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PLANT ANIMAL INTERACTIONS

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Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance

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Abstract This research tested the long-term effects of defoliation on aspen chemistry and growth in relation to genotype and nutrient availability. We grew saplings of four aspen genotypes in a common garden under two conditions of nutrient availability, and subsequently subjected them to two levels of artificial defoliation. Artificial defoliation suppressed plant growth, and saplings of the four genotypes did not show evidence of genetic variation in tolerance to defoliation. Phenolic glycoside concentrations did not respond to defoliation, but were influenced by genotype and nutrient availability. Condensed tannins responded to defoliation and varied among genotypes. Although defoliation affected condensed tannins, plant quality was not altered in a manner important for gypsy moth performance. Regression analyses suggested that phenolic glycoside concentrations accounted for most of the variation in insect performance. The lack of a strong response important for herbivores was surprising given the severity of the defoliation treatment (nearly 100% of leaf area was removed). In this study, plant genotype was of primary importance, nutrient availability was of secondary importance and long-term induced responses were unimportant as determinants of insect performance.

Keywords Plant-insect interactions · Tolerance · Genotype × environment · Defoliation · Nutrient availability

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Introduction

The effects of defoliation on foliar chemistry and plant growth can persist into seasons following the defoliation event. Measurement of such plant characteristics allows for evaluation of both long-term induced chemical responses and genetic variation in tolerance to defoliation. Long-term phytochemical responses and tolerance to herbivory are influenced by plant genotype, plant environment and interactions between genotype and environment. In this study, we investigated the relative roles of plant genotype and nutrient availability in mediating long-term induced responses and tolerance.

Long-term induced phytochemical responses of plant secondary compounds have been especially well studied in deciduous trees (e.g., Haukioja and Neuvonen 1985; Bryant et al. 1987a, 1993; Neuvonen et al. 1987; Hanhimaki 1989; Ruohomäki et al. 1992; Ruohomäki et al. 1996; Kaitaniemi et al. 1998, 1999; Mutikainen et al. 2000), notably because the coupling of resistance traits with a time delay could contribute to population cycles of forest insects. A fundamental question associated with long-term induced chemical responses is whether they represent active defensive responses by the plant, or passive responses driven by altered carbon-nutrient balance (Haukioja and Neuvonen 1985). Because foliage feeding by insects removes nutrients and is thought to elicit passive responses due to altered carbon-nutrient balance, nutrient addition should ameliorate such responses (Haukioja and Neuvonen 1985). In addition, nutrients, plant genotype and interactions of nutrients and genotype are thought important in mediating long-term induced responses (Mutikainen et al. 2000). For these reasons, we were interested in evaluating the effects of both nutrients and genotype on long-term induced responses to defoliation.

An alternative defensive strategy to chemical induction is tolerance, defined as the degree to which plant fitness is affected by herbivory relative to fitness in the undamaged state (Strauss and Agrawal 1999). By using various compensatory mechanisms to recover from herbivory

(e.g., enhanced photosynthesis and/or growth, altered allometric relationships, mobilization of carbon stores), "tolerant" plants are better able to grow and compete in the face of herbivore onslaught (Strauss and Agrawal 1999; Haukioja and Koricheva 2000). Genotypic variation in tolerance has been observed for a number of plant species (Fineblum and Rausher 1995; Mauricio et al. 1997; Stowe 1998; Agrawal et al. 1999). Moreover, tolerance responses are thought to be influenced by environmental conditions (Strauss and Agrawal 1999; Stowe et al. 2000). Soil nutrients, in particular, likely affect the ability of a plant to tolerate defoliation (Gertz and Bach 1995; Mutikainen and Walls 1995; Coley and Barone 1996; Houle 1999). For these reasons, we were interested in testing the effects of plant genotype and nutrient availability in mediating tolerance responses.

Quaking, or trembling, aspen (*Populus tremuloides*) and the gypsy moth (*Lymantria dispar*) were used as our experimental system. Quaking aspen grows in a variety of habitats, spanning the range from nutrient-poor to nutrient-rich soils (Dickmann and Stuart 1983; Mitton and Grant 1996). This early successional species is also highly genetically variable; variation is observed in growth rate and in susceptibility to disease and herbivores (Barnes 1969; Dickmann and Stuart 1983; Perala 1990; Mitton and Grant 1996). Variability in phenolic glycoside concentrations among genotypes is known to be important in determining the performance and preference of aspen-feeding insects such as gypsy moths (Lindroth and Hwang 1996; Osier and Lindroth 2001).

Although aspen foliage contains compounds with strong anti-herbivore properties, it is attacked by over 100 species of insects, a number of which are prone to population outbreaks (Perala 1990). The larvae of several insect herbivores [gypsy moth, forest tent caterpillar (*Malacosoma disstria*), aspen blotch leafminer (*Phyllonorycter tremuloidiella*), large aspen tortrix (*Choristoneura conflictana*)] cause extensive defoliation to stands of aspen during outbreak periods. Thus we endeavored to evaluate two aspects of aspen response to defoliation. First, we assessed whether aspens respond to such attack with an induced phytochemical response in the long-term and whether this response in turn affected gypsy moths. Although several studies have investigated short-term induced response in *Populus* species (Clausen et al. 1989; Lindroth and Kinney 1998; Havill and Raffa 1999; Roth et al. 1998; Osier and Lindroth 2001), no published studies have investigated long-term phytochemical responses. That nutrients may play a role in mediating long-term induced responses in aspen is suggested by the fact that nutrient addition ameliorated induced responses of condensed tannins in the short-term (Osier and Lindroth 2001). Second, we determined the degree to which tolerance to herbivory varies among aspen genotypes and whether such responses are mediated by nutrient availability. We expected that tolerance might be an important defensive strategy because aspen possesses characteristics shared by many tolerant plants (e.g., high growth and photosynthetic rates) (Strauss and Agrawal 1999; Haukioja and Koricheva 2000). We sought to test the effect of soil nutrients on tolerance responses in aspen because nutrients have been found important in mediating tolerance responses in previous studies (Strauss and Agrawal 1999).

