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FOLIAGE DAMAGE DOES NOT AFFECT WITHIN-SEASON TRANSMISSION OF AN INSECT VIRUS

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Abstract. Gypsy moth defoliation of oak trees has been shown to lead to increased tannin levels, which, in turn, lead to reduced gypsy moth growth and fecundity. In laboratory experiments, increased tannin levels can interfere with the transmission of a virus that is consumed by larvae on oak foliage, and high mortality rates of larvae in the field are sometimes associated with low levels of defoliation. These latter results have led to the suggestion that gypsy moth defoliation may cause reduced mortality attributable to the virus by elevating oak tannin levels. In a series of field experiments, we directly tested the hypothesis that gypsy moth defoliation of oaks leads to reduced virus transmission rates. In each of three study years, in oak forests with almost no naturally occurring gypsy moths or virus, we measured virus transmission rates in gypsy moths feeding on oaks, with and without experimental defoliation. By carefully synchronizing our experiments with the phenology of natural gypsy moth populations, we mimicked natural virus transmission processes during that part of the gypsy moth life cycle when virus transmission occurs. In our experiments, there was no effect of gypsy moth defoliation on tannin levels; consequently, virus transmission in both the field and the lab was unaffected by defoliation. Although we did observe increased tannin levels on more severely defoliated oak trees in one of two naturally defoliated oak stands late in the season, virus transmission had virtually ceased by that time. Our results suggest that gypsy moth defoliation does not affect tannin levels early enough in the larval season to have a measurable effect on the interaction between the gypsy moth and its nuclear polyhedrosis virus.

Key words: damage-induced responses; disease transmission; foliage chemistry; foliage quality; gypsy moth; inhibition of virus transmission; Lymantria dispar; *nuclear polyhedrosis virus;* Quercus rubra; Quercus velutina.

INTRODUCTION

Numerous studies have shown that foliage that has been damaged by herbivores changes in ways that can influence herbivore growth or survival (Karban and Myers 1988). One of the more prominent examples of such induced responses is provided by changes in the levels of secondary compounds of oaks following herbivory by the gypsy moth, *Lymantria dispar* (L.). Leaves on red oak (*Quercus rubra*) trees that have experienced significant defoliation contain higher levels of hydrolyzable tannins (Schultz and Baldwin 1982, Rossiter et al. 1988), and these high tannin levels lead to reduced gypsy moth growth rates (Rossiter et al. 1988). More recently, however, this classic story has been complicated by evidence for an effect of tannins on disease transmission, in that tannins reduce infection rates of a virus that is consumed with oak foliage (Keating et al. 1988). Because the evidence for this effect is entirely based on either lab data or field observations, we set out to experimentally test for an effect of gypsy moth defoliation of oak foliage on virus transmission in the field.

In previous experiments demonstrating an effect of tannins on virus transmission in the lab, gypsy moth larvae have been fed virus, at particular doses, on oak foliage taken from the field. Such data have shown that infection is less likely if the contaminated foliage has high tannin levels and/or if the foliage has been obtained from heavily defoliated trees (Keating et al. 1988, Hunter and Schultz 1993). Also, in one case, larvae on more heavily defoliated trees in the field showed lower rates of virus mortality (Hunter and Schultz 1993). There are, however, several difficulties with these data. First, the strength of the effect in the lab depends on the dose of the virus to which larvae are exposed, whereas the doses to which larvae are exposed in the field are unknown. Second, the field data show only an observed correlation between mortality rates and defoliation rates; in fact, the causation could be reversed, in that high mortality rates from virus may well cause low defoliation rates, rather than vice versa. Finally, induction of tannins in oaks defoliated by gypsy moths in the northeastern United States is usually reported in July (Schultz and Baldwin

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1982, Hunter and Schultz 1995), whereas nearly all virus transmission occurs in May and June. Our experiments were designed to mimic natural transmission processes as closely as possible, and were timed to coincide with transmission in natural populations.

