

Responses of condensed tannins in poplar roots to fertilization and gypsy moth defoliation

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Received September 30, 2005; accepted March 7, 2006; published online September 1, 2006

Summary We examined the effects of fertilization and gypsy moth defoliation on condensed tannin concentration (%CT) of hybrid poplar (*Populus × canadensis* cv ‘Eugeneii’) fine roots in the summers of 1997 and 1998. This factorial experiment included two defoliation treatments (defoliated and a foliated control) and fertilization treatments (100 kg nitrogen (N) ha⁻¹ and an unfertilized control). Gypsy moth (*Lymantria dispar* L.) populations were experimentally increased to obtain defoliation in the summers of 1996, 1997 and 1998; fertilization subplots were supplemented with NH₄NO₃ (100 kg N ha⁻¹) in the spring of each year. Despite the severity of defoliation, the effects were small, and significant on only two sampling dates: in May 1997, when fine root %CT was 23% lower in the defoliated trees, and in November 1997, when trees in the defoliated unfertilized plots had 35% higher root %CT than trees in all other plots. Defoliation effects on root %CT did not follow the same seasonal pattern as defoliation effects on root starch content, N uptake capacity or leaf %CT. Regulation of root condensed tannin concentration appeared to be partially uncoupled from these traits. The small transient effects on root defense reflect the resilience of this early successional tree to severe early season defoliation.

Keywords: *Lymantria dispar*, nitrogen, phytochemistry, *Populus*, root defense, short-rotation, soil nitrogen, woody biomass.

Introduction

A large body of research has established that herbivore damage to individual leaves can induce the production of defensive compounds in other leaves (Coley and Barone 1996, Baldwin and Preston 1999, Siemens et al. 2002, Arnold et al. 2004). While the effects of above-ground herbivory are well known, the effect of leaf damage on defensive compounds in roots has received much less attention. Herbivore damage elicits signals that may be translocated through the phloem to root and shoot

sinks remote from the site of damage. Shoot damage clearly alters root secondary metabolism in tobacco, where nicotine is synthesized in roots and translocated to shoots (Baldwin et al. 1998), and in rye, where defoliation leads to an increase in hydroxamic acid in roots (Collantes et al. 1999).

Defoliation alters resource allocation within the plant. Carbon allocation to roots has been shown to decrease with severe defoliation (Bassman and Dickmann 1985), and differences among shoots in source–sink relationships have been shown to alter condensed tannin synthesis in response to defoliation (Arnold and Schultz 2002). Whole-plant nitrogen (N) status can decline with severe defoliation (Bryant et al. 1983). Plant allocation to leaf defense varies with nutrient availability (Bryant et al. 1983, 1993, Herms and Mattson 1992), although this pattern does not hold in all cases (see Riipi et al. 2002).

Does plant allocation to root defenses also change with nutrient availability? Increasing soil N availability increases root turnover in the systems that have been studied (Pregitzer et al. 1993, Kubiske et al. 1998, Johnson et al. 2000), although there is, as yet, no evidence for a direct link between root defensive compounds and rates of root turnover. Nutrient availability can affect tree root defense; in many cases, root phenolic or condensed tannin concentrations increase as N availability decreases (Muller et al. 1989, Entry et al. 1998, Gebauer et al. 1998, Moore et al. 2000, Kraus et al. 2004).

In this paper, we discuss the individual and combined effects of severe defoliation by gypsy moth (*Lymantria dispar* L.) and of N fertilization on fine root condensed tannin concentration of hybrid poplar. Based on leaf responses to defoliation, several outcomes were possible. Defoliation could induce a whole-tree response to herbivory, with increased concentrations of condensed tannins in both roots and leaves. Although herbivory-induced increases in the synthesis of phenylpropanoid compounds are common in leaves, the decline in leaf area and increased carbon (C) demand for leaf regrowth would likely decrease C supply to roots, by reducing root synthesis of condensed tannins. Fertilization would be expected to

mitigate any increase in condensed tannins following defoliation (Bryant 1993). To our knowledge, this is the first field study to examine the combined effects of N fertilization and defoliation by insects on root tannins.