Materials and methods

Overview of experimental design

The experiment employed a completely randomized, fully factorial design, with aspen genotype (four), soil nutrient availability (low and high) and defoliation (none and severe) as treatments. Each treatment combination was replicated with six saplings (a total of 96 saplings, grown individually in pots).

Aspen genotypes

The four genotypes used in this study were chosen to span the known range of constitutive resistance against foliar-feeding Lepidoptera such as the gypsy moth, forest tent caterpillar, large poplar sphinx moth (*Pachysphinx modesta*) and Canadian tiger swallowtail (*Papilio canadensis*) (Hwang and Lindroth 1997; Hwang and Lindroth 1998). These genotypes were originally derived from root material collected from several sites in south-central Wisconsin and maintained in a common garden on the University of Wisconsin campus. Genotypes identified as: "A", "B", "C" and "D" in this study were the same as those in Osier and Lindroth (2001) and correspond to Wau1 (Waushara County), Dan1,2 (Dane County) and Sau3 (Sauk County) of Hwang and Lindroth (1997).

Aspen propagation and nutrient treatment

Saplings were propagated from root material (as in Hwang and Lindroth 1997) collected from common garden plants in summer 1996. After sprouting, suckers were planted individually and grown outside throughout the summer and early autumn of 1996. After leaf drop, saplings were bare-rooted and over-wintered in moist peat moss at 4°C. In early spring 1997, saplings were potted individually in 36-liter pots containing a 70% sand, 30% local field soil mixture.

For the nutrient treatment, we applied two levels that spanned the range from very poor growth (our low nutrient level) to optimal growth without over-fertilization (our high nutrient level) (Hemming and Lindroth 1999). The soil of saplings from the high nutrient treatment was amended with Osmocote 8–9 month slow release fertilizer (18:6:12 N-P-K + micro-nutrients), added at a rate of 3.5 g/l to pots in spring 1997, 1998 and 1999. The soil of low nutrient plants received no amendment.

Artificial defoliation

We used defoliation levels that spanned the range of defoliation experienced by aspen in the field (0% and 100% of leaves on the plant damaged). Saplings were mechanically defoliated from 11 to 15 May 1998, to coincide with the expected period of attack by outbreaking herbivores such as gypsy moth or forest tent caterpillars. In mechanically simulating herbivore feeding, we sought to remove leaf area to produce carbon-limiting conditions (mimicking leaf area removed by feeding), and to maximize damage along the cut edge [assuming the cue for induction would come from the remaining damaged portion of the leaf (Mattson and Palmer 1988)]. To accommodate both criteria, every leaf on a defoliated sapling was cut across the midrib near the leaf base, using hair-

thinning shears. This procedure removed 90% of leaf area, yet produced a long and ragged damaged edge.

Plant growth

We recorded root collar diameter and stem length of each experimental sapling before planting. To estimate initial mass of these saplings, eight additional saplings of each genotype were similarly measured, dried and weighed to obtain size: weight ratios. We estimated initial dry mass of the experimental saplings by using regression equations based on relationships of size [(stem length) × (root collar diameter^2)] to dry mass for the sacrificial saplings of each genotype (R^2 values for such relationships were 0.61, 0.51, 0.69, 0.73 for genotypes A, B, C and D, respectively). Saplings from each genotype were randomly assigned to their respective nutrient and defoliation treatment.

To determine plant growth, destructive harvest of saplings began on 1 July 1999, 13 1/2 months following artificial defoliation and 26 months after planting and application of the nutrient treatment. Saplings were partitioned into leaves, stems and roots, dried at 70°C to constant mass and weighed. We calculated growth increment (GI) as [(sapling final dry mass)–(sapling estimated initial dry mass)].

Chemical analyses

We analyzed the foliage for several phytochemical constituents, including those: (1) known important for insects feeding on aspen (phenolic glycosides, nitrogen, water), (2) traditionally thought important in plant-insect interactions (condensed tannins), and (3) that could indicate the carbon-nutrient status of the plant (nitrogen and starch). Foliage was collected once from experimental saplings on 7 June 1999, approximately midway through the insect bioassays (described below) and 1 year following defoliation. Fifteen leaves were collected per sapling by snipping cleanly at the petioles; removing leaves in this way has been shown not to induce a response from the sapling (Mattson and Palmer 1988). After removal, leaves were transported to the laboratory in plastic bags on ice, flash-frozen in liquid nitrogen, freeze-dried in a cooled specimen chamber (−10°C), ground through number 40 mesh in a Wiley Mill and stored at −20°C until analysis for phenolic glycosides, condensed tannins, nitrogen and starch. Treatment of aspen leaf material in this manner preserves labile compounds such as phenolic glycosides and condensed tannins (Lindroth and Koss 1996).

