Reports

Oak Leaf Quality Declines in Response to Defoliation by Gypsy Moth Larvae

Abstract. *Leaves of red oak trees that had been defoliated by gypsy moth larvae during the previous year and again during the period of the study had higher values of tanning coefficients, total phenolics, hydrolyzable and condensed tannins, dry matter contents, and toughness than did leaves of undamaged trees. These changes may influence larval growth and alter the course of further outbreaks.*

The gypsy moth, *Lymantria dispar* L., is a forest pest throughout the eastern United States. Since gypsy moth populations undergo episodic outbreaks and declines that do not seem to be related to food abundance (*1*), factors other than food are generally considered to bring about epidemic population "crashes" (*2*). However, there is evidence that the food quality of some plants may decline after herbivore attack (*3–6*). For example, gypsy moth larvae grow more slowly, produce smaller pupae and fewer eggs, and decline in "vigor" when fed leaves of oak trees defoliated by gypsy moths, even after one or more years of defoliation (*7*). We now report evidence of a mechanism that could produce these results; chemical changes in the leaves of oak trees defoliated by gypsy moths are great enough to reduce larval growth and influence the course of outbreaks.

During the summer of 1981, we examined leaves from 19 red oak saplings (*Quercus rubrum*, a preferred host) growing in a single stand in eastern Vermont (*8*). We analyzed the leaves for traits known to influence the growth of defoliating insects (*9, 10*). Ten of the trees, the "treatment" group, were nearly completely defoliated by gypsy moth larvae in 1980 and again in 1981. The other nine trees, which served as controls, grew 500 m north of the expanding larval population; these trees were not attacked in 1980, but were partially defoliated in 1981. The two groups were not significantly different in height, diameter, or phenology (*11*).

An entire branch on each tree was protected through the defoliation period in 1981 (*12*). Whole leaves were removed from each protected branch for analyses on three dates, one before, one during, and one after the current year's defoliation (*13*). In addition, newly flushed leaves, produced after defoliation, were sampled on the third date (*14*).

Leaves from treatment and control trees differed quantitatively and qualitatively in ways that could influence insect feeding and growth. Treatment leaves were significantly tougher and lower in water content on the second and third dates than were controls (Table 1). The tanning coefficients of the leaves of each treatment tree were much larger by the second date (Fig. 1). On this date, leaves of treatment trees were significantly more effective in precipitating proteins than any of the other leaves sampled and may have been less digestible to herbivores (*15*). This difference was lost after defoliation ceased, as both leaf groups declined in tanning capacity (Fig. 1).

Leaves from control and treatment trees also exhibited different seasonal changes in total phenol contents as estimated by the Folin-Denis method (Table 1). The two groups could not be distinguished statistically on the first date, but diverged during peak defoliation, and the difference was even greater by the third date. Newly flushed replacement leaves had higher phenolic contents than any of the other samples on any date and higher tanning coefficients than either control or treatment leaves on the third date (Table 1).

We also found significant differences in tannin composition among treatment, control, and replacement leaves. Treatment leaves were higher in both hydrolyzable and condensed tannins on the third date (Table 1). Hydrolyzable tannin contents had declined in control leaves, but increased in treatment leaves, by the third date. The replacement leaves had much higher hydrolyzable tannin contents than any of the other leaves sampled and had condensed tannin contents too low to measure; this composition is not associated with unusually high tanning coefficients (Table 1).

The relationships among our measures of tannin composition, total phenols, and tanning coefficients are complex. There is little or no correlation among mea-

Table 1. Red oak leaf traits assayed on three sampling dates during 1981. Values are means ± standard errors for treatment (T), control (C), and replacement (R) leaves. The number of leaves analyzed was 30 for T, 27 for C, and 12 to 22 for R; TAE, tannic acid equivalents; ROT, red oak condensed tannins. Different superscript letters indicate that values are statistically different at $P < .05$ (Student's t-test, Student's t-test modified for unequal variances, and Mann-Whitney U test). Percentages were arcsin-transformed for statistical treatment (*25*). See (*9*) for analytical methods. N.A., data not available (*9*).