Materials and methods

Experimental plots

Experiments were conducted in four replicate 1-ha blocks of hybrid poplars (*Populus × canadensis* 'Eugeneii') planted as cuttings in May 1989 (Marino and Gross 1998) on the Kellogg Biological Station Long Term Ecological Research site (KBS-LTER), located in southwestern Michigan, USA (42°24' N, 85°24' W). The 40 × 40 m study areas were in the northeastern corner of each block. Trees were planted in a 1 × 2 m array. Weed growth was suppressed from the time of planting by twice yearly (May and July) applications of glyphosate (2% v/v, Roundup, Monsanto Corp., St. Louis, MO). The plots are on a Kalamazoo sandy loam soil (Typic Hapludalf).

Prior to 1996, gypsy moth was present in the plots in low, but detectable numbers, having invaded the area within the last decade (S. Gage, Michigan State University, personal communication). To create densities sufficient to cause significant defoliation, we introduced large numbers of gypsy moth egg masses into the plots beginning in 1996, while removing caterpillars from the foliated (control) plots (see Parry 2000 and Kosola et al. 2001 for details). Briefly, first instar larvae were released into defoliation plots in 1996, followed by additional egg masses in 1997. In 1998, densities were high enough to cause severe defoliation without the addition of supplemental egg masses. Following the 1998 season, the gypsy moth infestation of our plots was low, and little or no defoliation occurred in 1999. The experiment utilized a split-plot design, with two defoliation treatments as the main plots (defoliated and control), each split into a fertilized (100 kg ha⁻¹ N as NH₄NO₃) and unfertilized subplot. Fertilizer was applied within one week of bud break in 1996, 1997 and 1998.

Defoliation varied among years, with about 10% defoliation in 1996, 75–100% defoliation in 1997 and 50–100% defoliation in 1998 (see Parry 2000 and Kosola et al. 2001 for details). Changes in canopy density due to defoliation were clearly detectable in measurements of canopy light penetration (Figure 1).

Root condensed tannins

We collected root samples at least four times during each growing season: early May (initial leafout), late June (peak defoliation), mid-August (coinciding with full canopy recovery of defoliated trees) and late October (after leaf senescence). On each occasion, soil cores (25 cm deep, 6 cm diameter) were taken from each treatment in randomly selected locations, and stored on ice until all cores were processed. The Ap soil horizon was approximately 25 cm deep in the poplar plots; preliminary samples showed that most roots were found within this horizon. We extracted roots from the soil using hydropneumatic root elutriation. This method typically achieves recov-

ery of over 95% of fine roots (Smucker et al. 1982). We collected live fine roots (< 2 mm diameter) for analysis of condensed tannin concentration. Live roots were distinguished from dead roots by assessing color, tissue integrity and mechanical strength. All soil cores from each block were collected and processed on the same day. Roots were extracted and sorted within 8 h of sampling, frozen in liquid nitrogen and stored in a -20 °C freezer until they were lyophilized. In 1996 and 1997, we collected four cores from each treatment, with the exception of August 25, 1997, when only one core was collected from each treatment. In 1998, a single core was collected from each treatment on each sampling date. All replicate root samples were bulked together by treatment within plots before they were ground. Lyophilized roots were stored at room temperature in sealed plastic bags with desiccant. Desiccant packs were changed every 6 months.

Root tissues were analyzed in 2001 for condensed tannin concentration by the acid butanol colorimetric assay (Czochanska et al. 1980). Both variation among dates in a growing season and treatment-induced variation in leaf condensed tannin composition have been reported to affect the response of this assay (Appel et al. 2001). To check for variation in condensed tannins among samples, standards were purified from composite samples using the method of Hagerman and Butler (1980). Standards were purified from each treatment, with suf-