To control for the effects of leaf age in chemistry collections and insect bioassays (below), we collected foliage from only the initial leaf flush (which comprises >95% of available leaves in the spring), and avoided new leaves at indeterminately growing shoot tips. For both insect bioassays and chemical analyses, foliage was removed in a haphazard manner from the upper third of each sapling.

Concentrations of phenolic glycosides (salicortin and tremulacin) were determined by high performance thin-layer chromatography as described by Lindroth et al. (1993) and the cumulative amount (percent dry mass) is presented in the text. Salicortin and tremulacin purified from aspen leaves served as standards. Condensed tannins were exhaustively extracted from leaf tissue in 70% acetone at 4°C (with 10 mM ascorbic acid as an antioxidant), and quantified by the butanol-HCl method (as in Porter et al. 1986). As the standard, we used condensed tannins purified from aspen by the method of Hagerman and Butler (1980). For nitrogen determinations, Kjeldahl acid digestions were conducted (as in Parkinson and Allen 1975), followed by micro-Nesslerization (as in Lang 1958). Glycine *p*-toluene-sulfonic acid (5.665% nitrogen) was digested and served as the standard. Starch concentrations were determined by hydrolysis of starch to glucose and quantification via the dinitrosalicylic acid method (as described in Lindroth et al. 2001). We determined percent water in foliar samples gravimetrically.

Gypsy moth bioassays

Bioassays were conducted with fourth stadium gypsy moth larvae 1 year following defoliation, beginning on 28 May 1999 (approximately 4 weeks after budbreak). At the time of the bioassays, the foliage was of appropriate age and toughness (fully expanded and nearly fully mature) for larval gypsy moths of this stage (Osier, personal observations). Due to quarantine restrictions, bioassays were conducted using excised foliage in Percival growth chambers within the University of Wisconsin Biotron. Growth chambers were maintained at 15:9 h LD photoperiod and 25:10°C to mimic early summer conditions in Madison, Wisconsin, USA (National Climatic Data Center).

Gypsy moth egg masses were provided by USDA—APHIS (Otis Air National Guard Base, Massachusetts). Egg masses were surface sterilized in a solution of 0.1% sodium hypochlorite with 1% Tween 80 (Sigma Chemical, St. Louis, Missouri) as a surfactant. Larvae were reared until the end of the third stadium on aspen foliage known to contain low concentrations of plant secondary compounds. Upon molting into the fourth stadium, larvae were assigned randomly among the 96 individual saplings. Multiple larvae (subsamples) were reared individually in petri dishes on foliage from each of the six replicate saplings per treatment combination: three larvae were reared per sapling for genotypes A, B and C, and six larvae per sapling for genotype D. (More larvae were used per sapling for genotype D because of anticipated mortality due to high levels of constitutive resistance). In all cases, mean larval performance was calculated among the subsamples grown on foliage for each replicate sapling. To maintain leaf turgor and freshness, floral waterpiks were used and foliage was changed at least every 3 days; treatment of foliage in this way has been shown to maintain foliar concentrations of aspen compounds important for insect feeding (Lindroth, unpublished data).

We restricted larvae in the bioassays to females. With the experimental design employed (relatively low replication and minimal sub-sampling), a high probability existed of losing experimental cells due to non-uniform distribution of males and females across replicates (i.e., all males or females on a sapling). Sex of newly molted fourth instar larvae was determined by use of known weight distributions from previous studies. This approach was successful, as >90% of the larvae used in the experiment were females. At the conclusion of the study, sex was determined by inspection of the genital pores of the fifth stadium larvae. Males were eliminated from the study.

Stadium duration and the final larval mass of newly molted fifth instar larvae were recorded. Herbivore relative growth rate (RGR) was calculated as in Waldbauer (1968), but was modified to use initial biomass rather than average biomass as the relative term (Farrar et al. 1989).

Statistical analyses

Fixed-effects analysis of variance [PROC MIXED (Version 8) SAS 1999] was used to analyze the effects of aspen genotype, soil nutrient availability, defoliation and their interactions on aspen growth, phytochemistry and insect performance. For analysis of plant growth, we sought to improve the analysis of variance model by incorporating initial sapling mass as a covariate. The covariate was not found to relate to the dependent variable, and thus, in accordance with the model fitting guidelines of Littell et al. (1996), was not used. We also evaluated whether to apply insect initial mass as a covariate in the analysis of variance model for indices of insect growth and development. Differences in initial larval weights among saplings were very small after generating means for larval performance within the replicates (saplings). Due to the minimal variation in larval initial mass, the covariate was not related to any of the dependent variables. Thus, we did not use analysis of covariance for the analysis of herbivore performance variables (contrary to the recommendation of Raubenheimer and Simpson 1992).

