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Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates, and root demography of poplars

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Abstract Large-scale outbreaks of defoliating insects are common in temperate forests. The effects of defoliation on tree physiology are expected to cascade through the entire forest ecosystem, altering carbon, nitrogen, and water fluxes, and subsequently affecting nitrogen cycling and plant-herbivore interactions. If these post-defoliation changes are largely driven by N deficiency, tree root system responses to defoliation should be central to regulating the long-term effects of defoliation; N fertilization should reverse the effects. We examined these phenomena in a 3-year large-scale replicated manipulative field experiment in a hybrid poplar plantation, where we regulated defoliation by gypsy moths as well as nitrogen availability. To our knowledge, this is the first manipulative field experiment at this scale to examine the effects of severe insect defoliation on whole-tree physiology. Defoliation decreased tree growth and increased the rate of top dieback in the stand. Defoliation led to transient declines in carbon allocation to starch in fine roots, trunk, and twigs in the year of heaviest defoliation. Root production and root mortality were unaffected by the heaviest defoliation, but nitrate and ammonium

uptake were strongly depressed. N fertilization increased tree growth, but did not alter defoliation effects on starch accumulation or top dieback. Defoliation and fertilization treatments did not interact. In this system, defoliation effects on tree recovery of leaf nitrogen lost to herbivory were primarily driven by effects on nitrogen uptake, rather than effects on root production or mortality.

Keywords *Lymantria dispar* · Root demography · Starch · Ammonium · Nitrate

Introduction

Large-scale outbreaks of herbivorous insects are a common feature of temperate forest landscapes. At this scale, defoliation effects on tree physiology are expected to cascade through the entire forest ecosystem, altering carbon, nitrogen, and water fluxes, and subsequently affecting nitrogen cycling and plant-herbivore interactions (Schowalter et al. 1986). Trees not only lose photosynthetic capacity during defoliation, but lose the resources, most notably nitrogen, contained in the leaves consumed by herbivores. Physiological changes in defoliated trees may determine the susceptibility of trees to future defoliation (Bryant et al. 1993; Karban and Baldwin 1997) and alter forest ecosystem nutrient cycling (Kielland et al. 1997; Ruess et al. 1998; Schowalter et al. 1986).

Carbon-nutrient balance theory predicts that many of these post-defoliation changes are due to N deficiency (Bryant et al. 1993; Tuomi et al. 1990; Koricheva et al. 1998). The extent of N deficiency is likely to be influenced by tree root system responses to defoliation (Bryant et al. 1993) and should be reversible by N fertilization, even in the presence of decreased root capacity for N acquisition (Tuomi et al. 1990). Carbon allocation to roots declines rapidly following defoliation, with a concomitant depletion of stem and root reserves, both in young trees (1- to 2-year-old *Populus*; Bassman and Dickmann 1982, 1985; Bassman and Zwier 1993; Tschaplinski and Blake 1994) and in mature trees (sugar maple, Wargo et al. 1972; oaks, Parker and Patton 1975;

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Douglas fir, Webb 1981). If the decline in carbon allocation to roots leads to decreased tree capacity to acquire nutrients, recovery of nutrients lost to defoliation will be slowed (Bryant et al. 1993).

Decreased root growth, increased root mortality, a decline in the rate of NO_3^- and NH_4^+ uptake, and decreased mycorrhizal colonization are all potential consequences of declining carbon allocation to roots which could depress tree capacity for N acquisition. Although root total nonstructural carbohydrate (TNC) pools in mature trees decline after defoliation (Wargo et al. 1972; Parker and Patton 1975), it is not clear whether root growth and nitrogen acquisition are strongly affected by defoliation in mature trees. Older trees have larger storage pools of C and N than the small trees used in most studies (Pregitzer and Friend 1996). Lower rates of root production were observed in browsed stands of early successional taiga trees (alder, birch, and willow) by Kielland et al. (1997) and Ruess et al. (1998); root mortality was also higher in these stands (Ruess et al. 1998). Contrary to these observations, Dickmann et al. (1996) did not observe substantial increases in root mortality during the first growing season following decapitation of dormant 4-year-old hybrid poplar trees. They hypothesized that accumulation of carbon stores in roots during the fall was sufficient to supply all the energy needed for normal root growth and maintenance the following season in decapitated plants. It is not known if insect defoliation effects on root demography are similar to the effects of either winter browsing or decapitation.

In this paper, we report the effects of gypsy moth (*Lymantria dispar* L.) defoliation and nitrogen fertilization of hybrid poplar (*Populus × canadensis* cv Eugeneii) on TNC content (glucose, fructose, sucrose, and starch) in root, twig, and trunk samples, tree growth and vigor, nitrate and ammonium uptake capacity, and root production and mortality. To our knowledge, this is the first large-scale, replicated manipulative field experiment to examine the effects of severe insect defoliation on whole-tree physiology. The use of mature trees growing in the field allows us to bridge the gap between detailed studies of young trees in pots and studies of natural forest ecosystems.