Because defoliation can affect oak tannin levels, and because oak tannin levels can affect virus transmission, the effect of gypsy moth defoliation on virus transmission has been cited as evidence for a tritrophic interaction among gypsy moths, oaks, and virus (Schultz and Keating 1991). Such an effect would be especially important for gypsy moth population dynamics, because the virus has been the most important factor causing the crash of outbreaking gypsy moth populations (Elkinton and Liebhold 1990). If defoliation does reduce virus transmission, however, the induced foliage changes in red oak could effectively prevent the virus from causing gypsy moth populations to crash. The possibility of this kind of tritrophic interaction is, therefore, of great significance for gypsy moth population dynamics.

Materials and Methods, Overview

In most of the northeastern United States, gypsy moth larvae hatch in early May. Within a season, virus epidemics in gypsy moth populations begin when larvae become infected while hatching from virus-contaminated egg masses. Because larvae only become infected by consuming the virus, subsequent transmission occurs when these infected hatchlings die on foliage, which is then consumed by other larvae. Also, because the virus prevents infected larvae from molting to the next larval stage (O'Reilly and Miller 1989), larvae infected as hatchlings typically die in the first instar, but the disease is then transmitted to larvae in the second or third instar. This secondary transmission occurs from mid-May to late June in the northeastern United States. In our field experiments, we mimic the natural process of secondary transmission by placing infected first-instar larvae on oak foliage inside mesh bags along with uninfected third instar larvae (which we refer to as test larvae). After a week, the test larvae are removed to individual cups of artificial diet in the lab. Because the virus takes ~10–14 d to kill in the field, this protocol ensures that only one round of virus transmission occurs. Test larvae that die are autopsied under a light microscope to verify the presence of the virus (Woods and Elkinton 1987). In previous work, we have demonstrated that transmission in these experiments is only slightly affected by rainfall (D'Amico and Elkinton 1995), and the bags have no measurable effect on the concentration of phenolics or hydrolyzable tannins in the confined foliage (Rossiter et al. 1988, Hunter and Schultz 1993).

Virus controls

In each experiment that we report here, we tested for naturally occurring virus by putting a bag on foliage of uncontaminated branches of experimental trees. These control bags contained only healthy larvae, and no virus-infected cadavers. Because we were careful to perform our experiments in areas where wild gypsy moths were extremely sparse, there was almost never any virus infection in any of these bags; the maximum virus infection rate in control bags never exceeded 3%. Consequently, we make no further mention of these controls.

Infecting larvae in the lab

We infected larvae for field experiments by feeding them virus in the lab (Hughes et al. 1986). We used doses sufficient to produce infection rates of ≥95%, and because only uninfected larvae molt to the second instar, the few uninfected larvae could be easily identified and discarded before an experiment began. After infection, larvae were held in the lab for 5 d (at 28°C, this is 1 d after molting and ~1 d before death), and then were placed in the bags on foliage 5 d before each experiment began. This approach guaranteed that all infected larvae were dead by the start of an experiment. The mesh bags were made of Reemay (Kleen-Test Products, Red Deer, Wisconsin, USA) and, in most cases contained 40 ± 5 leaves.

Study sites.—In our 1993 experiments, we used mature black oak trees (*Quercus velutina* L.) in a forest composed of black oak, white oak (*Q. alba*), and pitch pine (*Pinus rigida*) (Otis Air National Guard Base, Falmouth, Massachusetts, USA). In 1994 and 1995, we used 4–6 m high red oak saplings in a forest composed primarily of red oak and red maple (*Acer rubrum*) (Cadwell Memorial Forest, Pelham, Massachusetts). Neither forest had experienced significant defoliation since gypsy moth outbreaks in the early- to mid-1980s (Otis ANGB, 1986; Cadwell, 1981). In 1993, we used mature trees that were part of the canopy; in 1994 and 1995, we used saplings that were mostly in full sunlight along clearings or next to a logging road.