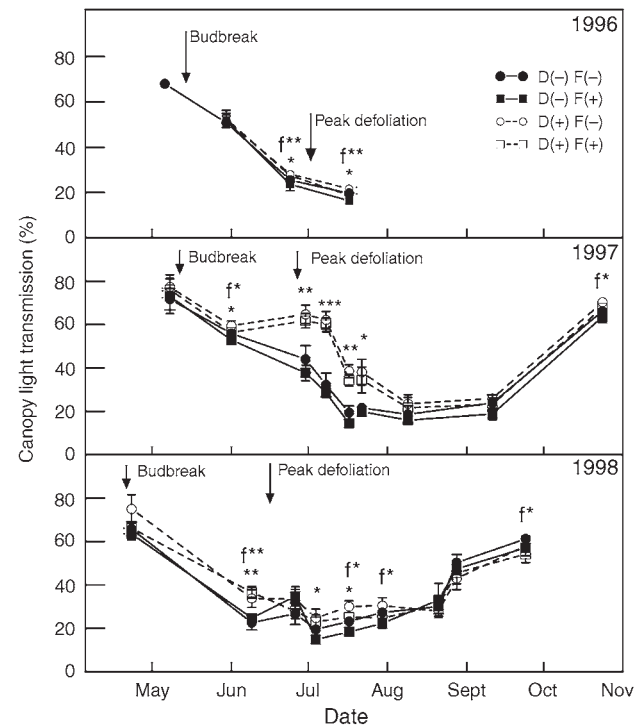


Figure 1. Canopy light transmission (% of total photosynthetically active radiation) at observation dates throughout the growing season in 1996, 1997 and 1998. Values are means \pm SE, $n = 4$. Error bars not shown are smaller than the symbol. Significance: * = $P < 0.05$; ** = $P < 0.01$; and *** = $P < 0.001$ for defoliation effects on light transmission; * = $P < 0.05$; and ** = $P < 0.01$ for fertilizer effects on light transmission.

efficient root mass remaining after the initial set of analyses. Composite samples for each treatment were composed of equal masses of lyophilized tissue from each of the four blocks. This same set of root samples was also analyzed for total nonstructural carbohydrates (starch, glucose, sucrose and fructose) by an enzyme-linked colorimetric assay (Hendrix 1993, Kosola et al. 2001).

Statistical analysis

All analyses were run as a mixed-model analysis of variance (ANOVA) with the SAS statistical software package (SAS v.8.80, 2001), with the Kenward-Roger estimation of denominator degrees of freedom (Littell et al. 1996). Defoliation and fertilizer treatments were analyzed as fixed effects; blocks were analyzed as random effects.

Results

Treatment and sampling date effects on standard curves for root condensed tannins were negligible, with no significant difference in the relationship between condensed tannin concentration and response of the acid butanol assay for any of the samples (data not shown).

There were no effects of defoliation or fertilization on fine root condensed tannin concentration (%CT) in 1996 or 1998 (Figure 2). The only two dates with a significant treatment effect were May 1997, when both defoliated treatments were lower in %CT than the undefoliated treatments (23% lower than foliated plots), and November 1997, when %CT was significantly greater in the defoliated unfertilized treatment (35% greater than in the defoliated, unfertilized treatment, Figure 2; Table 1). There was a significant interaction between defoliation and fertilization in November 1997 (Figure 2; Table 1). There was a marginally significant increase in %CT in both defoliated treatments (12% greater than control plots) in July 1997 (Figure 2; Table 1).

Discussion

Responses of root %CT to defoliation and fertilization were small and transient, consistent with effects of defoliation and fertilization on mycorrhizal colonization (transient and small effect, Kosola et al. 2004), root turnover and root system age structure (no effect, Kosola et al. 2001), root growth (no effect, Kosola et al. 2001), fine root starch (transient decline, Kosola et al. 2001) and N uptake (transient decline, Kosola et al. 2001). Although the root CT response to defoliation and fertilization was transient, as seen for these other root traits, timing of the response was different. Defoliated trees lagged in late-season accumulation of root starch in August 1997, during leaf canopy regrowth after defoliation (Figure 3). Treatment effects on root CT were observed in May 1997 (Figure 2, after the first season's light defoliation), in July 1997 (Figure 1, at peak defoliation) and in November 1997 (Figure 2, after starch in roots from defoliated plots had recovered to concentrations similar to those observed in foliated trees, Figure 3). The lower