Materials and methods

Experimental plots

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

Prior to 1996, gypsy moth was present in the plots at low but detectable levels, having invaded the area within the last decade (S. Gage, personal communication). To create densities sufficient

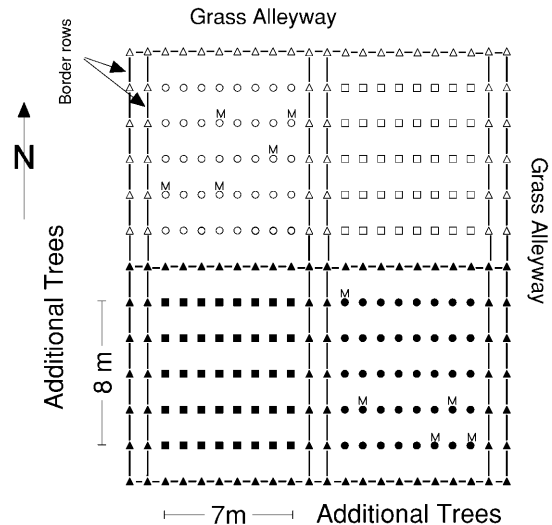


Fig. 1 Representative plot map. Closed symbols (●, ■, ▲) are undefoliated, open symbols (○, □, △) are defoliated, circles (●, ○) are unfertilized, squares (■, □) are fertilized, triangles (▲, △) are border row trees (M tree with minirhizotron)

to cause significant defoliation, we introduced large numbers of gypsy moth egg masses into the plots beginning in 1996, while removing caterpillars from the undefoliated (control) plots (see Parry 2000 for details). First-instar larvae were released into defoliation plots in 1996, followed by additional egg masses in 1997. In 1998, egg mass densities were high enough that supplemental egg masses were not added. Following the 1998 season, the gypsy moth population in our plots fell to very low levels; few gypsy moth were present 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 ($100 \text{ kg ha}^{-1} \text{ N}$ as NH_4NO_3) and unfertilized subplot (Fig. 1). Two of the four replicate blocks were randomly chosen to have the defoliated plot on the northern, exposed portion of the block, and the other two had the defoliated plot on the inner, southern portion of the block. Placement of the fertilized subplot on the eastern, exposed portion of each main plot was similarly randomly chosen for two of each of the defoliated and control plots, and then assigned to the inner western portion of the other two plots of each defoliation treatment. This procedure was followed to maximize both randomization and dispersion of treatments (Hurlbert 1984).

Minirhizotron observations of root production and mortality

Minirhizotrons (1.8 m long, 5.08 cm inside diameter) were installed in January and February of 1996 at a 60° angle to the soil surface; expandable-wall minirhizotrons (Kosola 1999) were installed in March 1997. We placed minirhizotrons next to five trees in each treatment; only data from the unfertilized plots with and without defoliation will be presented here. The five minirhizotron trees were randomly chosen from the subset of trees in each block that were within one standard deviation of the mean trunk diameter measured 15 cm above the soil surface. The top of each minirhizotron was 30 cm from the trunk, with the buried portion of the tube directed towards the trunk. Minirhizotrons were scribed with frames on the upper surface that matched the field of view of the video camera (BTC-100, Bartz Technology, Santa Barbara, Calif., USA), allowing us to locate and videotape the same roots repeatedly. We videotaped root growth on the upper surface of the minirhizotron tubes approximately every month during the growth season, starting just before bud break and ending after leaf fall. The data presented here were collected from two of the trees in each

replicate plot on 20 May and 10 December 1996; and 4 April, 9 June, 10 July, 5 August, and 6 September 1997. Expandable-wall minirhizotrons were observed for these trees on the dates in 1997. The choice of minirhizotrons for analysis was based on random selection among the minirhizotron trees which remained healthy throughout the experiment; in some plots, up to three out of the five minirhizotron trees experienced top dieback by 1997. We used the program MSU-ROOTS (W. Enslin, K.S. Pregitzer, and R.L. Hendrick, MSU-ROOTS version 8-10-93, Michigan State University, East Lansing, Mich., USA) to analyze the minirhizotron video images (Hendrick and Pregitzer 1992a). The data from the two minirhizotrons in each replicate plot were pooled for analysis of root production (Hendrick and Pregitzer 1992b). Survivorship of the cohorts of roots produced at each observation period was determined from minirhizotron data pooled from all plots.

Tree total nonstructural carbohydrates

We collected root samples at least four times each growing season: early May (initial leafout), late June (peak defoliation), mid-August (after leaf regrowth), and late October (after leaf senescence). On each occasion, 25-cm-deep, 6-cm-diameter soil cores were taken from each treatment in randomly selected locations, and stored on ice until all cores were processed. The Ap 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 gives >95% recovery of fine roots (Smucker et al. 1982). We separated roots from each core into four classes: coarse (greater than or equal to 2 mm), fine (less than 2 mm), very fine (less than 0.5 mm) and dead roots (all diameters). 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 after sampling, frozen in liquid nitrogen, and stored in a freezer at -20°C until they were lyophilized. In 1996 and 1997, we collected four cores from each treatment, with the exception of 25 August 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 each plot before they were ground.

We collected 2.5-cm-long, 4-mm-diameter trunk samples at 15 cm height from the ground with an increment hammer (Haglof Inc., Madison, Miss., USA) from four trees at each root sampling date in 1997 and 1998, storing them on ice for up to two hours until freezing in liquid N. Twice in 1997 (June and July) and three times in 1998 (May, June, and July) we collected samples of current-year twigs from the top 2 m of the canopy with a pole pruner, freezing them in liquid N within 30 min of sampling. Frozen trunk and twig tissue were stored in a freezer at -20°C until they were lyophilized. Twig samples and trunk samples were each pooled by treatment within each plot before they were ground.

We stored lyophilized tissue at room temperature in plastic bags containing desiccant, which was changed regularly. Dried tissue was ground to fit through a 40-mesh screen in a miniature Wiley mill (Thomas Scientific, Swedesboro, N.J., USA). We measured TNC (starch, glucose, fructose, and sucrose) in the lyophilized ground tissue by enzyme-linked colorimetric assays (Hendrix 1993).

