered (0.45 μm). Filtered samples were analyzed by HPLC as described above. A tannic acid standard curve was constructed from a series of tannic acid solutions prepared by serial dilution. Artificial diet contained nontannin compounds that coeluted with the lower-molecular-weight components of tannic acid (peaks a and b, Figure 1A below). In order to quantify higher-molecular-weight tannins (peaks c and d, Figure 1A below), areas of nontannin peaks (identified in control samples) with retention times greater than 4.0 min were subtracted from corresponding peak areas in chromatograms of extracts of tannin-containing samples.

Tannic Acid Levels Along the Gut. We dissected samples of the contents of foreguts (0.2–4.0 mg dry wt), anterior midguts (0.8–1.4 mg dry wt), posterior midguts (0.7–1.8 mg dry wt), and hindguts (0.4–2.2 mg dry wt) of chilled (−20°C, 7 min) larvae that had been fed either a diet containing 3% tannic acid ($N = 11$) or a tannin-free diet ($N = 3$). Fresh weight-to-dry weight conversion factors for foregut, midgut, and hindgut samples were calculated from control larvae. Samples of gut contents were extracted in 70% acetone (1.0 ml) and centrifuged (10,000*g*, 10 min). Supernatant solutions (0.5 ml) were partially evaporated in a stream of nitrogen, lyophilized, redissolved in mobile phase (1.0 ml), filtered (0.45 μm), and the high-molecular-weight component d was quantified using HPLC, as described above.

Gallic Acid Budgets. Two- to 3-day-old final-instar *M. disstria* larvae ($N = 13$), reared at 22° and 24°C, were fed artificial diet containing 2% dry wt gallic acid (Sigma) for three days followed by gallic acid-free diet. Egested gallic acid was recovered by collecting all dark brown or black frass. Frass and diet samples were collected daily, frozen (−20°C), and lyophilized. Control larvae ($N = 5$) were maintained on the gallic acid-free rearing diet. Control and gallic acid-containing diet (25–30 mg) and frass (5–30 mg) samples were extracted in 50% methanol (3 × 1.0 ml, 50°C, 30 min) and analyzed for gallic acid using the rhodanine assay (Inoue and Hagerman, 1988) as described in Barbehenn and Martin (1992). The percent of ingested gallic acid remaining in the frass was calculated using the formula: (mg frass × % GA in frass)/mg ingested diet × % GA in diet), with the amount of ingested diet calculated as above assuming an AD of 55.5%.

Diet and frass samples collected for the measurement of a tannic acid budget (above) were also analyzed for gallic acid to determine the extent to which tannic acid was hydrolyzed by *M. disstria.* All samples were assayed for gallic acid using the rhodanine assay and a budget was calculated as described above.

Quantification of Phenol Oxidation. To compare the extent to which tannic acid is oxidized in larvae of *M. disstria* and *O. leucostigma*, the amount of brown pigment (oxidation products) that could be extracted from the frass of larvae consuming tannin-free and tannin-containing diets was determined. Final-instar larvae were assigned at random to a control (tannin-free) diet ($N = 24$ *M. disstria*, $N = 10$ *O. leucostigma*) or tannin-containing (1% tannic acid) diet ($N = 27$ *M. disstria*, $N = 10$ *O. leucostigma*). Food and frass samples were collected and dried (60°C, >5 days) daily during the entire instar. After grinding with a mortar and pestle, 10 to 15 mg subsamples were extracted in 70% acetone (1.5 ml, 50°C, 1 hr) in a shaker. Samples were centrifuged (13,500*g*, 5 min) and the absorbance was measured with a Zeiss spectrophotometer (420 nm) (Cilliers and Singleton, 1989). The background absorbance (A_{420}/mg sample) from each diet extract was subtracted from each frass extract to give a net

production of brown pigment per milligram of frass. Means were compared by Mann-Whitney U-tests.

The formation of brown pigments (A_{420}/mg sample) was also measured in frass of final-instar *M. disstria* larvae fed 2% gallic acid-containing diet. Methanolic extracts used for the measurement of gallic acid were diluted with 50% methanol (3.5 ml), and the absorbance of each solution was measured with a spectrophotometer. Measurements of background absorbance in control and gallic acid-containing diets were made and used to correct levels of brown pigments extracted from frass. Measurements of A_{420} per milligram of frass were multiplied by 4.33 (dilution factor) to allow their comparison with measurements of tannic acid oxidation.