Manipulating levels of defoliation.—The basic experiments that we report here consisted of manipulations of the extent and timing of defoliation experienced by the trees on which the larvae fed, to see how defoliation affects virus transmission. Details of these experiments are given in Table 1; here, we describe only the more significant details. The experiments differed first in that we used black oaks in 1993, and red oak, the tree species on which most previous work has been carried out, in 1994 and 1995. Secondly, the timing of our experiments varied slightly from year to year, although all experiments took place at the time of the larval season when virus transmission occurs in natural populations (see Table 1 for dates). In all of these experiments, we used test larvae that were in the same larval stage as larvae in local, natural populations. (third instars in 1993; second instars in 1994 and 1995). New trees were used in each year; we were only concerned with quantifying within-season effects.

TABLE 1. Summary of field experiments and laboratory bioassays for of gypsy moth mortality after feeding on oak leaves. HT, hydrolyzable tannins; TP, total phenolics.

Date	Tree species	Description of leaf treatment	Mean (±1 SE) fraction of larvae dying		Differences in mortality between treatments detected by ANOVA?		Differences in tannin levels?
			Field test	Leaf assay	Field test	Leaf assay	
June 1993	*Quercus velutina*	previously damaged	0.48 (0.05)	...	no: $F_{1,36} = 0.21$, $P = 0.65$	...	...
		not previously damaged	0.44 (0.04)				
May 1994	*Quercus rubra*	previously damaged	0.92 (0.02)	0.89 (0.03)	no: $F_{1,28} = 3.45$, $P = 0.06$	no: $F_{3,28} = 0.17$, $P = 0.92$	HT; no, $P = 0.09$ TP; no, $P = 0.48$
		not previously damaged	0.84 (0.03)	0.90 (0.03)			
		foliage cut with scissors	...	0.91 (0.01)			
		foliage cut 8 h prior	...	0.93 (0.02)			
May 1995	*Quercus rubra*	concurrently damaged	0.60 (0.04)	0.48 (0.03)	no: $F_{1,28} = 0.55$, $P = 0.47$	no: $F_{2,27} = 0.56$, $P = 0.57$	HT; no, $P = 0.27$ TP; no, $P = 0.22$
		not concurrently damaged	0.64 (0.04)	0.52 (0.04)			
		induction control	...	0.47 (0.02)			
July 1994	*Quercus rubra*	10 larvae/80 leaves	0.33 (0.08)	...	no treatment effect: $F_{1,29} = 3.78$, $P = 0.06$; no tree effect: $F_{5,29} = 0.22$, $P = 0.95$; no interaction†: $F_{5,29} = 2.34$, $P = 0.08$	...	...
		20 larvae/80 leaves	0.32 (0.09)	...			
		40 larvae/80 leaves	0.35 (0.05)	...			
		80 larvae/80 leaves	0.46 (0.04)	...			
		160 larvae/80 leaves	0.46 (0.06)	...			

† Because the interaction effect was not significant, the ANOVA was redone, using a model with no interaction term, to test for main effects.

Because larvae must consume the virus on foliage in order to become infected, defoliation is an intrinsic part of the transmission process. In order to manipulate defoliation levels, we therefore imposed additional defoliation above and beyond what occurred during virus transmission. To do this, in each experiment, we established a "damaged" treatment and an "undamaged" treatment, such that trees in the damaged treatment were exposed to defoliation not only from the larvae used to measure virus transmission, but also from additional larvae used only to impose defoliation (we refer to these as defoliating larvae). The difference in virus transmission rates in the two treatments is a measure of the effects of defoliation on transmission.

The most important differences among experiments were in the extent of defoliation and in the timing of defoliation relative to transmission. In 1993, we imposed defoliation only at the level of the individual branch (~50% defoliation *within* bags). In 1994, we defoliated at both the level of the individual branch and at the level of the entire tree (30–50% defoliation *inside and outside* the virus treatment bags). Finally, in 1995, we caused defoliation only at the level of the entire tree (30–50% of the tree defoliated *outside* the virus treatment bags). Hereafter, we refer to these experiments as the branch-level, branch- and tree-level, and tree-level experiments, respectively. In the first two experiments, we imposed defoliation only for the two days before transmission began, but in the third experiment, we began imposing defoliation five days before transmission and continued throughout the transmission period. Because defoliation occurred over a longer period in the third year than in the second year, the overall effect was that we increased overall defoliation from experiment to experiment. In other words, as we accumulated negative evidence for any effects of defoliation on transmission, we imposed increasingly higher levels of defoliation in an attempt to demonstrate a positive effect.