Nitrate and ammonium uptake by excised roots

To determine whether defoliation affected the capacity of the trees to acquire nitrogen, we measured ^{15}N -labelled nitrate and ammonium uptake from 200 $\mu\text{mol l}^{-1}$ KNO_3 or NH_4Cl solution by excised roots collected from trees in unfertilized plots with and without defoliation 17 July 1997 and 31 July 1998. This concentration was determined to be saturating for NO_3^- and NH_4^+ uptake by measuring the kinetics of NO_3^- and NH_4^+ influx from solution by

excised roots from a single unfertilized undefoliated plot. Measurements for kinetics estimates were made at concentrations of 10 $\mu\text{mol l}^{-1}$, 20 $\mu\text{mol l}^{-1}$, 50 $\mu\text{mol l}^{-1}$, 100 $\mu\text{mol l}^{-1}$, and 200 $\mu\text{mol l}^{-1}$ NO_3^- or NH_4^+ .

At least three 15-cm-deep soil cores were collected from each treatment. Roots were washed from cores bulked together by treatment and plot within one hour of harvesting. We selected live roots 1 mm diameter or less, cut them into 2-cm lengths, and divided the roots into subsamples, each about 2 g fresh weight. Each subsample was placed into 250 ml aerated 2 mmol l^{-1} CaSO_4 for recovery from excision for 30–50 min. The solution was replaced once about halfway through this equilibrium period. The solutions were contained in 500-ml wash bottles, vigorously aerated through the wash bottle nozzle, and suspended in a 21°C ($\pm 0.1^{\circ}\text{C}$) water bath. The assay was run at 21°C to match the mean soil temperature at 5 cm depth in our plots in July 1996. At the start of the labeling period, the equilibration solution was replaced with 250 ml fresh 2 mmol l^{-1} CaSO_4 . $\text{K}^{15}\text{NO}_3^-$ or $\text{K}^{15}\text{NH}_4^+$ stock solution (99 atom% enrichment) was added to the solution to bring the solution to the desired final concentration. After 15 min, the labeled solution was poured from the bottle and replaced with 250 ml ice-cold unlabeled wash solution (200 $\mu\text{mol l}^{-1}$ NH_4^+ for all NH_4^+ solutions, and 200 $\mu\text{mol l}^{-1}$ NO_3^- for all NO_3^- solutions). After 15 min in the wash solution, roots were removed from the solution, frozen in liquid N and later lyophilized. Lyophilized roots were ground to a fine powder with a dental amalgamator (Wigg-L-Bug, Crescent Dental, Lyons, Ill., USA) and analyzed for ^{15}N enrichment (Harris and Paul 1989).

Artifacts may be introduced to measurements of nutrient acquisition by excised roots if the labeling period is sufficiently long that root carbohydrate pools become depleted. To test for effects of time on excised root carbohydrate pools, we measured TNC in roots before labeling (about 1 h after excision) and after labeling (3–4 h after excision).

Tree measurements

Light transmission through the canopy in each of the replicate blocks was measured on overcast days with a 1-m-long light (photosynthetically active radiation, PAR) sensor (Sunfleck Ceptometer, Decagon Devices, Pullman, Wash., USA). Reference full-light measurements were taken outside of the plot before and after measuring canopy light transmission. Measurements were taken near the center of each subplot under healthy trees. Each reading was the average of 20 PAR measurements; 5 PAR measurements were taken while facing each of the cardinal directions, with the north-south measurements within rows and the east-west measurements between rows.

Tree diameter (DBH) was measured at 1.4 m with a diameter tape (1996) or with a digital calliper (1997 and 1998 – Forestry Suppliers, Jackson, Miss., USA). The measurement points were marked on the bark with permanent marker, permitting consistent tape or caliper placement at each measurement date. Two caliper diameter measurements were taken for each tree – one north-south and one east-west – and averaged. We also recorded qualitative visual observations of tree vigor at intervals during each growing season. Three tree health classifications were used: tree dead (no live branches), top dead, and healthy. Tree heights were measured with a graduated measuring pole in December 1998; at this time, we also measured the height of terminal bud scars on the leader of each tree for the 1995, 1996, and 1997 growing seasons.

Statistical analysis

All analyses were run as a mixed-model analysis of variance in SAS Proc Mixed (Littell et al. 1996), with defoliation and fertilizer treatments as fixed effects, and blocks as random effects. Block averages for TNC and light transmission data at each date were analyzed as a split-plot design. Tree diameter growth and survivorship measurements were analyzed as a repeated measures,

split-plot design, with measurements on individual trees nested within each subplot; compound symmetric covariance structure with heterogeneous variances (CSH; Littell et al. 1996) was used for the error structure of the repeated measure. Since top survival of an individual tree was a binary variable (alive or dead), we used the general linear mixed models macro (glmm612.sas) to transform the data set to fit the assumptions for repeated-measures ANOVA (Littell et al. 1996). Root production data for 1997 were analyzed as a repeated-measures mixed-model ANOVA. Root production data for 1996 were analyzed as a one-way mixed-model ANOVA. All root production data were log-transformed to increase homogeneity of variances. Root survivorship curves were compared by the Gehan-Wilcoxon test for right-censored survivorship data (Pyke and Thompson 1986). The apparent affinity (apparent K_m) and apparent saturated rate of uptake (apparent V_{max}) for NO_3^- and NH_4^+ uptake were determined by linear regression of data transformed by the Wolf-Augustinsson-Hofstee method (abscissa= $V/[S]$, ordinate= V ; Segel 1976).

Results

Canopy light transmission and tree growth

Measurements of light transmission through the canopy in each plot gave an index of defoliation intensity, as light transmission is directly related to leaf area index (Buckley et al. 1999). The pattern and intensity of defoliation effects were different each year. Defoliation was moderate in 1996, uniformly severe in 1997 and varied among blocks from moderate to severe in 1998 (Fig. 2; Parry 2000). We did not quantify defoliation in 1999, as gypsy moth populations were negligible. We found significant effects of defoliation on canopy light transmission at the time of peak defoliation in each year (Fig. 2). In 1997, peak defoliation at the end of June eliminated most of the canopy (Fig. 2). Leaf regrowth was rapid, but canopy densities in the defoliated plots were reduced until August (Fig. 2).