Gut Acid-Base and Redox Conditions. To compare the acid–base and redox conditions in the guts of *M. disstria* and *O. leucostigma*, pH and redox potentials were measured in the foreguts and midguts of larvae that had been chilled (−20°C, 7 min) prior to dissection. pH was measured using a microneedle pH electrode (Microelectrodes MI-408P) and a silver–silver chloride reference electrode (Microelectrodes MI-401). Redox potentials were measured using a 0.02-in. platinum electrode (Microelectrodes MI-800) and a silver–silver chloride reference electrode. To minimize exposure of the gut contents to air, the microelectrodes were inserted together through small adjacent holes cut through the gut wall. Because of the greater stability of the redox potential measurements, pH measurements were made first. All measurements were made using a Metrohm/Brinkman (model 103) millivolt and pH meter. Observed redox potentials were converted to standard redox potentials (E_h) by adding 200 mV, and pe (electron availability) was calculated as $E_h/59.2$ (Appel and Martin, 1990). Overall redox conditions were summarized by the "redox parameter" (pH + pe) (Appel and Martin, 1990).

RESULTS

In order to test the permeability of the peritrophic envelope of *M. disstria* to tannic acid, we fed larvae a diet containing tannic acid and tested for the presence of tannic acid in the incubating solutions surrounding excised guts in which small holes had been cut to expose the peritrophic envelope. A chromatogram of the tannic acid included in the larval diet is illustrated in Figure 1A. Commercial tannic acid is composed of galloyl esters of glucose along with some unesterified gallic acid. Peak a is gallic acid; peaks b–e are galloyl esters of glucose, which differ in the number of galloyl groups attached to the glucose moiety. None of the higher-molecular-weight components of tannic acid (peaks c–e) could be detected in the incubation solution after a 2-hr incubation period (Figure 1C). Indeed, chromatograms of incubating solutions surrounding the

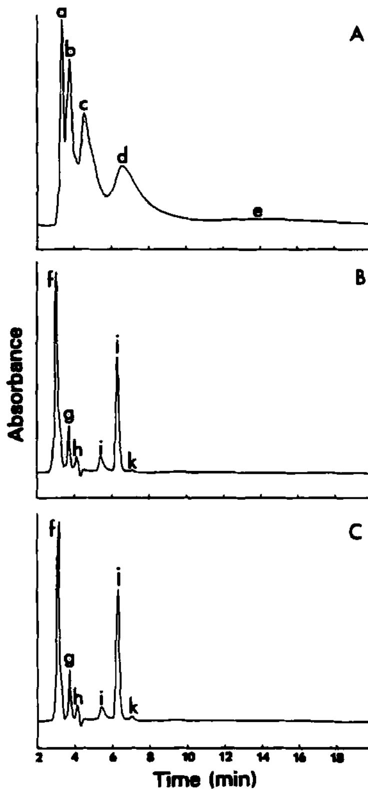

FIG. 1. *Malacosoma disstria* peritrophic envelope permeability to ingested tannic acid. (A) Chromatogram of tannic acid; (B) representative chromatogram of the incubating solution surrounding the gut of a larva fed a tannin-free diet ($N = 3$); (C) representative chromatogram of the incubating solution surrounding the gut of a larva fed a tannic acid-containing diet ($N = 11$). No compounds eluted before 2 min.

excised guts of larvae fed control (Figure 1B) and tannin-containing diets (Figure 1C) were virtually identical. The midguts of larvae fed the 3% tannic acid diet contained an average of 50 μg of peaks c and d of tannic acid. The sensitivity of the analytical method was such that we could have detected 0.00125 μg of tannic acid per microliter of incubating solution. Thus, we calculate that no more than 5% of the ingested tannic acid penetrated the peritrophic envelope during the 2-hr incubation period. Peaks f and g (Figure 1C) include compounds in the incubation solution but could also include small amounts of gallic acid (peak a) and a low-molecular-weight polyphenol (peak b) from ingested tannic acid.

This experiment was repeated using procedures that would ensure the presence of larger amounts of tannic acid in the midgut and the detection of smaller quantities in the incubating solution. When larvae were fed an 8% tannic acid diet, none of the polyphenols c or d could be detected in the incubating solutions, indicating that less than 0.3% of these polyphenols in the midgut penetrated the peritrophic envelope.