To impose defoliation at the level of individual branches, we added defoliating larvae only to the virus transmission bags. Specifically, we added 40 fourth-instar larvae to the bags on the damage treatment trees after the initially infected larvae had died. These larvae were removed and discarded after two days, and then test larvae were added to the bags. Because larvae do not avoid virus-contaminated oak foliage (V. D'Amico, *unpublished data*), the defoliating larvae did not affect the density of virus in bags in the damage treatment. To impose defoliation at the level of the entire tree, we

added additional bags (containing only defoliating larvae) to the trees in the damage treatment (40 fourth instars, no virus, no transmission larvae). To analyze data from these experiments, we performed one-way ANOVAs on the arcsine square-root transformed virus mortality.

Manipulating larval density.—In a final experiment, we manipulated defoliation within trees, rather than across trees, to look for an interaction between the effect of branch-level defoliation and the effect of differences among individual trees. We did this by manipulating the density of test larvae in the virus transmission bags, using treatments of 10, 20, 40, 60, 80, or 160 larvae in bags containing 40 infected cadavers and 80 red oak leaves. The effect of these treatments was to impose different levels of defoliation among bags on a tree, but to maintain a moderate level of defoliation on all trees. We refer to this as the larval density experiment. Each tree contained a bag of each larval density, as well as a no-virus control bag at one of the five host densities. To test for differences between mean mortality in each treatment, we transformed the fraction of insects dying in each bag, using the arcsine square-root transformation (Sokal and Rohlf 1981); treatments were compared with an ANCOVA (SAS Institute 1985).

Laboratory bioassays.—In order to better compare our results to the original work demonstrating effects of tannins on virus bioassays in the laboratory, we performed a bioassay in conjunction with the tree-level defoliation experiment and the branch- and tree-level defoliation experiment. At the end of transmission in these defoliation experiments, we took samples of some of the unconsumed foliage from the virus test bags, and used them to measure larval infection rates with known doses. First, we surface-sterilized the foliage samples from the two treatments (rinsing thoroughly with distilled water after the bleach treatment), and then added 5 μL of virus (10^6 occlusion bodies/mL) to small (8 mm diameter) disks cut from these samples within 1–2 h after removal from the field. Each disk was then fed to an uninfected, starved, third-instar larva. To ensure the efficacy of the bleach treatment, we also fed control larvae disks with 5 μL of distilled water instead of bleach. To ensure that all larvae received the same total amount of virus, any larva that did not consume the entire leaf disk after 36 h was discarded. The remaining larvae were reared on artificial diet for 2 wk in the usual fashion to assess levels of infection (Bell et al. 1981). We also assayed trees, from outside each experiment, that had experienced no defoliation whatsoever. Finally, in the branch- and tree-level experiment, we looked for an extremely short-term effect of oak foliage chemistry by assaying leaves that originated on trees from outside the experiment. We cut these leaves in half with scissors 8 h before the assay.

Tannin assays.—In addition to bioassays, we assayed tannin levels in leaves from the branch- and tree-level and tree-level defoliation experiments. We also assayed leaves from undefoliated, unmanipulated trees, some of which we used as induction controls, and some of which we cut in half with scissors 8 h before the assay to look for a short-term induction response. Also, because of the lack of any evidence of induced tannins in our defoliation experiments, we looked for elevated tannin responses in trees that had been defoliated by naturally occurring populations of gypsy moth. To do this, we measured tannin levels in defoliated and undefoliated trees in two areas of patchy gypsy moth outbreaks in 1995. Although the two sites were ~64 km (40 miles) apart, gypsy moth densities in that part of Massachusetts were high throughout the entire area. We sampled from a site near Southborough, Massachusetts, USA on 29 June, and from a site near Bellingham, Massachusetts, on 6 July. Gypsy moth larvae at these sites were either either fifth or sixth instars or pupae. At each site, we collected five leaves at random from each of 10 oaks that had been severely defoliated (~70% tree-wide defoliation), and from each of 10 oaks on the same ridge that had been only slightly defoliated (~5% tree-wide defoliation). Leaf area consumed on collected leaves ranged from 40% to 95% for leaves from severely defoliated trees, and from 0% to 10% on leaves from lightly defoliated trees. We immediately placed these leaves on dry ice and froze them at −70°C when we returned to the laboratory.