Fertilization increased tree diameter growth in 1996, 1997, and 1998 (Table 1, Fig. 3). DBH increment was significantly lower in defoliated plots in 1997, 1998, and 1999 (Table 1, Fig. 3). The significant interaction terms between measurement date and both fertilization and defoliation (Table 1) are due to within-season variation in growth response to these treatments. Analysis of simple effects (not shown) indicated that, after their first appearance, fertilizer and defoliation effects on early-season growth were maintained during the rest of the growing season (Fig. 3). The three-way interaction between defoliation, fertilization, and date in 1997 is due to a delay in the response of fertilized plots to defoliation; significant effects of defoliation were seen first in unfertilized plots (24 June), and later in fertilized plots (14 July). The patterns of tree height growth are consistent with the patterns seen in DBH growth, with defoliation significantly decreasing growth in 1996 and 1998 (Table 2).

We monitored the health of individual trees throughout the experiment, recording top survival and qualitative estimates of tree vigor beginning in 1997. As expected for stands undergoing self-thinning (Oliver and Larson 1996), initial tree size (diameter October 1995) had highly significant effects on top survivorship

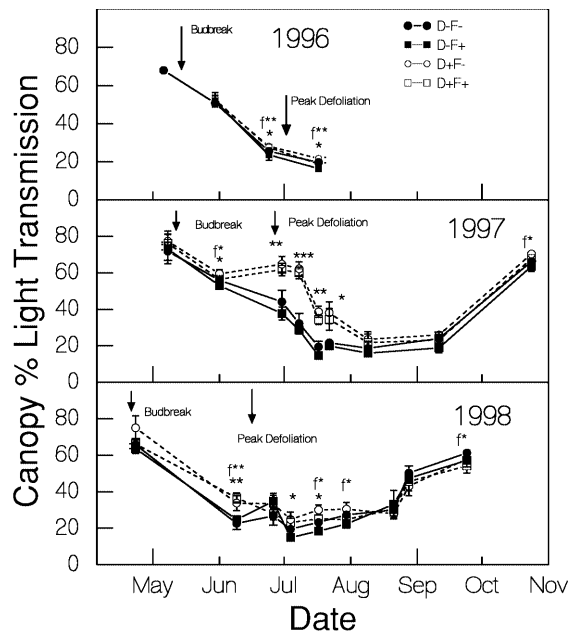


Fig. 2 Canopy light transmission (percent of total photosynthetically active radiation, PAR) at observation dates throughout the growing season in 1996, 1997, and 1998. Values are mean \pm SE, $n=4$ for all data. Error bars not shown are smaller than the symbol (\bullet -defoliation -fertilizer, \circ +defoliation -fertilizer, \blacksquare -defoliation +fertilizer, \square +defoliation +fertilizer). * $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$ for defoliation effects on light transmission, f* $P\leq 0.05$ for fertilizer effects on light transmission

Table 1 Split-plot repeated measures ANOVA of diameter growth in 1996, 1997, 1998, and 1999. Data for 1999 are from a single end-of-season measurement, so date effects for that year are not included in the analysis

Source	df	F			
		1996	1997	1998	1999
Date	3	106.19* ⁴	166.69* ⁴	68.69* ⁴	
Defoliation	1	8.44	32.92* ⁴	20.17* ⁴	8.82* ¹
Date \times Defoliation	3	0.99	25.89* ⁴	12.46* ⁴	
Fertilization	1	6.96 ^a	10.54* ²	2.91 ^a	0.40
Date \times Fertilization	3	4.21* ²	9.14* ⁴	1.48	
Defoliation \times Fertilization	1	1.34	0.75	0.07	0.56
Date \times Defoliation \times Fertilization	3	0.62	4.41* ²	0.12	

^a $P\leq 0.1$, * $P\leq 0.05$, ** $P\leq 0.01$, *** $P\leq 0.001$, *⁴ $P\leq 0.0001$

throughout the experiment ($P\leq 0.001$); top dieback occurred faster in smaller trees. Defoliation experienced in previous years significantly affected top survivorship in 1999, a year with no gypsy moth herbivory (Fig. 4). In this year, there was a 60% decline in top survivorship in the defoliated plots relative to the control plots (Fig. 4).

Seasonal patterns in TNC, root growth and mortality

We observed typical seasonal patterns in tissue TNC pools (Nguyen et al. 1990), with seasonal low values in

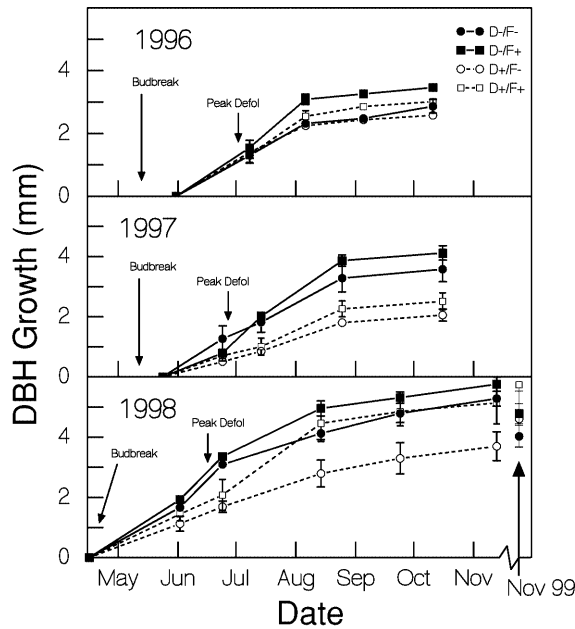


Fig. 3 Cumulative trunk diameter growth at 1.4 m height during the 1996, 1997, 1998, and 1999 growing seasons. Symbols and error bars as in Fig. 2