Leaf trait	Leaf type	Sample date		
		10 June	30 June	28 July
Toughness (g/cm^2)	T	227.1 ± 43.2^a	287.5 ± 45.2^b	325.5 ± 48.8^c
	C	187.6 ± 51.2^a	234.1 ± 41.5^a	268.7 ± 42.8^d
	R			275.23 ± 79.3^c
Water (%)	T	66.3 ± 1.9^a	57.0 ± 1.8^b	55.1 ± 5.9^c
	C	66.8 ± 4.9^a	59.2 ± 1.5^c	57.3 ± 0.9^c
	R			63.2 ± 7.6^a
Total phenols (% TAE)	T	9.20 ± 0.4^a	7.65 ± 0.3^c	7.73 ± 0.6^c
	C	9.01 ± 0.4^a	6.09 ± 0.6^b	3.40 ± 0.2^d
	R			16.24 ± 1.1^c
Hydrolyzable tannins (% TAE)	T	23.30 ± 1.0^a	N.A.	27.05 ± 1.4^b
	C	23.36 ± 0.9^a	N.A.	19.54 ± 0.9^c
	R			59.74 ± 4.7^d
Condensed tannins (% ROT)	T	0.25 ± 0.1^a	N.A.	1.02 ± 0.2^b
	C	0.32 ± 0.1^a	N.A.	0.54 ± 0.1^c
	R			0.0 ± 0.0^d
Tanning coefficient (% TAE)	T	33.99 ± 2.9^a	53.77 ± 2.7^b	23.29 ± 1.6^c
	C	44.49 ± 5.3^a	36.97 ± 2.4^a	21.16 ± 1.6^c
	R			44.12 ± 2.4^a

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sures of total phenols, "hydrolyzable or condensed tannins," and protein binding (Table 1). Thus, commonly used methods to estimate hydrolyzable or condensed tannins do not necessarily reflect tanning activity in vitro (*16*).

Whether the observed differences were due solely to a previous defoliation or to the defoliation occurring during the experiments cannot be ascertained. A normal pattern of seasonal decline in total phenols appeared to be inhibited in defoliated trees (Table 1), and similar declines in other measures were reversed (hydrolyzable tannins; Table 1) or altered (tanning coefficients; Fig. 1). None of our measurements of leaf quality differed on the first sampling date, suggesting that previous defoliation did not alter early season values. No shifts in overall growth phenology were observed in treatment trees. Replacement leaves resembled early season leaves in some respects (water content and tanning coefficients), but differed strongly in others (hydrolyzable and condensed tannins, and total phenols), suggesting that their quality does not simply reflect developmental age but may be influenced by defoliation history.

Low water content and increased toughness of the treatment leaves should have a negative influence on larval growth. However, the differences in our study are smaller than those observed by others (*17*), so that their impact may be less. Red oak tannins do retard gypsy moth larval growth at concentrations above 0.5 percent tannic acid equivalents (TAE) in artificial diets (*18*); this concentration was exceeded in 90 percent of the leaves in this study (Table 1). Feeny (*19*) and Chan *et al.* (*20*) found that incremental increases in tannin contents from 0.1 to 1.0 percent dry weight significantly reduced the growth of larval Lepidoptera. Thus, the differences in tanning between trees that were defoliated and trees that were not defoliated should have a strong impact on larval growth.

Consistent differences were also observed for total phenols and hydrolyzable tannins, which may be toxic to insects and other organisms (*21*). Wallner and Walton (*7*) found that gypsy moths reared on previously defoliated black oaks (*Q. nigra*) not only grew more slowly and produced lighter-weight pupae than did controls (possibly due to tannins), but also experienced 80 percent nondisease mortality after the third instar. The greatly elevated total phenols seen in treatment trees during this portion of the growth season may explain Wallner and Walton's observation.

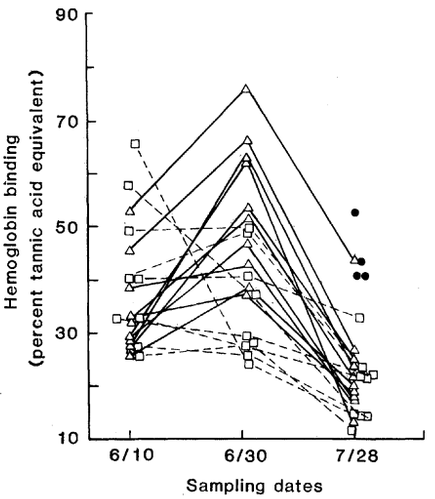

Fig. 1. Tanning coefficients of individual trees on three sampling dates during 1981, for (□) control, (△) treatment, and (●) replacement leaves. Each point represents a mean value for three leaves per tree. The mean for the treatment population was greater than that of the control only on 30 June (Table 1). Units are percent (dry weight) tannic acid equivalents obtained from a standard curve (*10*).

Moreover, the very high phenol and hydrolyzable tannin contents of the replacement leaves may help to prevent a second defoliation during a single season.

These results indicate that the role of the host plant must be considered in studies of episodic population phenomena. Not only may reduced food quality retard pest growth, but it can also make the pests more susceptible to disease, parasites, and predators (*22*). This could explain why so many different hypotheses about the initiation and decline of outbreaks appear reasonable (*23*). The induction of lowered host quality by herbivory, followed by herbivore population decline and relaxation of plant responses could result in cyclic pest population explosions driven in part by the responses of the host plant (*4, 6, 24*). One important consequence is that outbreaks of pests such as the gypsy moth may normally decline under natural circumstances.