In preparation for processing in the lab, leaves from all experiments were freeze-dried, ground through a 0.25-mm screen, and stored in sealed containers over silica gel at −70°C until analyzed. We extracted 50 mg of each sample with 10 mL of 70% acetone at 45°C for 15 min in a test tube flushed with nitrogen. The acetone was evaporated with a stream of N_2, and water was added to give a volume of 10 mL. The contents were centrifuged to separate particulates from the supernatant. Total phenolics in the supernatant were determined using the Folin-Denis procedure (AOAC 1990); hydrolyzable tannins were analyzed using a procedure described by Bate-Smith (1977).

Results

Manipulating defoliation and larval density.—No significant differences were seen between virus-caused larval mortality in the damage and no-damage treatments in any field experiment, whether defoliation occurred at the branch level, the tree level, or at both levels. Similarly, there was no effect of larval density on virus mortality in the larval density experiment, and there was no significant interaction between larval density and tree. Results of statistical tests and the mean mortality for each experiment are summarized in Table 1. In several of these experiments, there was a nonsignificant trend toward increasing virus mortality with increasing insect density or foliage damage. This is the opposite trend from that expected; insects at higher densities cause greater levels of defoliation, and in-

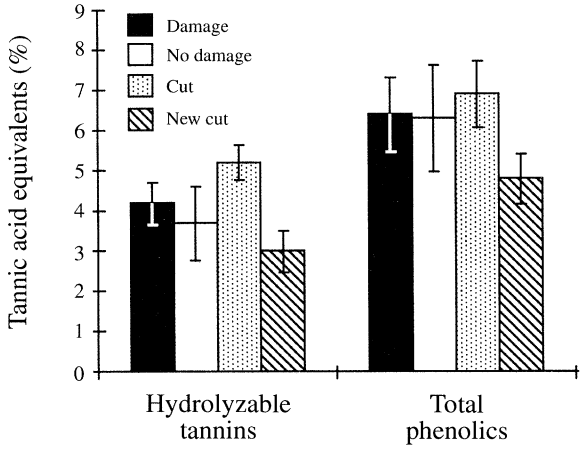

FIG. 1. Levels of hydrolyzable tannins and total phenolics in red oak leaves from the branch- and tree-level defoliation experiment (mean ± 1 SE). There were no significant damage effects on levels of hydrolyzable tannins ($F_{3,28} = 2.45$, $P = 0.085$) or total phenolics ($F_{3,28} = 0.95$, $P = 0.482$).

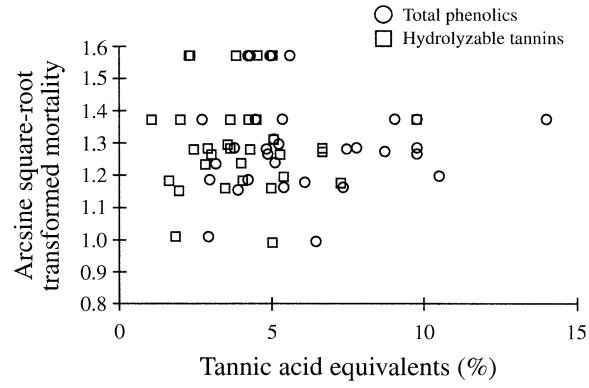

FIG. 2. Arcsine square-root transformed gypsy moth larval mortality plotted against levels of hydrolyzable tannins and total phenolics in red oak leaves from the branch- and tree-level field experiment. There were no significant correlations between mortality and hydrolyzable tannins ($r^2 = 0.0005$; df =1, 30; $P = 0.9071$) or total phenolics ($r^2 = 0.0001$; df = 1, 30; $P = 0.9648$).

duced chemistry effects would be expected to protect them from virus mortality.