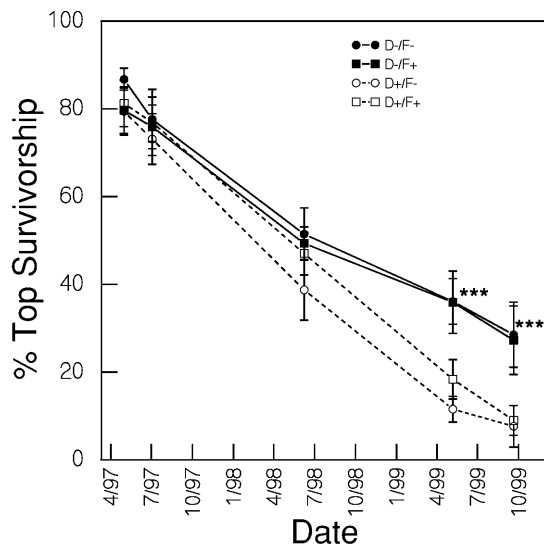


Fig. 4 Proportion of trees in plot with live tops (% top survivorship), 1997 to 1999. Symbols and error bars as in Fig. 2

Table 2 Total seasonal height growth (m). Measured in November 1998; height of bud scars from previous years used to determine height growth in each season. Values are mean \pm standard error; $n=4$. Defoliation significantly decreased tree height growth in 1996 and 1998 ($P\leq 0.05$)

Treatment	1996	1997	1998
Not defoliated, not fertilized	0.70 \pm 0.08	0.35 \pm 0.03	0.26 \pm 0.01
Not defoliated, fertilized	0.84 \pm 0.06	0.42 \pm 0.10	0.33 \pm 0.02
Defoliated, not fertilized	0.59 \pm 0.10	0.28 \pm 0.07	0.23 \pm 0.05
Defoliated, fertilized	0.63 \pm 0.11	0.34 \pm 0.05	0.21 \pm 0.04

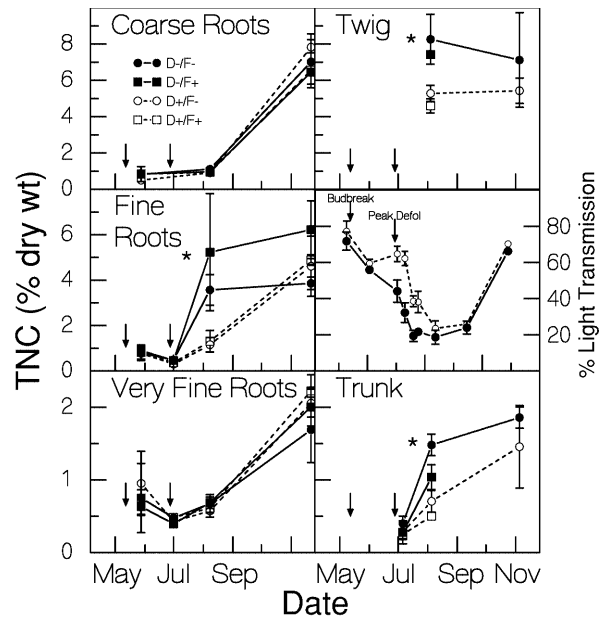


Fig. 5 Treatment effects on total nonstructural carbohydrates in 1997; data on light transmission are included to demonstrate timing of defoliation and refoliation. Arrows indicate the dates of bud break and peak defoliation. Symbols and error bars as in Fig. 2

the spring and summer, with an increase to seasonal highs in the fall (Fig. 5). There were no significant treatment effects on either coarse root or very fine root TNC at any of the sampling dates (Fig. 5). Fertilization did not affect TNC, starch, or soluble sugar content in any tissue at any sampling date during the 3-year period (Fig. 5). We did see defoliation treatment effects on TNC in fine roots, trunk cores, and twigs in August of 1997, when TNC was lower in tissues from defoliated plots (Fig. 5). The increase in TNC was due entirely to increased starch content, as there was no significant treatment affect on soluble sugar contents at any sampling date. By November, TNC concentrations in trees from defoliated plots were indistinguishable from TNC concentrations in trees from control plots. There was a significant effect of fertilizer and a significant interaction between defoliation and fertilizer effects on twig TNC on 29 July 1998, again due to increased starch concentrations. Defoliation only led to lower starch levels in unfertilized plots at this date (twig % starch: unfertilized plots, defoliated, 10.74 \pm 1.13, control 16.15 \pm 1.40; fertilized plots, defoliated, 15.94 \pm 1.39, control 14.70 \pm 1.02; mean \pm SE).

