Effects of Paper Birch Condensed Tannin on Whitemarked Tussock Moth (Lepidoptera: Lymantriidae) Performance

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ABSTRACT This research tested the effects of paper birch, *Betula papyrifera* Marshall, condensed tannin on larval performance of the whitemarked tussock moth, *Orgyia leucostigma* (J. E. Smith). We conducted laboratory bioassays on fifth stadium larvae. Larvae were reared on one of three diets: control (no condensed tannin), moderate condensed tannin (8.8% dry mass), and high condensed tannin (17.6% dry mass). Although survivorship was not different between the treatments, larvae fed diets amended with condensed tannin exhibited increased stadium duration, decreased relative growth rate, and decreased food conversion efficiencies. Prolonged development times enabled larvae to compensate for low consumption and growth rates such that insects on tannin diets ate more and grew larger than insects on the control diet. Analysis of tannin levels in food, frass, and body tissue indicated that larvae do not metabolize condensed tannin, but concentrate and egest it. Our results show that paper birch condensed tannin has both positive and negative effects on the performance indices of whitemarked tussock moths. However, whether the benefits of increased final size (and possibly fecundity) outweigh the risks of increased development time and prolonged exposure to natural enemies remains unclear.

KEY WORDS *Orgyia leucostigma, Betula papyrifera,* whitemarked tussock moth, condensed tannin, paper birch

CONDENSED TANNINS ARE common secondary metabolites of dicotyledonous plants, occurring in ≈80 families (Bate-Smith and Metcalfe 1957, Mole 1993). Although long believed to function as plant defenses against herbivory, recent studies demonstrated that the affects of condensed tannins on insect performance vary by tree species, and are determined by tannin structure and gut physiology (Ayres et al. 1997). For example, insect performance was positively correlated with red oak condensed tannin (Rossiter et al. 1988, Kleiner and Montgomery 1994), minimally or not correlated with trembling aspen condensed tannin (Hemming and Lindroth 1995; Hwang and Lindroth 1997, 1998), and negatively correlated with quebracho condensed tannin (Manuwoto and Scriber 1986).

In this study we used a generalist insect herbivore, the whitemarked tussock moth, *Orgyia leucostigma* (J. E. Smith), to evaluate the effects of paper birch, *Betula papyrifera* Marshall, condensed tannin on insect performance. The whitemarked tussock moth is a common herbivore of eastern forests and feeds on over 140 species of plants, including paper birch (Covell 1984). Condensed tannin is a major secondary metabolite in paper birch (Roth and Lindroth 1994). Research has shown that environmental factors influence levels of condensed tannins in various *Betula* species (e.g., Bryant et al. 1993; Roth and Lindroth 1994; Lindroth et al. 1995; Agrell et al. 1999, 2000). For example, high light and enriched CO_2 increased condensed tannin concentrations in paper birch, and these changes were correlated with decreases in tussock moth performance (survival, pupal mass, development time) (Agrell et al. 2000). Whether increases in condensed tannin levels were responsible for the decrease in insect performance, however, could not be determined. The purpose of this study, therefore, was to evaluate the effect of paper birch condensed tannin on whitemarked tussock moths, a representative generalist insect herbivore.

Materials and Methods

Whitemarked tussock moths were obtained as egg masses from the Canadian Forest Service (Sault St. Marie, Ontario, Canada). Egg masses were surface-sterilized with a solution containing 0.1% sodium hypochlorite and 1% Tween 80. Insect rearing studies were conducted in a Percival (Boone, IA) growth chamber maintained at 26:18°C and a photoperiod of 16:8 h (L:D) h.

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Artificial Diet. To determine the effects of condensed tannin on tussock moth performance we used an artificial diet (gypsy moth diet, ICN Biomedicals, Aurora, OH) to which we added purified tannin. We assayed three diets: control (no condensed tannin), moderate condensed tannin (8.8% dry mass; 1.5% wet mass), and high condensed tannin (17.6% dry mass; 3% wet mass). The latter two represent low and high levels found in natural birch foliage (Kopper and Lindroth 2001).