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8. The stand, 1 mile north of Hartland, Vt., was dominated by red and white oak (*Q. alba*), and white pine growing on a gulley-bisected esker of Hadley very fine sandy loam. Staghorn sumac (*Rhus typhina*) dominated the 50-m-wide gulley separating the treatment from control trees. Slopes and aspects were identical. Sample trees were approximately 15 years old.
9. Leaves were extracted under N_2 in aqueous methanol and analyzed for protein binding capacity with hemoglobin as a binding substrate, and for total phenols by the Folin-Denis method (*10*). Water content was determined gravimetrically, and toughness measurements were made using a penetrometer (Chatillon, No. 516-500, R. C. Schwarz & Sons, Inc., Rochester, N.Y.). Hydrolyzable tannins were determined by a modified iodate technique [E. C. Bate-Smith, *Phytochemistry* **16**, 1421 (1977)]; results are expressed as percent dry weight tannic acid equivalents from a tannic acid standard curve. Diluted samples were kept on ice; absorbances were read at 550 nm 40 minutes after 0.1 ml of KIO_4 was added. Condensed tannins were determined by the proanthocyanidin method [E. C. Bate-Smith, *Phytochemistry* **14**, 1107 (1975)] and are expressed as percent dry weight red oak condensed tannin from a standard curve for purified red oak tannin [B. R. Brown, C. W. Love, W. R. C. Hadley, *Report on Forest Research* (Her Majesty's Stationary Office, London, 1972)]. Because these methods are used with different standards or different reactions, higher values may be obtained for condensed or hydrolyzable tannins than for total phenols. Unfortunately, the 30 June samples were lost before hydrolyzable and condensed tannin analyses could be performed. Nitrogen was determined using the micro-Kjeldahl technique and did not differ significantly among dates or groups.
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11. All trees broke buds on 11 or 12 May 1981. Heights of control and treatment trees (mean ± standard deviation, 3.52 ± 2.0 and 3.12 ± 2.0 m, respectively) and diameters at breast height (3.57 ± 3.24 and 3.07 ± 1.58 cm, respectively) were not significantly different ($P > .6$; Student's t-test). One control tree refoliated after dropping its leaves during a late frost and exhibited altered phenological development; it was eliminated from the analysis.
12. Leaves were protected with a plastic mesh screen bag (65 by 45 cm) through the second gypsy moth instar (25 May) and with cotton, aluminum foil, and Tanglefoot collars thereafter.
13. Three leaves were removed from each sample branch for each analysis class on 10 and 30 June and on 28 July. Egg masses hatched within 3 days of leaf flush. By 4 June, treatment trees had noticeable damage. By 30 June, we estimated treatment trees were 50 percent defoliated, and controls had lost less than 10 percent of their foliage. Larvae pupated during the first week of July.
14. Three newly flushed leaves [see G. H. Heichel and N. C. Turner, in *Perspectives in Forest Entomology*, J. F. Anderson and H. K. Kaya, Eds. (Academic Press, New York, 1976), p. 31] were removed from each of four treatment trees on 28 July.
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26. We thank M. Montgomery, M. Martin, and C. D. Schlichting for permission to cite unpublished work. M. Montgomery provided purified red oak tannin for use as a standard. R. T. Holmes read and improved the manuscript. D. B. Munson and J. B. McKoy III helped with chemical analyses, P. J. Nothnagle located the study site, and M. J. Richards drew the figure. Supported by NSF grant DEB-8022174 (J.C.S. and R. T. Holmes) as part of our continuing studies of herbivory at the Hubbard Brook Experimental Forest.

8 February 1982

Gut Reactions of Radioactive Nitrite After Intratracheal Administration in Mice

Abstract. *Intratracheal administration to mice of radioactive nitrite labeled with nitrogen-13 ($^{13}NO_2^-$) (half-life, 9.96 minutes) in dosages that do not cause pharmacological perturbation reveals that oxidative and reductive reactions occur in different organs. Oxidation of $^{13}NO_2^-$ to radioactive nitrate ($^{13}NO_3^-$) predominates in the blood and liver. Reduction of $^{13}NO_2^-$ occurs in those mice that harbor intestinal microflora; this reduction does not occur in germ-free mice. The intestinal reduction products include ammonium, glutamate, glutamine, and urea. With a detection limit of about 0.01 percent of the instilled nitrogen-13, no labeled nitrosamines were detected within 30 minutes. Reduced nitrogen-13 is transported out of the intestine into the circulatory system and appears in the urine along with $^{13}NO_3^-$. The biological half-period for $^{13}NO_2^-$ destruction is about 7 minutes, and both oxidation and reduction products are formed.*

The presence of nitrogen dioxide (NO_2) and various nitrate (NO_3^-) compounds in indoor and outdoor air is well established (*1*). These chemical species are either known or suspected to form nitrite (NO_2^-) in vivo (*2*). Nitrite is known to form carcinogenic *N*-nitroso compounds in vivo by reaction with amines in the stomachs of experimental animals (*3*) and in vitro under the mediating influence of intestinal microflora (*4*). The formation of nitrosamines has been reported in rodents that were first gavaged with a precursor amine and then allowed to inhale NO_2 (*5*). Nitrous acid was postulated as an intermediate, although the mechanism or site of nitrosation was not determined. Pathogenesis attributed to NO_2^- alone has been reported, but this conclusion is still a source of debate (*6*).