Table 1 Correlation matrix of relationships among aspen phytochemicals from the 16 combinations of plant genotype, nutrient availability and defoliation. Pearson product-moment correlations are based on the mean values for each treatment

	Phenolic glycosides		Condensed tannins		Nitrogen		Starch	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Condensed tannins	−0.185	0.493						
Nitrogen	−0.602	0.014	−0.006	0.983				
Starch	−0.361	0.170	0.005	0.986	−0.089	0.743		
Water	−0.674	0.004	−0.271	0.310	0.054	0.843	0.643	0.007

To relate gypsy moth performance to quantitative variation in aspen phytochemistry, we used stepwise multiple regression [PROC REG (Version 8) SAS 1999]. Stepwise regression in SAS uses a combination of forward selection (α=0.10) and backward elimination (α=0.10) to fit a model. We used group means for each defoliation × genotype × nutrient combination (n=16). Concentrations of many of the phytochemicals were substantially correlated in a pairwise fashion (Table 1) [PROC CORR (Version 8) SAS 1999] so interpretation of individual coefficients of determination (partial R^2) as independent values is inappropriate (Sokal and Rohlf 1995). Because relationships of insect performance and phenolic glycosides were of special interest, we conducted univariate regressions between insect performance and phenolic glycoside concentrations and report these values in the text.

Results

Sapling growth

Aspen growth increment (GI) was depressed by defoliation, strongly variable among genotypes and greatly enhanced by nutrient addition (Fig. 1). Among the three treatment types, defoliation had the weakest effect on GI; defoliated saplings were, on average, 9% smaller than the controls. Averaged across the environmental treatments, growth of the best performing genotype was 35% greater than that of the poorest. GI of high nutrient plants was 450% that of low nutrient plants. Moreover, the nutrient effect depended upon genotype, as genotypic variation was greater under high nutrient conditions.

Phytochemistry

Aspen phytochemistry was universally affected by genotype and often affected by nutrient availability but was less commonly affected by defoliation (Figs. 2, 3). Concentrations of the two suites of plant secondary metabolites measured (phenolic glycosides and condensed tannins) responded differently to defoliation and nutrient addition, whereas both varied widely across genotypes (Fig. 2). Concentrations of phenolic glycosides were not affected by defoliation or nutrient addition. In contrast, condensed tannin concentrations were induced (21%) by defoliation and the effect of nutrients differed among genotypes. Concentrations of primary metabolites (nitrogen, starch and water) were rarely affected by defoliation, but were universally affected by plant genotype and nutrient availability (Fig. 3). Foliar nitrogen was unaffected by defoliation, varied among genotypes and increased 15% with nutrient addition. Starch concentrations were unaffected by defoliation, were variable among genotypes and were 15% lower under high nutrient availability. Foliar water concentrations responded to each of the treatments, but the responses were small compared to those of other phytochemicals measured (Fig. 3).

Performance of gypsy moths

Gypsy moth performance variables were seldom affected by defoliation, usually affected by nutrient availability and always affected by genotype (Fig. 4). Insect relative growth rate (RGR) did not respond to defoliation, but

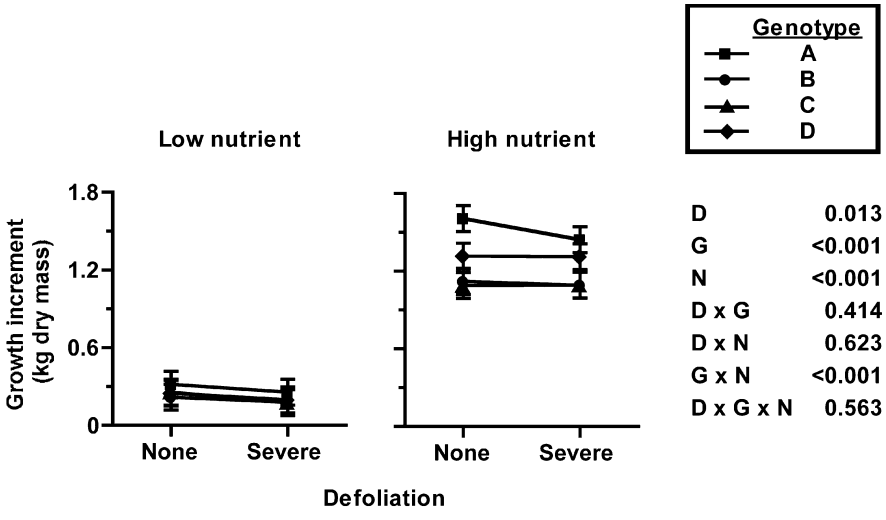

Fig. 1 Norm of reaction plots for growth of aspen saplings in relation to nutrient availability and defoliation. *P*-values indicate the results of 3-way ANOVA: genotype (*G*) *df* =3; nutrient availability (*N*) *df*=1; defoliation (*D*) *df*=1; *G* × *N* *df*=3; *G* × *D* *df*=3; *N* × *D* *df*=1; *G* × *N* × *D* *df*=3. Each line represents a single aspen genotype. *Vertical lines* represent 1 SE of the mean (based on pooled error estimates)

Table 2 Phytochemical components accounting for variation in gypsy moth performance. (Stepwise multiple regression, α=0.10 was used as the criterion for acceptance to, or rejection from, the model. *CT* condensed tannins, *N* nitrogen, *PG* phenolic glycosides, *S* starch, *W* water)

Parameter	Stepwise regression model			Partial components		
	Equation	R^2	*P*	Variable	R^2	*P*
RGR	Y=−0.44−0.01(PG)+0.21(N)+0.05(S)	0.923	<0.001	PG	0.783	<0.001
				N	0.078	<0.018
				S	0.062	0.009
Final mass	Y=−29.42−0.55(PG)+16.03(N) +3.61(S) +0.57(CT)	0.924	<0.001	PG	0.739	<0.001
				N	0.082	<0.029
				S	0.052	0.046
				CT	0.051	0.020
Developmental time	Y=−52.25+0.70(PG) +0.90(W)−1.04(S)	0.936	<0.001	PG	0.806	<0.001
				W	0.099	<0.003
				S	0.030	<0.036

varied widely among the aspen genotypes. RGR of herbivores feeding on genotype A was over five times higher than those on genotype D (Fig. 4). Fertilization enhanced RGR an average of 42%. Treatment effects on insect final mass were similar to those on RGR with the exception that defoliation tended to suppress final mass (Fig. 4). Aspen genotype and nutrient availability independently and interactively influenced developmental time of larvae; the genotype effect again exceeded the nutrient effect (Fig. 4).