Condensed tannin was isolated from paper birch leaves collected at the Harshaw experimental farm (U.S. Forest Service) in Oneida County, WI. Leaves were excised at the petiole and stored under crushed ice in the field. Upon return to the laboratory (≈3 h from first leaf excision), leaves were flash-frozen in liquid nitrogen, freeze-dried in a Virtis specimen freeze drier (model 24DX24, Virtis, Gardiner, NY), ground and stored at −20°C. Tannins were purified by absorption chromatography (Hagerman and Butler 1980). They were added to the diets immediately before setting of the agar (≈55°C) to avoid degradation at high temperatures. Diets were refrigerated until used in bioassays. Fresh diets were made every 5 d.

Insect Bioassays. First to fourth instars were reared on artificial diet containing no supplemental tannin. Upon molting to the fifth instar, 25 larvae were randomly assigned to each of the three diet treatments. Single, newly molted larvae were weighed and placed in a Petri dish (15 by 2.5 cm) containing a cube of preweighed diet, and a damp cotton wick to maintain humidity. To preserve freshness, diet was added at 3- to 4-d intervals until the fifth stadium was completed. Males pupated after the fifth instar and were discarded. Females were frozen upon molting to the sixth instar. As a check, we determined the sex of "female" larvae by viewing external pits on the ventral surface of the abdomen. We report data performance data only for female larvae (8–13 per treatment). Larvae, uneaten diet, and frass were freeze-dried, weighed and analyzed for condensed tannin.

We calculated nutritional indices using standard ratio-based formulas (Waldbauer 1968, Scriber 1977), except that initial rather than average dry mass was used for calculating relative growth rate (RGR) and relative consumption rate (RCR) (Farrar et al. 1989). Initial dry mass for larvae was calculated based on the proportional dry mass of 15 newly molted fifth instars. We also calculated duration of fifth instar, average growth rate (AGR), final biomass, total food consumed (TC), and average consumption rate (ACR) for use in analysis of covariance (ANCOVA) (Raubenheimer and Simpson 1992).

Condensed Tannin Analysis. Condensed tannin concentrations in the diet, frass, and newly molted sixth instars were measured using the butanol-HCl method of Porter et al. (1986). Purified paper birch condensed tannin served as the reference standard.

Statistical Analysis. We used a one-way analysis of variance (ANOVA, PROC MIXED, Littell et al. 1996) with treatment as a fixed effect for statistical analysis of RGR, RCR, approximate digestibility (AD), and efficiencies of conversion of digested (ECD) and ingested (ECI) food. Raubenheimer and Simpson (1992) suggested that the use of ANCOVA is more appropriate than use of ratio variables for analysis of nutritional indices. Thus, we performed one-way ANCOVA using treatment as a fixed effect and initial biomass as the covariate (PROC MIXED, Littell et al. 1996) for duration, AGR, final biomass, TC, and ACR. We conducted further ANCOVA analyses to check our results for the ratio-based processing indices AD, ECD, and ECI. For AD, we used frass produced as the dependent variable and consumption as the covariate. For ECD, we used biomass gained as the dependent variable and utilization (consumption –frass) as the covariate. Finally, for ECI, we used biomass gained as the dependent variable and consumption as the covariate. Because results for the covariate analyses of AGR, ACR, and the ratio-based processing indices were qualitatively similar to those for the respective ratio-based parameters, we report and discuss only the ratio-based variables.

Comparisons of condensed tannin concentrations in the diet, frass, and larvae were analyzed using a nonparametric test (Kruskal-Wallis) (PROC NPAR1WAY, SAS Institute 1989) because the variance was heterogeneous across treatments.

Results

Survivorship. Larval survivorship was high (>84%) across all treatments (data not shown). Survivorship rates did not vary among treatments.

Development and Growth. Diet treatment influenced the development, growth rate, and final biomass of fifth-instar tussock moths (Table 1). Larvae reared on the moderate and high tannin diets took longer to develop (44 and 25%, respectively) than those on the control diet. Relative growth rates for larvae reared on moderate and high tannin diets were lower (42 and 29%, respectively) than for larvae reared on the control diet. Perhaps due to the extended development time, larvae reared on the moderate and high tannin diets achieved a higher final biomass (26 and 31%, respectively) than did control larvae.