We quantified the amount of polyphenols c and d in tannic acid (Figure 1A) in the food and frass of *M. disstria* larvae to construct a tannic acid budget. Tannic acid was stable in the artificial diet; the efficiency of recovery of tannic acid from diet changed from virtually 100% soon after it was prepared to 94% after it was incubated for 24 hr under the conditions of the feeding experiment. Of the ingested polyphenols, only 21% (±2.4 SE) could be accounted for in the frass (Table 1). The remaining 79% was chemically transformed into substances that could not be detected using HPLC analysis.

The region of the digestive tract of *M. disstria* larvae in which the chemical transformation of tannic acid is most extensive was determined by a comparison of the percent of polyphenol d (Figure 1A) in the contents of the foregut, anterior midgut, posterior midgut, and hindgut (Table 2). There is a steady decrease in the percent of this tannic acid component as food passes from the foregut to the hindgut. Assuming that no food is assimilated from the foregut (Dow, 1986), but that 55.5% is assimilated during its passage through the midgut (Karowe, 1989), it can be calculated from the data summarized in Table 2 that only 29.0% of the tannic acid present in the food is still present in the posterior midgut (containing 0.27% polyphenol d). In the hindgut, only 18.7% of the tannic acid present in the food is still present (Table 1). These results clearly identify the midgut as the region in which the transformation of most of the tannic acid occurs.

Hydrolysis and oxidation are two chemical transformations that might reduce the levels of tannic acid in the guts of *M. disstria*. Although the measurement of gallic acid in the frass of an insect fed tannic acid might provide evidence for the hydrolysis of tannic acid, the amount of gallic acid present may be affected by either assimilation (Bernays et al., 1983; Kato, 1978) or oxidation (this study). Nonetheless, the results summarized in Table 1 suggest that hydrolysis accounts for the disappearance of only a small fraction of the tannic acid ingested by *M. disstria*. The tannic acid used in our experiments contained free gallic acid as an impurity (7%). Given that the larvae, therefore, ingested 0.14 mg of free gallic acid and that the hydrolysis of pure tannic acid generates 70% of its weight in gallic acid, and assuming that gallic acid is assimilated or oxidized with an efficiency of 93.6% whether it is ingested or produced by hydrolysis, then the 0.012 mg of gallic acid present in the frass can be accounted for by the hydrolysis of only 4% of the tannic acid ingested.

Phenol oxidation generates brown pigments (Hathway and Seakins, 1957;

TABLE 1. DRY MASS BUDGETS FOR TANNIC ACID AND GALLIC ACID INGESTED BY FINAL-INSTAR *Malacosoma disstria* LARVAE

	Tannic acid			Gallic Acid		
Diet (*N*)	Ingested	Egested	Remaining (%)	Ingested	Egested	Remaining (%)
3% TA (13)	1.05 ± 0.17[a]	0.25 ± 0.05[a]	21.0 ± 2.4	0.14 ± 0.02[c]	0.012 ± 0.002[c]	7.9 ± 0.8
3% TA (11)	0.030 ± 0.004[b]	0.006 ± 0.001[b]	18.7 ± 4.1			
2% GA (11)				2.52 ± 0.15	0.16 ± 0.02	6.4 ± 0.7

[a]Tannic acid peaks c and d; the total amount of tannic acid ingested (peaks b–e) is 1.72 mg. Data are presented as mean ± SE.
[b]Tannic acid peak d in fresh samples of diet and frass pellets dissected from the hindgut.
[c]Free gallic acid present as an impurity (7%) in tannic acid.

Thomson, 1962; Leatham et al., 1980; Igarashi and Yasui, 1985; Cilliers and Singleton, 1989). In *M. disstria* larvae fed a tannin-containing diet, the contents of the mid- and posterior midgut change from light tan to dark brown or green–brown, suggesting the oxidation of tannins. Pigment formation in the guts of tannin-fed larvae was quantified by measuring the absorbance at 420 nm of extracts of the frass of *M. disstria* larvae fed tannin-free and tannin-containing artificial diets. The amount of brown pigment in the frass of *M. disstria* larvae fed a diet containing tannic acid is significantly greater ($P < 0.0001$) than the amount in the frass of larvae fed a tannin-free control diet (Figure 2). Brown pigments were present in larger amounts in the frass of male than of female

TABLE 2. PERCENT TANNIC ACID IN DIET AND GUTS OF FINAL-INSTAR *Malacosoma disstria* LARVAE[a]

Sample location	Percent tannic acid (N)
Artificial diet	0.99 ± 0.07 (4)
Foregut	0.86 ± 0.08 (11)
Anterior midgut	0.80 ± 0.11 (11)
Posterior midgut	0.64 ± 0.09 (11)
Hindgut	0.42 ± 0.09 (11)

[a]Tannic acid component d (Figure 1A). Data are presented as mean ± SE.