Relationship of gypsy moth performance to phytochemistry

Gypsy moth performance was related to a number of the phytochemicals measured (Table 2). Indices of insect performance (RGR, final mass and developmental time) were universally related to phenolic glycoside concentrations. For all three indices, phenolic glycosides were the first phytochemical to enter the regression model and in univariate regressions explained >70% of the variation in herbivore performance. In univariate regressions, phenolic glycosides were related negatively to RGR (R^2 =0.783, P<0.001) and final mass of larvae (R^2 =0.739, P<0.001)

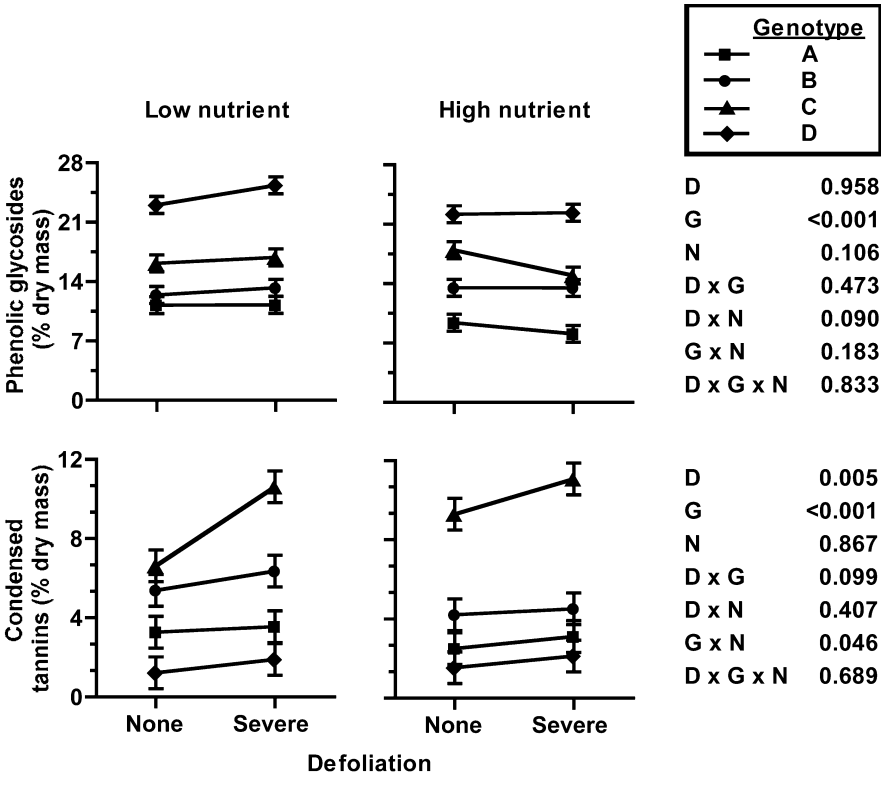

Fig. 2 Norm of reaction plots for phenolic glycoside and condensed tannin concentrations of aspen foliage in relation to nutrient availability and defoliation. Figure format as in Fig. 1