Consumption and Processing. Birch tannins also influenced consumption and processing of food by tussock moths (Table 2). Tannins decreased relative consumption rates (RCR) by 20% for larvae fed the moderate tannin diet, but did not affect consumption rates for larvae fed the high tannin diet. Possibly due to the extended development time, total consumption by larvae fed moderate and high tannin diets was greater (21 and 26%, respectively) than that by larvae fed the control diet.

No differences were detected among the three treatments for approximate digestibility (AD). In contrast, larvae reared on the moderate and high tannin diets had lower efficiencies of conversion of digested (ECD) and ingested (ECI) food (36 and 29%; 26 and 29%, respectively) than did larvae on the control diet.

Condensed Tannin Determinations. As a check to determine the actual amount of condensed tannin fed

Table 1. Effects of condensed tannin on development and growth of fifth-instar whitemarked tussock months [mean ± SE (*n*)]

Diet	Nutritional indices		
	Duration (d)	RGR (mg/mg × d)	Final biomass (mg)
Control	5.2 ± 0.2a (13)	0.55 ± 0.03a (13)	30.8 ± 2.3a (12)
Moderate tannin	7.5 ± 0.2c (9)	0.32 ± 0.03b (9)	38.9 ± 2.8b (9)
High tannin	6.5 ± 0.2b (13)	0.39 ± 0.03b (13)	40.3 ± 2.3b (12)
df for treatment	2, 31	2, 32	2, 29
F-value for treatment	29.72	11.71	4.82
P-value for treatment	<0.001	<0.001	0.016
df for covariate	1, 31	NA	1, 29
F-value for covariate	0.79	NA	16.14
P-value for covariate	0.380	NA	<0.001

Within a column, values with different letters are significantly different ($P < 0.05$; LSD test, Littell et al. 1996). Standard errors are calculated from the pooled variance. RGR, relative growth rate. NA, not applicable.

to larvae, we analyzed condensed tannin concentrations in the diets. Concentrations of condensed tannin were determined to be 0.1% dry mass (0.0% wet mass) in the control diet, 3.1% dry mass (0.6% wet mass) in the moderate tannin diet, and 7.1% dry mass (1.5% wet mass) in the high tannin diet. These measured levels were less than half the target concentrations. Low detection of condensed tannin in the diet likely resulted from reduced extraction efficiency of tannins bound to protein or other diet components.

Concentrations of tannins in insect frass differed significantly among treatments: 0, 6.3 and 13.7% dry mass for insects reared on the control, moderate and high tannin diets, respectively (Kruskal-Wallis, df = 2, $P = 0.001$). Levels of tannins in sixth instars at the conclusion of the feeding trials were below detection level (<0.1% dry mass) for all three treatments.

Discussion

We found that high (but naturally occurring) levels of condensed tannin negatively affected development time, growth rate (RGR), and assimilation efficiencies (ECD and ECI) of fifth instar whitemarked tussock moths. The reduction in growth rate can be explained primarily by a decrease in the efficiency with which digested food was converted to biomass. Thus, for tussock moths, a significant metabolic cost was detected for processing tannin in the diet. These results are similar to those of Manuwoto and Scriber (1986) who demonstrated that *Spodoptera eridania* (Cramer) larvae reared on tannin-amended diet had lower growth efficiencies than did larvae reared on control diet. Furthermore, when paper birch tannin was amended to leaves, at levels comparable to our moderate tannin diet, growth was significantly reduced for two species of chrysomelid beetle and slightly reduced for a species of papilionid butterfly (Ayres et al. 1997). With respect to ECD, the reason for the decrease is unclear but one possibility is that larvae were more active in the tannin-amended diets.