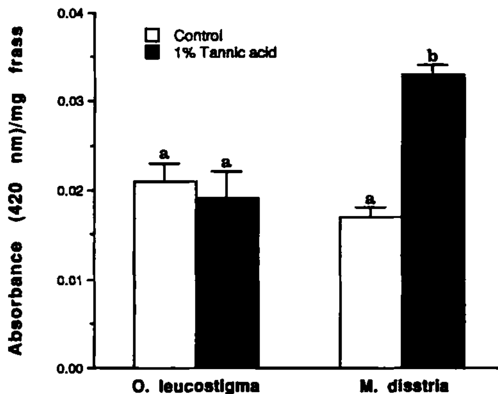

FIG. 2. Tannic acid oxidation in *Malacosoma disstria* (tannin sensitive) and *Orgyia leucostigma* (tannin tolerant). Data from male and female larvae are combined. Means within species were compared by Mann-Whitney U-tests. Different letters above bars denote $P < 0.0001$.

larvae fed both control ($P < 0.0005$) and tannic acid-containing ($P < 0.0004$) diets. Frass extracts from males and females fed control diets averaged 0.019 AU/mg (±0.001) and 0.014 AU/mg (±0.001), respectively. Frass extracts from males and females fed tannic acid-containing diets averaged 0.036 AU/mg (±0.002) and 0.027 AU/mg (±0.001), respectively. Ingested gallic acid was also oxidized by *M. disstria* larvae (Figure 3). Oxidation products (measured as absorbance at 420 nm/mg frass) are elevated eightfold compared to control frass. These measurements cannot be used to calculate the actual amount of phenol oxidized, since the relationship between the amount of absorption at 420 nm by the pigments formed and the amount of phenol oxidized has not been determined.

The concentration of brown pigments that leached through the peritrophic envelopes of *M. disstria* larvae containing tannic acid was elevated twofold compared with their concentration in control solutions (4200 ± 230 AU/μl (mean ± SE) and 2220 ± 70 AU/μl, respectively). Most of the brown compounds eluted between 3.0 and 4.5 min. However, the HPLC procedure we employed did not result in sufficient resolution of peaks to allow us to identify any new compounds generated during the oxidation of tannic acid.

In contrast to the production of brown pigments by *M. disstria* larvae fed a tannin-containing diet, when *O. leucostigma* larvae are fed a diet containing tannic acid their frass does not contain brown pigments in excess of those produced by larvae that consume a tannin-free diet (Figure 2). As noted earlier, tannic acid passes through the guts of *O. leucostigma* larvae without any chem-

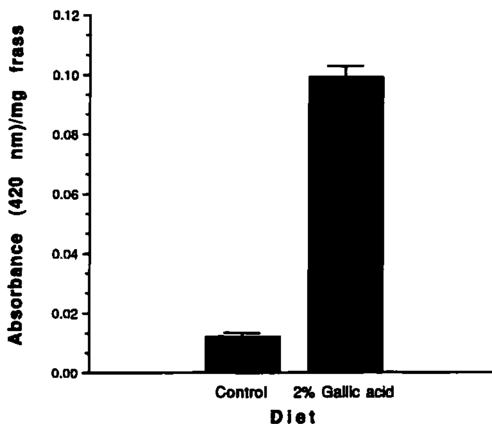

FIG. 3. Gallic acid oxidation in *Malacosoma disstria* larvae. Final-instar larvae were fed artificial diet containing 2% dry wt gallic acid or control diet.

ical modification (Barbehenn and Martin, 1992). These experiments show that phenols are oxidized in the guts of *M. disstria*, but not in *O. leucostigma*.

In seeking an explanation for the occurrence of tannic acid oxidation in the guts of *M. disstria* larvae and the absence of oxidation in the guts of *O. leucostigma* larvae, we compared the redox conditions in their gut lumens. The pH and redox conditions in the guts of *M. disstria* and *O. leucostigma* are similar (Table 3). The midgut pH in both species is close to 10, and as a consequence, ingested polyphenols are ionized. Likewise, the values of pe, a parameter that reflects electron availability (Lindsay, 1979; Appel and Martin, 1990), are similar in both the foreguts and midguts of the two species. As a consequence, the redox parameter (pH + pe), which is a composite measure of the redox status of a complex aqueous system giving equal weight to acid–base status and electron availability (Lindsay, 1979; Appel and Martin, 1990), is also similar in both species. *M. disstria* and *O. leucostigma* maintain weakly oxidizing conditions in their foreguts and strongly oxidizing conditions in their midguts. Thus, while these results are compatible with our observation of the occurrence of oxidation in the guts of *M. disstria* larvae, they provide no explanation for the absence of oxidation in *O. leucostigma*.