Laboratory bioassays.—In the leaf disk bioassays, there were no significant differences in mean mortality from virus between treatments. Mean mortality and results of statistical tests are summarized in Table 1.

Tannin assays.—For the branch- and tree-level defoliation experiment, a one-way ANOVA (see Fig. 1) could detect no significant treatment effects on hydrolyzable tannins (df = 3, 28; $F = 2.45$; $P = 0.085$), or between total phenolics levels in these same leaves (df = 3, 28; $F = 0.95$; $P = 0.482$). Linear regressions (Fig. 2) of tannin levels in the leaves used in the bioassay against transformed mortality in the bioassay showed no significant correlation for hydrolyzable tannins (df = 1, 30; $R^2 = 0.0005$, $P = 0.907$) or total phenolics (df = 1, 30; $R^2 = 0.0001$, $P = 0.965$).

For the tree-level defoliation experiment, a one-way ANOVA (Fig. 3) detected no significant differences in hydrolyzable tannin levels between the damaged and undamaged treatments (df = 2, 27; $F = 1.35$; $P = 0.275$), nor were any differences detected in levels of total phenolics (df = 2, 27; $F = 1.59$; $P = 0.222$). Linear regressions of transformed mortality in the field experiment against tannin levels in the leaves used in the field and bioassay experiments showed no significant correlation for total phenolics (df = 1, 18; $R^2 = 0.1$, $P = 0.173$; Fig. 4). There was, however, a nonsignificant trend for higher mortality of larvae due to virus at higher levels of hydrolyzable tannins (df = 1, 18; $R^2 = 0.159$, $P = 0.08$). Again, this trend is the opposite from that expected from a defoliation-induced effect of high density on virus transmission. No significant correlation was seen when transformed mortality in the leaf disk bioassay was plotted against tannin levels (hydrolyzable tannins: df = 1, 28; $R^2 = 0.029$, $P = 0.369$; and total phenolics: df = 1, 28; $R^2 = 0.014$, $P = 0.526$).

At the naturally defoliated sites, tannin levels in leaves from red oaks in late June were not significantly different, whether the trees had been severely defoliated or were essentially undamaged (Fig. 5 ANOVA, hydrolyzable tannins: df = 1,18; $F = 0.16$; $P = 0.698$; total phenolics: df = 1, 18; $F = 0.32$; $P = 0.576$). In early July, damaged leaves were found to contain higher levels of tannins (Fig. 5). A one-way ANOVA detected significant differences between tannin levels in damaged and undamaged leaves for both hydrolyzable tannins (df = 1, 18; $F = 4.67$; $P = 0.044$) and total phenolics (df = 1, 18; $F = 39.51$; $P < 0.001$).

DISCUSSION

Our results suggest that tannin levels are not affected by defoliation at the time when virus transmission oc-

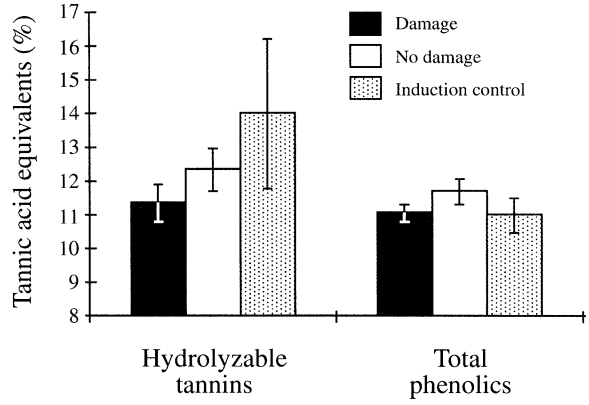

FIG. 3. Levels of hydrolyzable tannins and total phenolics in red oak leaves from the tree-level field experiment. There were no significant damage effects on levels of hydrolyzable tannins ($F_{2,27} = 1.35$, $P = 0.275$) or total phenolics ($F_{2,27} = 1.59$, $P = 0.222$).