A reduction in relative growth rate is typically accompanied by increased development time, reduced final biomass and total consumption. In this study, however, prolonged development enabled insects to over-compensate for reduced growth rates, leading to increased final biomass and total consumption. Thus, increased development time for tannin-fed larvae was not due primarily to feeding deterrence, as in the case of fed cotton condensed tannin (Klocke and Chan 1982, Reese et al. 1982). Rather, it was due to reduced efficiency with which digested food was converted into biomass. However, the reason for the apparent over compensation remains unclear.

Tussock moth larvae did not sequester condensed tannins, or simpler anthocyanidin products thereof, as

Table 2. Effects of condensed tannin on food consumption and processing by fourth-instar whitemarked tussock moths (means ± 1 SE *n*)

Diet (% dry mass)	Nutritional indices				
	TC (mg)	RCR (mg/mg × d)	AD (%)	ECD (%)	ECI (%)
Control	62.4 ± 3.6a (11)	1.74 ± 0.09a (11)	50.3 ± 2.4a (10)	63.9 ± 4.4a (10)	31.2 ± 1.8a (11)
Moderate tannin	75.5 ± 4.3b (9)	1.40 ± 0.10b (9)	51.3 ± 2.7a (8)	40.9 ± 4.9b (8)	23.0 ± 2.0b (9)
High tannin	78.7 ± 3.5b (13)	1.77 ± 0.08a (13)	50.6 ± 2.2a (12)	45.4 ± 4.0b (12)	22.3 ± 1.8b (13)
df for treatment	2, 29	2, 30	2, 27	2, 27	2, 30
F-value for treatment	5.45	4.66	0.04	7.41	7.36
P-value for treatment	0.010	0.017	0.965	0.003	0.003
df for covariate	1, 29	NA	NA	NA	NA
F-value for covariate	4.37	NA	NA	NA	NA
P-value for covariate	0.046	NA	NA	NA	NA

Within a column, values with different letters are significantly different ($P < 0.05$; LSD test, Littell et al. 1996). Standard errors are calculated from the pooled variance. TC, total consumption; RCR, relative consumption rate; AD, approximate digestibility; ECD, efficiency of conversion of digested food; ECI, efficiency of conversion of ingested food. NA, not applicable.

detectable by the acid butanol assay. Our results suggest that tannins were egested in a concentrated form. A doubling of tannin concentrations is what would be expected for undigested constituents in a diet with an approximate digestibility of 50%. We cannot rule out, however, the possibility that condensed tannin or tannin products were absorbed through the midgut, into the hemolymph and actively excreted via Malpighian tubules (Maddrell and Gardiner 1976). Analysis of tannin in the frass cannot discriminate between tannins egested versus tannins excreted.

In conclusion, we found that paper birch condensed tannin has multiple and somewhat contradictory effects on tussock moth performance. Birch tannin decreases growth rates via reductions in conversion efficiencies, but also causes larvae to consume more and grow larger than larvae reared on the control diet. The effect of tannin, however, might have been influenced by the high nutrient content of the diet, with different magnitudes of change possibly occurring with other, low-nutrient diets. If the results of this study apply under natural conditions, tussock moth larvae feeding on foliage containing moderate and high levels of condensed tannin may experience increases in development time, which could increase exposure to predation by natural enemies (Price et al. 1980). In addition, larvae may grow larger on birch foliage containing moderate and high tannin levels, which may translate into increased adult fecundity. Whether the benefits of increased fecundity outweigh the risks of increased development time is unclear. These data provide evidence that paper birch condensed tannins, which appear to have high biological activity compared with other condensed tannins (Ayres et al. 1997), tend to have mixed effects on the performance of an adapted herbivore. Our results are similar to Erelli et al. (1998), who found that birch condensed tannin (2–10% dry mass) had little effect on whitemarked tussock moth performance. Finally, because the differences in herbivore performance on the two tannin-containing diets were small (although concentrations in those diets varied two-fold), we suggest that the magnitude of variation in concentrations observed in the field (due to genetic or environmental differences among plants) will likely have relatively small effects on the performance of whitemarked tussock moths. Previously observed environmental effects on birch foliar quality and the resultant changes in whitemarked tussock moth performance (Agrell et al. 2000) are probably not due solely to changes in tannin levels.

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