Root production on the minirhizotron tubes between installation and the initial observation (May 1996) did not differ significantly among the treatments (data not shown). Significantly fewer new roots were observed in the defoliated plots in December 1996 (Fig. 6). Defoliation did not significantly affect root production in 1997, whether measured with standard minirhizotrons (installed in 1996; Fig. 6A) or with expandable minirhizotrons (installed in 1997; Fig. 6B). Contrary to our original hypothesis, defoliation did not lead to increased root

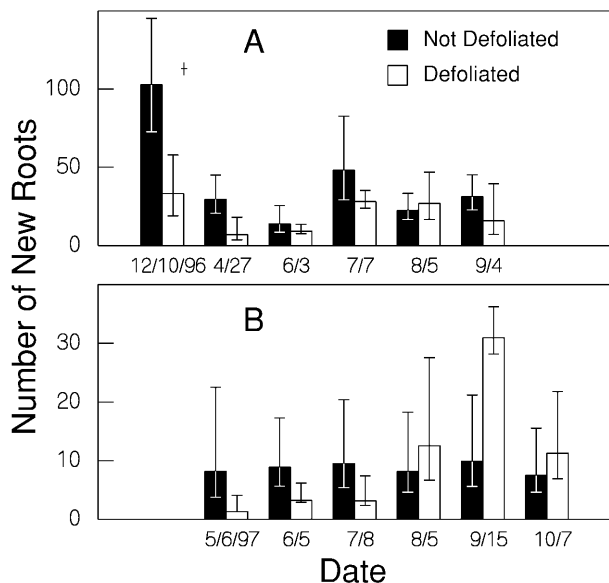


Fig. 6A, B Number of new roots produced during each observation interval as monitored by minirhizotron observations. Data are pooled for minirhizotrons within plots; values are mean \pm SE (based on log-transformed data), $n=4$ for all data. Data are for unfertilized treatments only; closed bars represent undefoliated control plots, open bars represent defoliated plots ($\dagger P\leq 0.1$). **A** Data from standard minirhizotrons, installed January–February 1996. Data for 10 December 1996 are surviving roots produced since 18 May 1996. **B** Data from expandable-wall minirhizotrons, installed March 1997

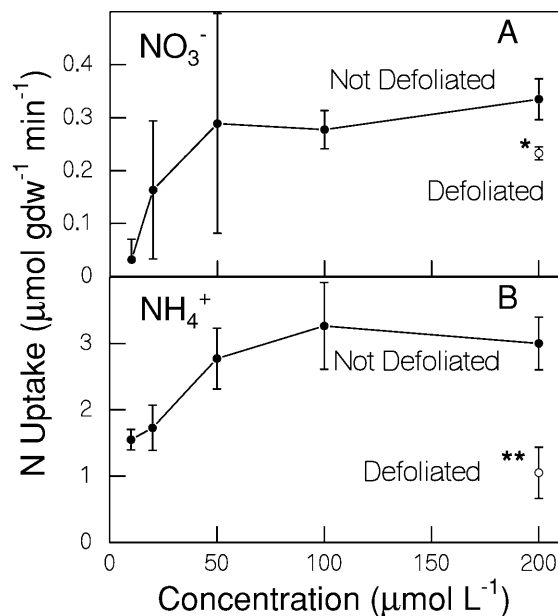


Fig. 8A, B Effect of defoliation on NO_3^- and NH_4^+ uptake by roots from unfertilized plots. **A** NO_3^- uptake ($\mu\text{mol g}^{-1} \text{DW min}^{-1}$) by excised roots as a function of NO_3^- concentration ($\mu\text{mol l}^{-1}$). **B** NH_4^+ uptake ($\mu\text{mol g}^{-1} \text{DW min}^{-1}$) by excised roots as a function of NH_4^+ concentration ($\mu\text{mol l}^{-1}$). Symbols and error bars as in Fig. 2

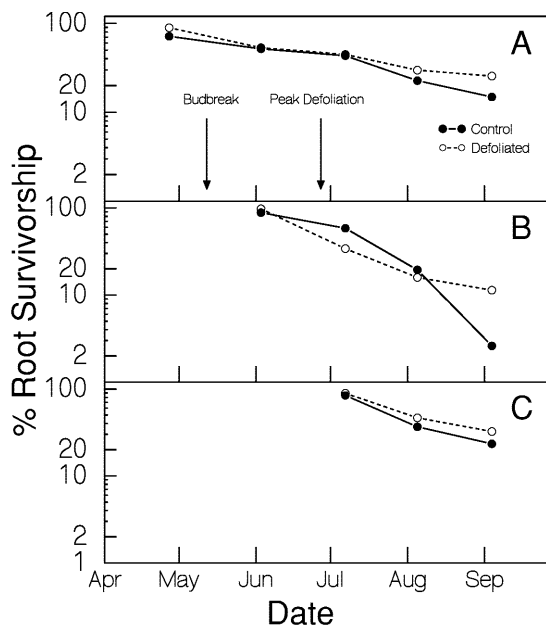


Fig. 7A–C Root survivorship during 1997 for three cohorts of roots. **A** May cohort of roots, **B** June cohort of roots, **C** July cohort of roots. Data from all four replicate plots are pooled together, and are for unfertilized treatments only. Symbols and error bars as in Fig. 2

mortality in cohorts of roots first observed in April, June, or July 1997 (Fig. 7). Root length and weight (data not shown) in the soil cores collected for TNC analysis were not significantly affected by defoliation.

For our root demographic data to be most comparable with other studies, we made the decision to only analyze data from healthy trees, rather than introducing complex interactions between tree vigor and defoliation. We did not see any evidence for effects of defoliation on top survivorship in 1996 or 1997 (Fig. 4), so excluding trees experiencing top dieback in these years is unlikely to have introduced substantial bias to our analyses.

Nitrate and ammonium uptake

Nitrate and ammonium uptake exhibited saturable kinetics (Segel 1976) within the range of concentrations measured (Fig. 8). The apparent V_{max} for nitrate uptake ($0.37\pm 0.05 \mu\text{mol gdw}^{-1} \text{min}^{-1}$) was about 10 times lower than the apparent V_{max} for ammonium uptake ($3.34 \mu\text{mol l gdw}^{-1} \text{min}^{-1}$). The apparent K_m for NH_4^+ uptake ($12.69\pm 3.53 \mu\text{mol gdw}^{-1} \text{min}^{-1}$) was slightly lower than the apparent K_m for NO_3^- uptake ($21.60\pm 8.54 \mu\text{mol gdw}^{-1} \text{min}^{-1}$)

Defoliation significantly decreased saturated rates of both ammonium and nitrate uptake in 1997; ammonium uptake was strongly affected, with rates in the defoliated plots 66% lower than in the undefoliated plots. (Fig. 8). NO_3^- and NH_4^+ uptake were not significantly affected by defoliation in 1998 (data not shown).