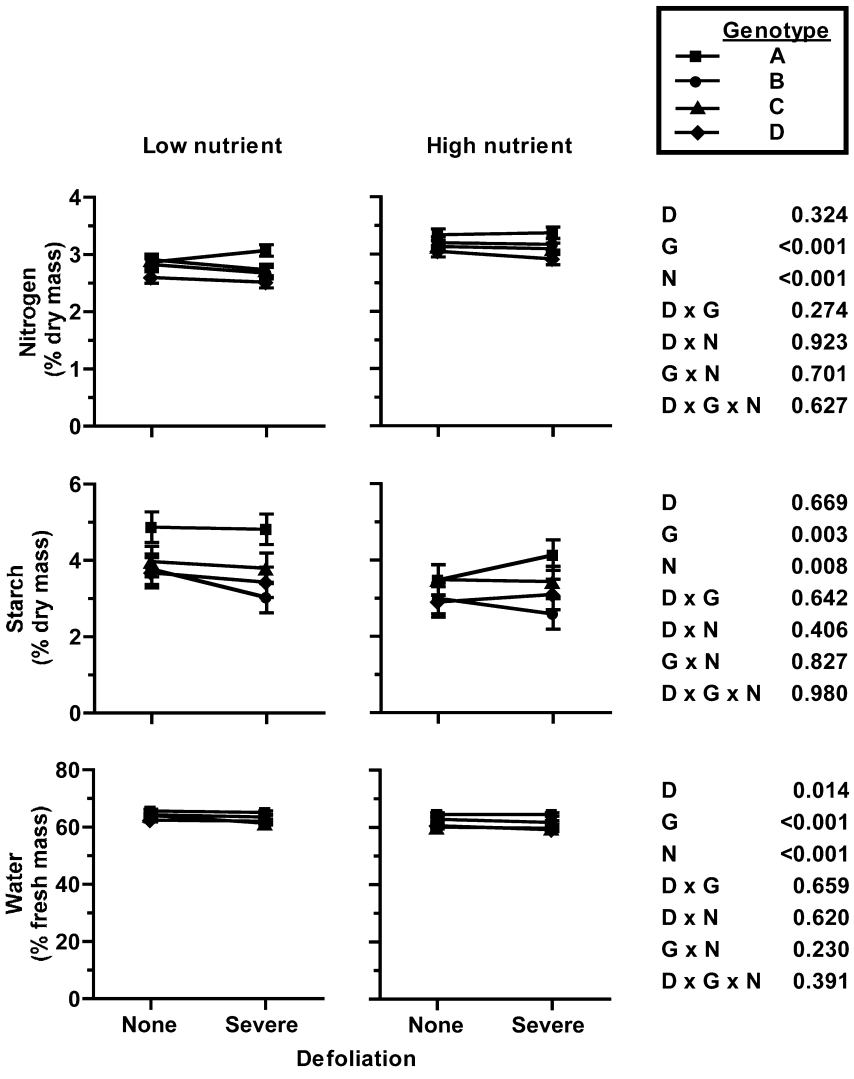

Fig. 3 Norm of reaction plots for nitrogen, starch and water concentrations of aspen foliage in relation to nutrient availability and defoliation. Figure format as in Fig. 1

and were related positively to developmental time (R^2 =0.806, P<0.001). Additional phytochemicals entered the model for RGR (nitrogen and starch), final mass (nitrogen, starch and condensed tannins) and developmental time (water and starch) and the full models explained >90% of the total variation in these insect growth and development indices.

Discussion

Defoliation, genotype and nutrient availability were all important in determining aspen growth. As expected, heavy defoliation significantly reduced growth. These results are consistent with a number of other studies with *Populus* species, where removal of ≥75% of leaf area resulted in growth loss (Hodson 1981; Bassman et al. 1982; Reichenbacker et al. 1996). Anecdotally, defoliation also had dramatic effects on aspen biomass distribution. Within 2 weeks of defoliation, saplings abscised most remaining (partial) leaves, and commenced growth with a flush of new leaves at the terminal ends of branches. Continued terminal branch growth occurred at the expense of radial growth, leading to a down-swept, weeping appearance.

As has been found previously (Mitton and Grant 1996; Lindroth et al. 2001), we observed variation in growth rate among the aspen genotypes. Our strongly contrasting soil nutrient conditions produced poor and excellent growth in the low and high nutrient treatments, respectively. These results are similar to those of Hemming and Lindroth (1999), after which our nutrient additions were modeled.

The aspen genotypes tested did not exhibit genetic variation in tolerance to defoliation (no significant defoliation × genotype or defoliation × genotype × nutrient interactions) even though both defoliation and genotype influenced growth. Our study was not appropriate to identify the presence or absence of particular tolerance mechanisms in aspen; rather it was our goal specifically to identify variation in tolerance responses among the genotypes. The absence of statistical interactions indicative of genetic variation in tolerance contrasts with results of other studies of the Salicaceae, in which genetic variation in tolerance to artificial defoliation has