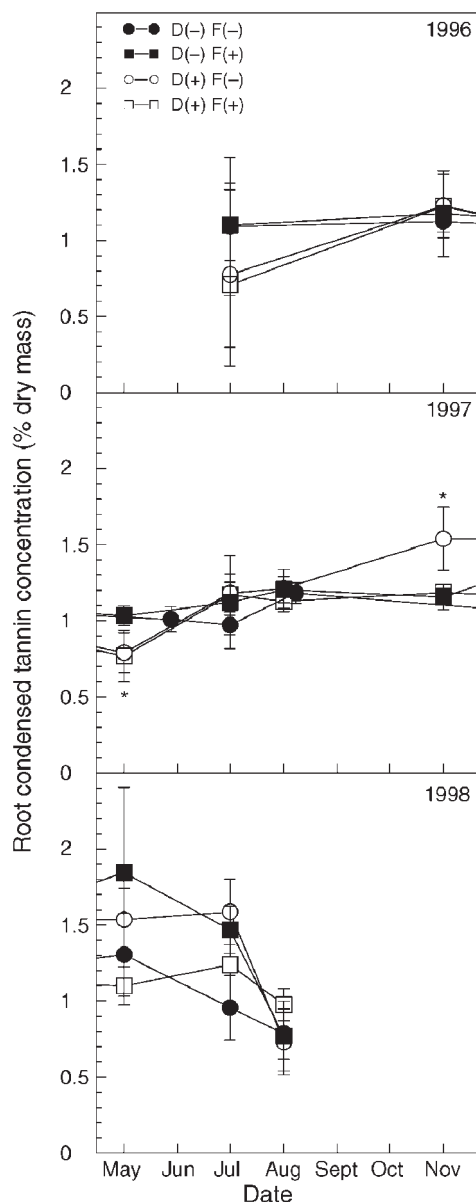


Figure 2. Fine root condensed tannin concentration (% root dry mass) at observation dates throughout the growing season in 1996, 1997 and 1998. Values are mean \pm SE, $n = 4$ for all data. Error bars not shown are smaller than the symbol. An asterisk (*) indicates $P < 0.05$, significant defoliation effect.

Table 1. Analysis of variance for effects of defoliation and fertilization (100 kg ha^{-1} N as NH_4NO_3) treatments on fine root condensed tannin concentration (% root dry mass) in 1997.

Effect	May 1997	July 1997	Nov 1997
Defoliation	$F = 4.74_{1,52}$ $P = 0.032$	$F = 3.99_{1,28.8}$ $P = 0.055$	$F = 10.05_{1,7.78}$ $P = 0.014$
Fertilization	$F = 0.02_{1,52}$ $P = 0.88$	$F = 0.60_{1,5.9}$ $P = 0.467$	$F = 1.60_{1,6.71}$ $P = 0.247$
Defol \times Fert	$F = 0_{1,52}$ $P = 0.99$	$F = 1.10_{1,28.8}$ $P = 0.303$	$F = 8.10_{1,7.78}$ $P = 0.022$

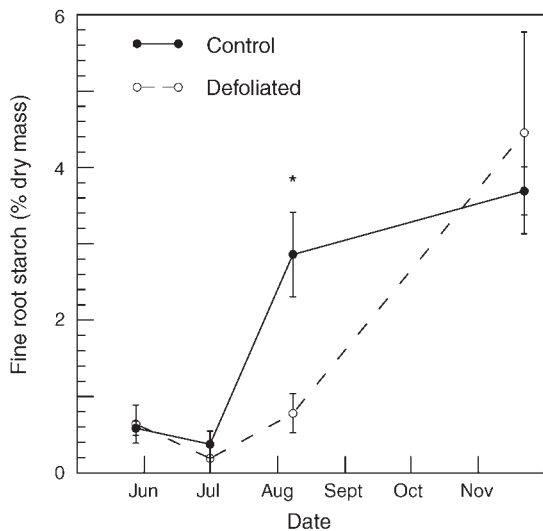


Figure 3. Defoliation effects on fine root (< 2 mm diameter) starch concentration in unfertilized treatments in 1997. Values are means \pm SE. Error bars not shown are smaller than the symbol. An asterisk (*) indicates $P \leq 0.05$, $n = 4$.