DISCUSSION

In this study we have demonstrated that the peritrophic envelope of the tannin-sensitive larvae of *M. disstria*, like the peritrophic envelope of the tannin-tolerant larvae of *O. leucostigma*, is impermeable to tannic acid. Clearly, the mere containment of tannic acid within the endoperitrophic space is insufficient to protect an insect herbivore from the potentially adverse effects of ingested tannins.

The impermeability of the peritrophic envelopes of *M. disstria* and *O.*

TABLE 3. pH AND REDOX CONDITIONS OF FOREGUTS AND MIDGUTS OF *Malacosoma disstria* AND *Orgyia leucostigma*[a]

	pH	Eh (mV)	pe	pH + pe
M. disstria				
Foregut (n = 6)	6.06 ± 0.10	96 ± 6	1.61 ± 0.11	7.67 ± 0.13
Midgut (n = 10)	10.23 ± 0.07	−30 ± 12	−0.51 ± 0.21	9.97 ± 0.18
O. leucostigma				
Foregut (n = 6)	5.58 ± 0.27	115 ± 6	1.94 ± 0.11	7.51 ± 0.30
Midgut (n = 14)	9.80 ± 0.11	−3 ± 11	−0.05 ± 0.19	9.75 ± 0.18

[a] Data are presented as mean ± SE.

leucostigma to the polyphenolic components of tannic acid presents an apparent dilemma, given that these molecules (approx. 3.5–4.7 nm diameter; Barbehenn and Martin, 1992) are smaller than the pore diameters in the peritrophic membranes of Lepidoptera, which have been reported to be greater than 7 nm (Adang and Spence, 1983; Santos and Terra, 1986; Wolfersberger et al., 1986). Two mechanisms might account for the containment of tannic acid within the endoperitrophic spaces of lepidopteran larvae: (1) binding by tannin-binding substances and (2) electrostatic exclusion. The binding of tannins by proteins or other substances (De Veau and Schultz, 1992; Ikeda et al., 1992) has the potential to form complexes with molecular dimensions that exceed the diameters of the pores of peritrophic membranes. Although the presence of tannin-binding proteins has not been examined in insects, they are produced by tannin-adapted vertebrate folivores (Austin et al., 1989). Secondly, the glycosaminoglycan component of the peritrophic membrane is ionized at physiological pH, forming a dense, negatively charged barrier (Miller and Lehane, 1993). Phenolic hydroxyl groups would also be ionized under the strongly basic conditions that prevail in the midguts of *M. disstria* and *O. leucostigma* (Hagerman, 1989). It is possible that electrostatic repulsion between anionic sites in peritrophic membranes and negatively charged polyphenolate ions prevent the diffusion of polyphenols through the peritrophic envelope. The permeability of other extracellular matrices containing glycosaminoglycans has been found to be reduced for dextrans that bear negatively charged groups (Chang et al., 1975).

Our results provide support for the hypothesis that the primary determinant of the effect of ingested tannins on insect herbivores is their chemical transformation in the gut (Appel and Martin, 1990; Appel, 1993). The essential difference between the larvae of *M. disstria* and *O. leucostigma* that explains their tannin sensitivity and tannin tolerance, respectively, appears to be the occurrence of tannin oxidation in *M. disstria*. Whereas 90–100% of the tannic acid ingested by *O. leucostigma* is recoverable (Barbehenn and Martin, 1992), only 19–21% of the tannic acid ingested by *M. disstria* can be recovered from the frass. Three observations suggest that tannic acid ingested by *M. disstria* is oxidized: (1) approximately 80% of ingested tannic acid is chemically transformed, (2) hydrolysis cannot account for the chemical modification of more than 4% of the ingested tannic acid, and (3) tannic acid and gallic acid produce brown pigments in the midguts of *M. disstria*, but not in *O. leucostigma*.