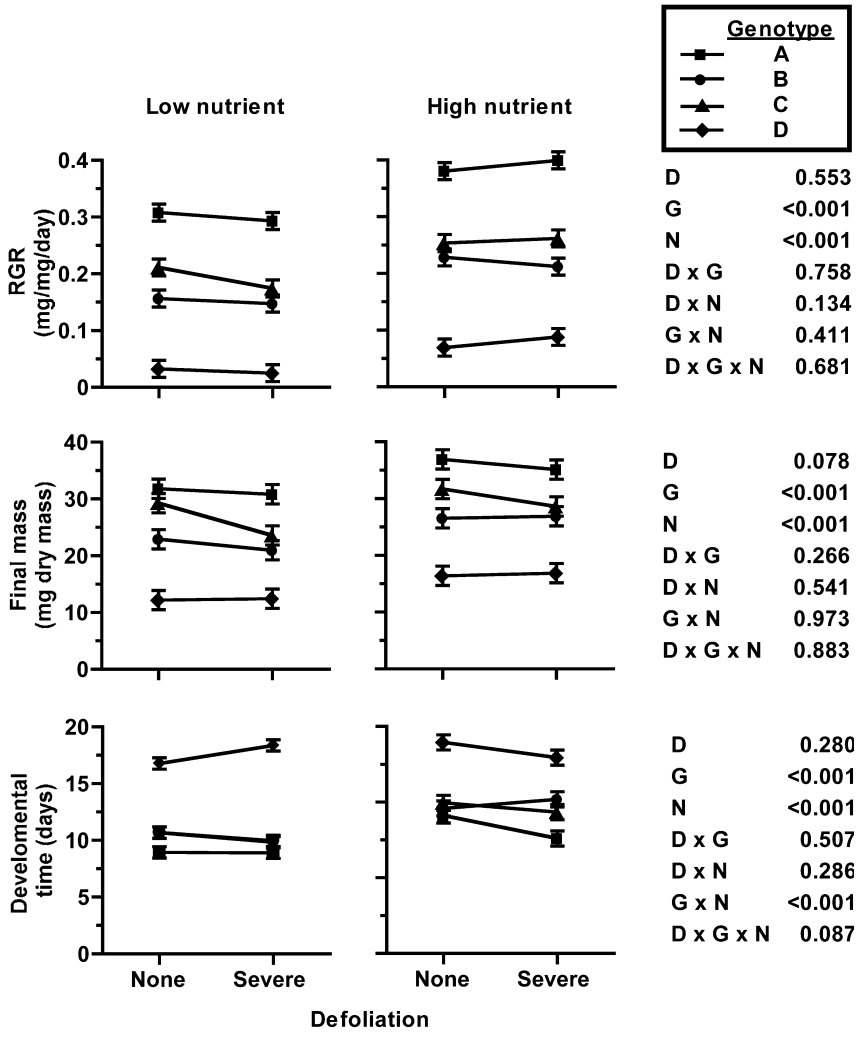

Fig. 4 Norm of reaction plots for gypsy moth relative growth rate, final mass and developmental time in relation to nutrient availability and defoliation. Figure format as in Fig. 1

been demonstrated (Robison and Raffa 1994; Shen and Bach 1997). More recent work has demonstrated genetic variation in tolerance of 12 aspen genotypes after 1 year of defoliation (Stevens and Lindroth, unpublished data).

Long-term effects of defoliation and nutrients had no direct impact on phenolic glycoside concentrations, although levels varied widely among genotypes. Long-term induced responses of phenolic glycosides have not been well studied in aspen. Short-term, damage-induced responses, however, have been observed in some studies (Clausen et al. 1989; Lindroth and Kinney 1998) but not in others (Roth et al. 1998; Osier and Lindroth 2001). Clausen (1991) suggested that long-term responses to defoliation in aspen might be an extension of the short-term response. Our results cannot refute or support this notion since we found no consistent induction response by phenolic glycosides in either the short- or long-term. The strong effect of genotype on phenolic glycoside concentrations is, however, consistent with several other studies of quaking aspen (Hemming and Lindroth 1995; Hwang and Lindroth 1997; Hwang and Lindroth 1998; Osier et al. 2000); genotype accounted for the vast majority of the variation in phenolic glycoside concentrations across treatments. Nutrient addition did not affect phenolic glycoside concentrations. Responses of phenolic glycosides to nutrient addition have not been consistent across previous studies; total concentrations have exhibited modest increases (Lindroth and Kinney 1998), decreases (Bryant et al. 1987b; Hemming and Lindroth 1999) or no change in response to nutrient addition (Kinney et al. 1997; Lindroth et al. 2001). Overall, concentrations of phenolic glycosides in this study were strongly genetically fixed and unresponsive to environmental conditions.

Condensed tannin concentrations, however, did respond to defoliation. We were interested in determining the mechanism driving the induced response: is the response an active defensive response by the plant (Rhoades 1979; Haukioja and Neuvonen 1985) or is it a passive adjustment as a result of altered carbon-nutrient balance due to nutrient removal via defoliation? Defoliation removes more nutrients than carbon, and should result in increased carbon-nutrient ratios and concentrations of plant secondary compounds in the following year (Bryant et al. 1983; Tuomi et al. 1988; Tuomi et al. 1991). The induction response of condensed tannins to nutrients varies among deciduous tree species; amelioration has been found in some studies (Bryant et al. 1987a, 1993), but not in others (Haukioja and Neuvonen 1985; Ruohomäki et al. 1996).

We expected that nutrient addition might eliminate the long-term induction response of condensed tannins because we observed amelioration of the response by nutrients in the short-term (Osier and Lindroth 2001). It appears that in this study, the induction of tannin is an active, rather than passive, response because nutrient addition did not ameliorate induction. Other phytochemical evidence supports this perspective. For example, foliar starch and nitrogen concentrations in defoliated saplings were similar to those in control plants, suggesting that defoliation did not markedly alter plant carbon to nutrient ratios in the year following defoliation.

As has been found previously, tannins varied among the genotypes (Hemming and Lindroth 1995; Hwang and Lindroth 1997, 1998; Osier et al. 2000). We observed no direct effect of nutrient addition on tannin concentrations in this study. Numerous studies (Bryant et al. 1987b; Kinney et al. 1997; Hemming and Lindroth 1999) have found tannins in aspen to respond to nutrient availability in the direction predicted by the carbon-nutrient balance hypothesis (Bryant et al. 1983), the growth-differentiation balance hypothesis (Herms and Mattson 1992) and the protein competition model (Jones and Hartley 1999). Why tannins did not respond directly to nutrients in this study is perplexing, since the nutrient levels used were similar to those of Hemming and Lindroth (1999), who found a strong effect of nutrient addition on tannin accumulation. Perhaps ontogenic changes in carbon partitioning (sensu Bryant and Julkunen-Tiitto 1995) played a role in the differences between our study and the others, as our trees were at least 1 year older and much larger than those used previously by our research group. To be sure, this result is not due to weak experimental treatments, as nutrient effects on plant growth were substantial.

Inconsistent responses of carbon-based secondary compounds to nutrient availability, such as we have observed in aspen, have helped spawn debate regarding the nature and generality of the carbon-nutrient balance hypothesis (Hamilton et al. 2001; Koricheva 2002; Lerdau and Coley 2002; Nitao et al. 2002). Regardless of the status of the carbon-nutrient balance hypothesis, we did not observe anything but the weakest responses to nutrient addition by tannins or phenolic glycosides. Such results showcase the role of genotype in driving variation in resistance in this system.