There was no difference in root TNC pools measured before or after the labeling period ($P=0.76$), indicating that artifacts due to depletion of carbohydrate reserves were negligible. Excised roots from defoliated plots

had significantly lower levels of starch (defoliated: $0.09 \pm 0.01\%$ starch, control: $0.22 \pm 0.03\%$ starch, $P=0.02$; mean \pm SE) and soluble sugars (defoliated: $0.14 \pm 0.005\%$ sugars, control: $0.16 \pm 0.01\%$ sugars, $P=0.05$; mean \pm SE).

Discussion

Defoliation by gypsy moth clearly had significant effects on poplar growth and vigor. Tree seasonal diameter increment and top survivorship were both lower in defoliated plots (Figs. 3, 4), as seen in many previous studies of tree growth during defoliation (Kulman 1971; Davidson et al. 1999; Schowalter et al. 1986; Wickmann 1978, 1980). Defoliation effects on growth were greatest during the period between peak defoliation in late June and completion of canopy replacement in the defoliated plots in August (Figs. 2, 3). Fertilizer effects on growth were also observed during this period (Figs. 2, 3). Relatively little tree growth took place after August (Fig. 3) even though the defoliated trees had recovered canopy densities similar to the control trees. This suggests that full refoliation may occur too late in the season for the defoliated trees to make up the difference in growth. Year-to-year variation in defoliation effects on growth (Fig. 3) were largely due to differences in the intensity of defoliation (Fig. 2).

Starch accumulation was delayed in defoliated plots until leaf regrowth was complete, but recovered by the post-leaf-fall sampling date (Fig. 5). Reichenbacher et al. (1996) also found that late-season TNC concentration in 2-year-old hybrid poplar was unaffected by manual defoliation. Carbon allocation to storage pools increases significantly in poplars after budset, when shoot extension and leaf production cease (Isebrands et al. 1983; Nelson and Isebrands 1983; Friend et al. 1994). Defoliation effectively leads to an extension of the period of leaf growth until refoliation is completed; this was about a 3-week shift in 1997 (Fig. 2). We speculate that the extension of leaf growth later in the growing season (Fig. 2) delays the shift in carbon allocation from leaf production to formation of starch, the primary storage carbohydrate in *Eugeneii* poplar (Nguyen et al. 1990).

Trees in the experimental plots were entering a phase of self-thinning, with top mortality a highly-visible sign of this stand-level effect (Fig. 4). Top dieback is common in poplar trees experiencing stress (Dickmann and Stuart 1983), accompanied by the production of epicormic branches on the trunk as apical control is lost (Zimmerman and Brown 1971). Defoliation significantly increased top mortality observed in 1999, lagging other defoliation effects on the trees. This apparent lag may be due to defoliation affects on apical bud formation, bud viability, or winter hardiness of buds and stems, as the buds for 1999 were formed in 1998. The delay in carbon allocation to storage in defoliated trees may have led to a decline in apical bud viability. Direct observation of bud numbers, bud viability, and apical twig carbon storage pools would be required to test this hypothesis.

Despite the importance of root growth in the dynamics of plant responses to defoliation (Richards 1993; Bryant et al. 1993), there are relatively few studies of both root growth and mortality in defoliated plants. No previous studies combine measures of nitrogen uptake, root composition, growth and mortality in mature trees defoliated by insects in the field. We had expected that carbon allocation to roots would decline in defoliated plots, leading to lower root production and increased root mortality. We observed significant but transient effects of defoliation on root production in 1996 (Fig. 6), consistent with a decline in carbon allocation to roots. Root mortality showed little response to defoliation (Fig. 7), consistent with previous observations of root demography in decapitated 4-year-old *Eugeneii* poplar (Dickmann et al. 1996). These data are in marked contrast to the increases in root mortality seen in browsed treatments in an early successional taiga community dominated by *Salix* sp. and *Alnus* (Ruess et al. 1998), in pruned treatments of citrus trees (Eissenstat and Duncan 1992), and in alfalfa following cutting (Goins and Russelle 1996).

Although carbon limitations to root survivorship are the most likely cause of root mortality in plants subject to herbivory, defoliation, browsing, and decapitation are all likely to cause shifts in hormonal and hydraulic signals from shoots to roots. It is not clear whether differences in root mortality following herbivory (e.g., no increase in mortality, this study, versus increased root mortality, Ruess et al. 1998) are due entirely to differences in carbon economy among the systems studied, or to differences among species in the relative sensitivity of fine root mortality to carbon limitations and altered hormonal signals from the shoot. If species vary in sensitivity of fine root mortality to herbivory, there are likely to be consequences for the induction of long-term resistance in these species (Bryant et al. 1993), which could contribute to inherent host species differences in the dynamics of outbreaks of insects. Other possible ecological consequences for variation in sensitivity of fine root mortality to herbivory include interactions between herbivory and plant nutrient competition. If preemptive exploitation of nutrients plays a role in competitive superiority in a plant community (Kosola and Gross 1999), there is likely to be selection for maintenance of extensive live root systems in the soil.