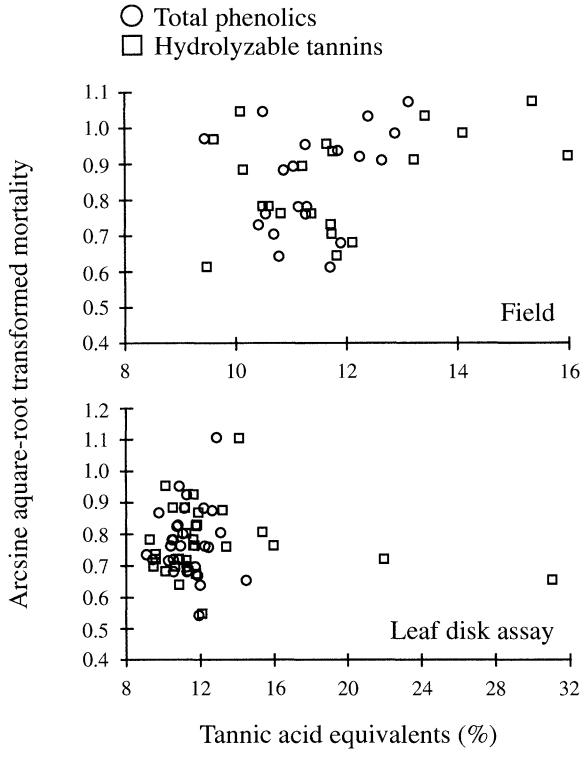

FIG. 4. Arcsine square-root transformed larval mortality in the field experiment and mortality in the leaf disk bioassay (performed using the same leaves), plotted against levels of hydrolyzable tannins and total phenolics in red oak leaves from the tree-level field experiment. There were no significant correlations (top panel) between mortality in the field experiment and hydrolyzable tannins ($r^2 = 0.159$; df = 1, 18; $P = 0.08$) or total phenolics ($r^2 = 0.1$; df = 1, 18; $P = 0.173$). There were no significant correlations (bottom panel) between mortality in the leaf disk bioassay and hydrolyzable tannins ($r^2 = 0.029$; df = 1, 28; $P = 0.369$) or total phenolics ($r^2 = 0.014$; df = 1, 28; $P = 0.526$).