root %CT observed in defoliated plots sampled in May 1997 was apparently a response to the initial defoliation during the previous (1996) growing season. The increases in root CT of defoliated trees seen in July and November 1997 were not coincident with treatment effects on total root N concentration. The timing of root CT responses to defoliation treatments suggests that carbon partitioning to root CT is decoupled from late-season starch deposition, N uptake or root and shoot growth. Both root CT synthesis and turnover play a role in determining measured pools of CT (Kleiner et al. 1999). The seasonal timing of root CT synthesis and turnover has not been determined for roots of any tree.

Fertilization alone did not decrease fine root %CT in this study. Trunk diameter growth rates increased significantly with fertilization (Kosola et al. 2001). Fertilization mitigated the increase in root condensed tannin observed in November 1997 (Figure 2), consistent in part with predictions of the carbon-nutrient balance theory (Bryant et al. 1983, Herms and Mattson 1992). In contrast to our observations of root %CT, leaf %CT was significantly elevated in defoliated treatments in midsummer 1996 and 1997 (Parry 2000), indicating that gypsy moth feeding induced increased CT synthesis in leaves. We were unable to conduct quantitative comparisons between root and leaf %CT because of differences in the methodology used for the root and leaf sample analyses.

It is unclear what mechanisms are responsible for the changes we observed in root %CT. It is possible that the decline in root %CT seen in the spring following the initial season of defoliation was caused by a decline in carbon allocation to roots. Root %CT was greater in defoliated trees at the peak of defoliation during the second growing season and in defoliated, unfertilized trees in the fall after the second growing season, consistent with increased CT synthesis. The lack of defo-

liation effects on root %CT in fertilized trees in the fall of 1997 may be due to alleviation of defoliation-induced N deficiency.

Clearly, root %CT is not highly responsive to defoliation. The root systems of these trees appear to be strongly buffered against the effects of even multiple years of severe defoliation. Vegetative propagation by root suckers is an important mode of reproduction in *Populus* species. Coppice regrowth from trunks is strong (e.g., Tschaplinski and Blake 1995), and is essential to recovery from shoot loss from beaver activity in the native riparian habitat. The ability to maintain a viable root system despite defoliation or even coppicing (Dickmann et al. 1996) may have been subject to strong selection in view of the substantial contribution of both shoot regrowth and root suckering to the success of many poplar species.

Fine root turnover and leaf litter contributions dominate forest soil carbon inputs; each is likely to contribute similar quantities of carbon to soil carbon pools (Luo et al. 2001). Factors that alter root litter quality and root turnover can alter rates of carbon and nutrient cycling in forest ecosystems (Hendricks et al. 2000). In our study, the hybrid poplars recovered quickly from defoliation, with only a transient effect on root %CT and no effect on root turnover (Kosola et al. 2001). Root contributions to soil carbon pools in healthy early successional forests are likely to be stable during outbreaks of early season defoliators like gypsy moth, in contrast to the large shifts in timing and composition of leaf litter inputs to soil C found during outbreaks. Future studies of ecosystem dynamics of soil C pools during outbreaks of herbivores should characterize the composition and quantity of both root and leaf litter inputs.

Acknowledgments

This work was supported by NSF Grant DEB 95-10044, the NSF LTER Program at KBS, by the Michigan Agricultural Experiment Station and the University of Wisconsin. Thanks to Kay Baergen, Emily Duncan, Christine Easley, Joshua Edwards, Marla Fisher, Mary Kellogg, Christy Lynn, Carolyn Miller, Justin Pittinaro, Helmut Stoyan and Bob Watts for technical assistance, Heidi Barnhill for advice on condensed tannin assays and Brent McCown, Kay Gross, Mike Klug for equipment. This is paper 1264 from the W.K. Kellogg Biological Station.

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