Herbivory has the strongest impact on plant growth and survival if there is a long delay between loss of photosynthetic tissue and leaf regrowth (Richards 1993). Defoliation by gypsy moths occurs while hybrid poplar trees are actively growing – they have active, intact meristems during the period of peak defoliation, and so are able to recover leaf area quickly. Even during the highest gypsy moth population densities of 1997, the leaf meristems and youngest, unexpanded leaves were not eaten by caterpillars, leaving a large potential leaf area ready to expand and photosynthesize as soon as pupation started. Consequently, trees were poised to recover very rapidly following defoliation, as shown by the rapid in-

crease in canopy density following defoliation in 1997 (Fig. 2). In contrast, browsing (Ruess et al. 1998; Bryant et al. 1991), pruning (Eissenstat and Duncan 1992), and alfalfa harvest (Goins and Russelle 1996), which all lead to increased root mortality, remove leaf and apical stem tissue, simultaneously decreasing the number of leaf meristems and the stored resources needed for growth of new leaves. Gypsy moth, as an early-season defoliator, does not remove leaves late in the season when poplar C allocation is primarily directed belowground (Isebrands et al. 1983; Friend et al. 1994). Physiological effects of gypsy moth defoliation on other trees such as the northern oaks, which produce a single flush of leaves each year in older trees, may depend upon the timing of defoliation relative to shoot development. The combination of plant growth traits and defoliator characteristics in the gypsy moth-poplar system is also seen in forage species tolerant of ruminant grazing (Richards 1984, 1993).

The effects of defoliation on nitrate and ammonium uptake in mid-July of 1997 (Fig. 8) may also be caused by shifts in carbon allocation to leaf regrowth and away from root metabolism. This speculation is supported by the observation that diurnal fluctuations in carbon flux to roots can lead to diurnal variation in NO_3^- uptake (Rideout and Raper 1994). Although we do not have data on the seasonal dynamics of N uptake capacity in defoliated plots, it seems likely that the decline in N uptake capacity was transient and would recover. This expectation is based on the observation that the effect of defoliation on starch accumulation was transient (Fig. 5). The lower maximum rates of N uptake we observed during peak re-foliation could lead to transient declines in N availability for new leaf production, consistent with observations of lower leaf N in defoliated trees sampled both at peak defoliation and after re-foliation (Parry 2000). However, foliar nitrogen levels in previously defoliated trees were not significantly different from controls in the following spring (Parry 2000), supporting our speculation that defoliation-induced declines in N uptake were transient.

Our data from 1997 apparently contradict previous findings that defoliation does not affect N uptake by red oak seedlings (Lovett and Tobiessen 1993). The difference in response of N uptake to defoliation may be due to differences in intensity of defoliation between the studies. This speculation is supported by the contrast between our data on N uptake in 1997 and 1998. Despite substantial herbivory in 1998, defoliation was less intense in this year (Fig. 2), and there was no effect of defoliation on NO_3^- or NH_4^+ uptake. Levels of tree carbohydrate stores at the time of defoliation may also play a role in determining the impact of defoliation on N uptake (Tuomi et al. 1990). Defoliation is likely to have the largest negative impact on nutrient acquisition if it occurs when root carbohydrate stores are low (Tuomi et al. 1990; Bryant et al. 1988).

The 10-fold greater V_{\max} for NH_4^+ uptake compared to NO_3^- uptake is striking (Fig. 8). The low capacity for NO_3^- uptake may reflect a lack of induction due to the low levels of NO_3^- found in the soil in our plots

(C. Russell, unpublished work). It is also possible that these trees have an inherently lower capacity for NO_3^- uptake. Our data are consistent with the finding that NO_3^- influx for *P. tremuloides* is consistently lower than NH_4^+ influx (Min et al. 1999).

Bryant et al. (1988, 1993) have suggested that increased root mortality following defoliation is one of the causes of prolonged tree nutrient deficiency and, consequently, delayed induced resistance. Our data demonstrate that tree capacity to acquire nutrients can be decreased by defoliation (Fig. 8) even in the absence of increased root mortality (Fig. 7). It seems likely that the physiological capacity to acquire nitrate and ammonium is relatively more sensitive to defoliation than is root demography. Therefore, systems where herbivory leads to increased root mortality (e.g., Ruess et al. 1998) are also likely to experience declines in nitrate and ammonium acquisition by surviving roots. This hypothesis assumes that declining root capacity for N uptake and increased root mortality are both driven by the same signal, most likely decreased carbon availability from the shoot.

The cultivar *Eugeneii* used in this study should have ecological characteristics of the parental species, *P. deltoides*, the North American cottonwood, and *P. nigra*, the European black poplar, both in section *Aigeiros* (Dickmann and Stuart 1983). Both are early-successional riparian species favoring floodplains (Braatne et al. 1996; Dickmann and Stuart 1983) and produce leaves continuously during most of the growing season (Eckenwalder 1996). Given conditions comparable to those seen in newly exposed soil on a floodplain, with high levels of light, nutrients, and water and little competition, hybrid poplars are capable of very high rates of growth throughout the growing season (Dickmann and Stuart 1983). Combining these growth characteristics with the brief duration of gypsy moth herbivory and lack of damage to newly formed leaves and leaf meristems, it is not surprising that the hybrid poplar in this study was relatively tolerant of defoliation.

The transient effect of defoliation on TNC (Fig. 5) and rapid recovery of growth and leaf N status in 1999 (Fig. 3) reveal the fundamental tolerance of healthy stands of hybrid poplar to herbivory due to outbreaks of defoliating insects. Declining carbon allocation to roots following defoliation did not lead to massive fine root mortality. These disturbance-tolerant trees are maintaining belowground structures necessary for recovery from loss of shoot tissues. We expect that other early-successional species with indeterminate shoot growth would show similar patterns following defoliation by gypsy moth.

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