Our results do not support the hypothesis that thermodynamic indicators of gut redox conditions, such as the redox potential (E_h) or the redox parameter (pe $\pm$ pH), are sufficient to predict the oxidation of phenols in lepidopteran larvae (Appel and Martin, 1990; Appel, 1993). Both *M. disstria* and *O. leucostigma* maintain strongly oxidizing redox conditions in their midguts, yet tannic acid is extensively oxidized in the former species but not in the latter. The thermodynamic favorability of oxidation (determined by oxidizing redox

conditions) does not necessarily imply that oxidation is kinetically favorable, i.e., that phenol oxidation will proceed at an observable rate. The absence of phenol oxidation under conditions in which oxidation is favored thermodynamically could be explained by the absence of necessary catalysts (e.g., polyphenol oxidase, laccase) (Felton et al., 1989), the presence of antioxidant systems (e.g., catalase, dehydroascorbic acid reductase, ascorbic acid, α-tocopherol) (Larson, 1988; Felton and Duffey, 1991, 1992; Summers and Felton, 1993) and/or low levels of essential oxidants (e.g., oxygen, hydrogen peroxide).

Although we are currently unable to distinguish among these potential mechanisms, we have made one observation consistent with the hypothesis that low oxygen levels in *O. leucostigma* may explain the absence of phenol oxidation in this species. The contents of the midguts of *O. leucostigma*, which are normally the same color as the diet (tan), turn dark brown in a few minutes after the gut is opened and its contents exposed to air. Oxygen tensions in the guts of insect herbivores have not been measured, to our knowledge. Such measurements might reveal important differences between tannin-sensitive and tannin-tolerant species.

The adverse effects of dietary tannic acid on *M. disstria* are different in early- and late-instar larvae. Early-instar larvae fed tannin-containing diets have low consumption rates, resulting in greatly prolonged development times and the failure to develop beyond the third or fourth instar (Karowe, personal communication; Barbehenn, unpublished). Feeding deterrence by polyphenols in early-instar lepidopteran larvae appears to be common (Isman and Duffey, 1982; Klocke and Chan, 1982; Manuwoto et al., 1985; Manuwoto and Scriber, 1986) and is even exhibited by early-instar larvae of the "tannin-tolerant" species *O. leucostigma* (Barbehenn, unpublished).

By contrast, when late-instar larvae of *M. disstria* are fed tannic acid, consumption and growth rates may be reduced, but little larval mortality occurs (Karowe, 1989). Instead, mortality occurs during the pupal stage and during the eclosion of adults (Karowe, 1989; Barbehenn, unpublished) and is associated with severe deformities in the wings of pupae and adults. Wings are commonly shortened to stubs in pupae (see Karowe, 1989) and are twisted and unexpanded in adults that are able to eclose. Similar wing deformities are the primary symptoms observed when many larval Lepidoptera are fed diets containing inadequate amounts of essential fatty acids (linolenic and/or linoleic acid) (Chippendale et al., 1964; Kato, 1978; Dadd, 1981). We hypothesize that since these polyunsaturated fatty acids are easily destroyed by oxidation (Lea, 1962), tannic acid acts as a prooxidant in *M. disstria*, generating reactive radical oxidants (e.g., superoxide and hydroxyl radicals) that bring about the oxidation of essential fatty acids. We note that, in contrast to *M. disstria*, *O. leucostigma* larvae fed *M. disstria* diet containing 3% tannic acid diet develop normal wings (Barbe-

henn, unpublished). This result demonstrates the adequacy of the *M. disstria* diet for wing formation when the tannic acid remains unoxidized.

Wing deformities have also been observed when *Spodoptera* (*Prodenia*) *eridania* and *Manduca sexta* larvae are fed diets containing L-dopa or L-canavanine, respectively (Rehr et al., 1973; Rosenthal, 1977). In *S. eridania*, the wing deformities closely resemble those observed in *M. disstria* fed tannic acid. The authors postulated that L-dopa may interfere with tyrosinase, resulting in incomplete cuticle hardening and darkening. However, the observations reported Rehr et al. (1973) do not rule out the possibility that the adverse effects of ingested L-dopa are a consequence of its potential to act as a prooxidant that produces a deficiency in an oxidizable nutrient. Although our nutrient deficiency hypothesis remains untested, it is clear that the nature of the detrimental effect of tannins on late-instar *M. disstria* is different from that described previously in other tannin-sensitive insects, in which lesions in the midgut epithelium result in death during the feeding period (Bernays et al., 1980; Berenbaum, 1983; Steinly and Berenbaum, 1985).

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