We chose artificial over herbivore-inflicted damage because we were interested in determining the defoliation responses over a range of nutrient and genotype combinations. Natural defoliation would not be adequately uniform among these treatments to determine the relative responses in which we were interested. Pragmatically, experience with the most well-defended genotype used in this study (genotype D) (Hwang and Lindroth, 1997, 1998) has shown that it would be impossible to inflict a substantial amount of damage on the foliage using insect herbivores.

We are aware that artificially inflicted damage does not always completely simulate herbivore damage when investigating short term induced responses (Baldwin 1990; Hartley and Lawton, 1991) and tolerance (Strauss and Agrawal 1999) in plants. Little exists in the literature to suggest how artificially inflicted damage will differ from natural damage in the long-term. In the short-term, artificial damage and damage from feeding herbivores generated foliage of statistically similar quality for gypsy moths on hybrid poplar (Havill and Raffa 1999); genotypes that responded to one induction cue tended to respond to others. It is unknown if the above pattern will translate into the long term. As for tolerance responses, Strauss and Agrawal (1999) found different responses by a number of genotypes of wild radish when defoliated artificially compared with natural defoliation by insects. The two types of defoliation inflicted, however; did not alter the magnitude of the variation in response among the genotypes; rather, the tolerance responses of individual genotypes were altered (Strauss and Agrawal 1999). Little is known about how the type of damage will impact tolerance in aspens, or trees of any species.

Defoliation did not strongly affect gypsy moth relative growth rate, final mass or developmental time, whereas genotype and nutrient availability significantly altered these performance parameters. Although long-term induced chemical responses have often been found to be important for tree-feeding insects (e.g., Haukioja and Neuvonen 1985; Bryant et al. 1987a, 1993; Neuvonen et al. 1987; Hanhimaki 1989; Ruohomäki et al. 1992, 1996; Kaitaniemi et al. 1998), the lack of a response by gypsy moths was not surprising given that phenolic glycosides were not induced by defoliation. Plant genotype was expected to be important in determining insect growth; aspen genotype has been found to have similar effects in a number of previous studies (Hemming and Lindroth 1995; Hwang and Lindroth 1997, 1998; Osier et al. 2000). Nutrient addition had a positive effect on insect growth, and this result is also consistent with other studies with aspen (Kinney et al. 1997; Hemming and Lindroth 1999). The most striking pattern in herbivore growth indices was that the environmental treatments (nutrients and defoliation) were much less important in determining insect performance than was plant genotype.

Concentrations of phenolic glycosides appear to be the single most important factor in determining host quality in this study (Table 2). The importance of phenolic glycosides on gypsy moth performance is not surprising given that similar results have been found previously (Hemming and Lindroth 1995; Hwang and Lindroth 1997, 1998; Osier et al. 2000). In addition to our documented effects of phenolic glycosides on gypsy moth performance, nearly all of the mortality of experimental insects (36 out of 38) occurred on the genotype (D) that contained the highest concentrations of phenolic glycosides. Such a result, although anecdotal, further confirms that phenolic glycosides are the most important aspen constituents for insect herbivores, and that variation in levels thereof are driven primarily by genetics rather than by environment.

Little evidence exists to suggest that aspen condensed tannins (Lindroth and Hwang 1996), or condensed tannins in general (Ayres et al. 1997), function as feeding

deterrents or toxins to insects. Because condensed tannin concentrations were the only phytochemical constituent to respond strongly to defoliation and because insects grew larger on foliage containing high concentrations of tannins, it is unlikely that long-term induced responses in aspen are important for insect herbivores. Absence of effective short- and long-term induction responses helps to explain why aspen forests are susceptible to sustained outbreaks of caterpillars such as gypsy moths, forest tent caterpillars, and large aspen tortrix. The lack of long-term changes in phytochemistry important for herbivores suggests that the forces producing population cycles are not bottom-up factors, at least with respect to deterioration of food quality. More likely top-down forces, perhaps coupled with food depletion, influence the population cycles of aspen-feeding insects, as is thought to be the case for the gypsy moth (Liebhold et al. 2000).

The goal of our study was to gain insight into the causes and consequences of high levels of defoliation that accompany outbreaks of aspen-feeding insects. Surprisingly, we found no evidence to suggest that long-term induced responses are important in mediating aspen—gypsy moth interactions. Our results indicate that other factors constrain population cycles of aspen-feeding insects.

The large degree of genotypic variation in resistance found in this study contrasts with the absence of genetic variation in tolerance. Differences in the magnitude of genetic variation in resistance compared to tolerance can perhaps be explained by the notion that selection may operate very differently on these two plant attributes. Roy and Kirchner (2000) suggest that selection may drive the fixation of tolerance genes in a population, because as the frequency of tolerance increases, intolerance becomes increasingly deleterious. In contrast, as the frequency of resistance increases in a population, the incidence of damage declines. This reduces the fitness advantage of resistant genotypes, thereby contributing to the maintenance of polymorphic resistance in a population. Results of this study are consistent with such ideas, as we found no evidence of genetic variation in tolerance but striking evidence of variation in resistance. The model of Roy and Kirchner (2000) is predicated on the notion that resistance is costly (i.e., disadvantageous in the absence of damaging agents), and such appears to be the case in aspen (Osier 2001).

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