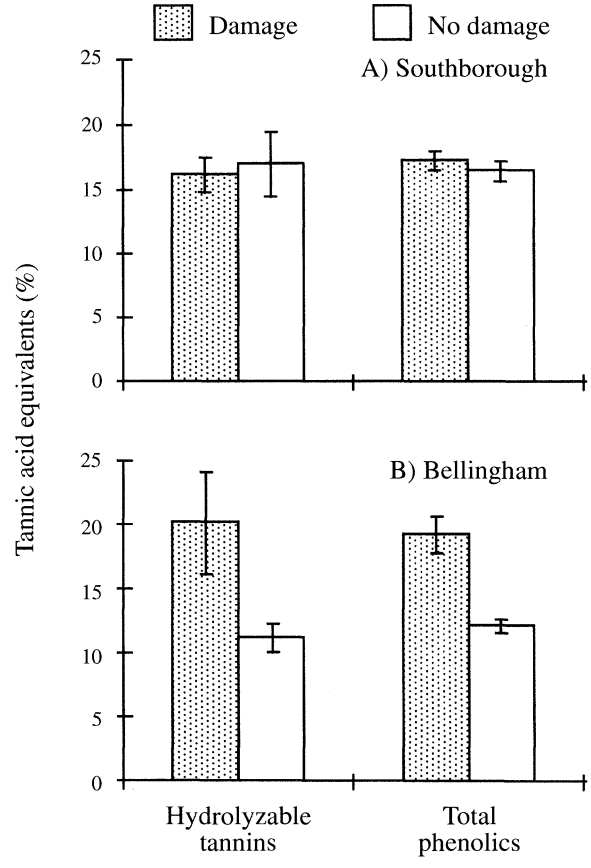

FIG. 5. Levels of hydrolyzable tannins and total phenolics in damaged and undamaged red oak leaves from two sites in Massachusetts (mean ± 1 SE). There was a significant damage effect at the Bellingham site (B) when leaves were collected in early July (hydrolyzable tannins, $F_{1,18} = 4.67$, $P = 0.044$; total phenolics, $F_{1,18} = 39.51$, $P = 0.000$), but not at the Southborough site (A) when leaves were collected in late June (hydrolyzable tannins, $F_{1,18} = 0.16$, $P = 0.698$; total phenolics, $F_{1,18} = 0.32$, $P = 0.576$).

curs in natural gypsy moth populations. Apparently, because of this lack of tannin induction, our virus transmission experiments did not show any effects of defoliation on virus-caused mortality, irrespective of the timing or intensity of defoliation, nor was there any effect of defoliation on virus mortality in our laboratory bioassays. In fact, we were only able to find a significant effect of defoliation on tannin levels at a naturally defoliated site in July. Even in that case, it is not certain whether gypsy moth larvae induced higher tannin levels or simply chose hosts with higher constitutive tannin levels. Also, it is likely that virus transmission had ceased by the time that elevated tannins appeared, because larvae were pupating and thus were no longer consuming contaminated foliage.

We emphasize that our protocols were designed to eliminate mechanisms that might obscure or counteract induced effects. First, although we are confident that the defoliating larvae did not concentrate virus in the experiments that included branch-level defoliation, because larvae do not avoid virus-contaminated foliage, any such avoidance effects would be irrelevant in the tree-wide defoliation experiment and the bioassays. Second, although there is uncertainty in the literature as to whether induction effects will occur at the level of the individual branch or the entire tree (Rossiter et al. 1988, Hunter and Schultz 1993), by manipulating defoliation at both levels, we allowed for either effect, or both. Finally, because our experiments were performed in synchrony with naturally occurring gypsy moth populations, we believe that they accurately mimicked natural virus transmission processes.

We do not question the finding that tree species affect the susceptibility of gypsy moth larvae to viral infection (Keating and Yendol 1987), or that foliar tannin content may explain differences in virus transmission among tree species. However, our results suggest that the evidence for induction of tannins in oaks is equivocal, especially in the early part of the season (May

and June), when most virus transmission occurs in gypsy moth populations. In fact, the results of previous studies have similarly reported low tannin levels in the early part of the gypsy moth season. For example, Schultz and Baldwin (1982) and Hunter and Schultz (1995) reported high levels of tannins in July, but not in mid-June.

We recognize that our data do not bear on the effects of defoliation between larval seasons. Although there is a suggestion of such an effect in previous work (Hunter and Schultz 1993), our previous experience with virus transmission in this system (Woods and Elkinton 1987) leads us to suspect that such complex effects also do not occur, because the first round of transmission in the gypsy moth larval season occurs on egg masses rather than on foliage.

Our finding that larval density also has no effect on transmission rates is relevant to our earlier work on mathematical models of disease transmission (Dwyer and Elkinton 1993, D'Amico et al. 1996). That is, classical mathematical disease models assume that disease transmission is a linear function of the densities of both host and pathogen. In previous experiments, we demonstrated that this assumption was incorrect, but it was not clear whether the nonlinearity arose in larval density, virus density, or both. The larval density treatment that we report here strongly suggests that the nonlinearity is not due to host density, because transmission was not affected by larval (i.e., host) density.

In summary, gypsy moths may induce tannin responses in oaks under some conditions, but both our results and the results of others suggest that those conditions may not commonly occur at the time of year when most virus transmission takes place. We therefore suspect that tritrophic interactions among gypsy moths, nuclear polyhedrosis virus, and oak foliage are unlikely.

Acknowledgments

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