Evaluation of the potential health hazard of inhaled or ingested NO_2, NO_2^-, or NO_3^- at concentrations likely to be encountered under realistic conditions has been complicated by Tannenbaum's discovery of NO_3^- synthesis in humans. This was originally attributed to the formation of NO_3^- by intestinal microflora (*7*), but more recent studies have revealed this to be a mammalian process (*8*). Saul *et al.* found that human fecal material destroys both NO_2^- and NO_3^- in vitro but did not identify any reaction product (*9*). Witter *et al.* reported that the presence of intestinal microflora decreased urinary NO_3^- excretion in rats; the speculation was offered that NO_2^- was a metabolite (*10*).

Parks *et al.* reported that radioactive ^{13}N (half-life, 9.96 minutes) as $^{13}NO_2^-$ or $^{13}NO_3^-$ (carrier dosage less than 100 ng per kilogram of body weight) was cleared from the lungs of mice to the blood and distributed into the gastrointestinal tract and other organs within 30 minutes (*11*). At this low concentration of added NO_2^- (2 to 3 n*M* in body fluids), $^{13}NO_2^-$ was 70 percent oxidized to $^{13}NO_3^-$ in blood within 10 minutes. Consequently, NO_2^- derived from nitrogenous air pollutants is partially converted to NO_3^- before reaching the gastrointestinal tract. However, mammals synthesize NO_3^- (*8*, *10*), and their oral (*12*), and possibly intestinal (*9*, *13*), microflora reduce it to NO_2^-. This presented an enigma with respect to the evaluation of health risk from either inhaled or ingested oxidized nitrogen compounds. The uncertainties regarding the metabolism of oxidized nitrogen compounds have persisted in part because analysis of trace NO_2^-, NO_3^-, or their metabolites in biological samples is difficult. Mass-spectrometric detection of stable ^{15}N allows tracer chemistry but requires dosages of ^{15}N compounds that may perturb normal biochemical processes. We have now determined that the sites of the most rapid metabolism of $^{13}NO_2^-$ in mice are the blood and the intestines. We report here the primary chemical fate in the gut and other tissues of intratracheally instilled $^{13}NO_2^-$.

In our metabolic studies, we used $^{13}NO_2^-$ to minimize any pharmacological action of the tracer (*11*). Labeled NO_2^- was prepared by the reduction of cyclotron-produced $^{13}NO_3^-$ (*14*) and concentrated into saline solution prior to instillation (*15*). All the experiments were done under "no carrier added" conditions, with specific activities of 1 to 7 Ci per micromole of NO_2^-. Consequently, total NO_2^- dosages ranged between 10 and 100 ng kg^{-1}. We determined the composition of the labeled components by using high-pressure liquid chromatography (HPLC) together with thin-layer electrophoresis (*16*).

Conventional (CV) BALB/c mice were given commercial feed and water without restriction. We assessed the effect of the microflora on NO_2^- metabolism by comparing CV mice with germ-free (GF) mice and specific pathogen–free (SPF) mice (*17*). Further comparisons were made on exenterated (small and large intestines removed) or penicillin-treated CV mice (*18*).

Table 1 shows the distribution of intratracheally administered $^{13}NO_2^-$ in the various mouse tissues and indicates the relative amounts of radioactivity in the NO_3^-, NO_2^-, and nonanionic (NA) fractions. The chemical determinations were done by anion-exchange chromatography (*16*). The NA components include all nitrogenous compounds not retained on the anion-exchange column. We determined the mean values and asymmetric error intervals about the means, using the logit transformation. This technique adequately estimates the central tendency of fractional values that are bounded by fixed margins (for example, 0 and 1) (*19*).

The fractions of whole body activity (WBA) in the various tissues of CV mice (Table 1) are consistent with a uniform distribution of ^{13}N on the basis of weight (*11*). These values show that 10 to 20 percent of the ^{13}N introduced into the lungs as NO_2^- is transported to the gut. The chemical distribution of radioactivity in the intestines of CV mice is dramatically different from that in the blood, acidic stomach contents, and other body fluids. This difference indicates that oxidation of $^{13}NO_2^-$ to $^{13}NO_3^-$ in blood is

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