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EFFECTS OF GENOTYPE, NUTRIENT AVAILABILITY, AND DEFOLIATION ON ASPEN PHYTOCHEMISTRY AND INSECT PERFORMANCE

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Abstract—Genetic and environmental variability, and their interactions, influence phytochemical composition and, in turn, herbivore performance. We evaluated the independent and interactive effects of plant genotype, nutrient availability, and defoliation on the foliar chemistry of quaking aspen (*Populus tremuloides*) and consequences for performance of gypsy moths (*Lymantria dispar*). Saplings of four genotypes were grown under two conditions of nutrient availability and subjected to three levels of artificial defoliation. Concentrations of all secondary and primary metabolites evaluated responded to at least one or more of the experimental treatments. Of the secondary metabolites, phenolic glycosides were affected strongly by genotype, less so by nutrient availability, and not induced by defoliation. Condensed tannins were strongly dependent upon genotype, soil nutrient availability, and their interaction, and, in contrast to phenolic glycosides, were induced by artificial defoliation. Of the primary metabolites, foliar nitrogen was affected by genotype and soil nutrient availability. Starch concentrations were affected by genotype, nutrient availability, defoliation and interactions among these factors. Foliar water content responded to genotype, nutrient availability, and defoliation, and the effect of nutrient availability depended on genotype. Herbivore performance on these plants was strongly influenced by plant genotype and soil nutrient availability, but much less so by defoliation. Although several of the compound types (condensed tannins, starch, and water) responded to defoliation, quantitative variation in these compounds did not contribute to substantive changes in herbivore performance. Rather, the primary source of variation in insect performance was due to plant genotype (phenolic glycoside levels), while nutrient availability (foliar nitrogen levels) was of secondary importance. These results suggest that genetic variation in aspen plays a major role in determining patterns of insect performance, whereas environmental variation, such as was tested, here is of negligible importance.

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Key Words—Plant–insect interactions, genotypic variability, fertilization, defoliation, quaking aspen, *Populus tremuloides*, gypsy moth, *Lymantria dispar*, phenolic glycosides, condensed tannins.

INTRODUCTION

Quantitative variation in both primary and secondary plant chemistry is largely responsible for patterns of herbivore performance and distribution across available hosts (Krischik and Denno, 1983; Schultz, 1988; Bernays and Chapman, 1994; Berenbaum, 1995). Variation in phytochemistry is due to plant genotype, environment, and interactions between genotype and environment (Karban, 1992). Numerous studies have evaluated the independent effects of genotype (e.g., Foulds and Grime, 1972; Bowers and Stamp, 1992; Orians et al., 1993; Hwang and Lindroth, 1997; Rousi et al., 1997; Han and Lincoln, 1997) and environmental factors such as nutrient availability (e.g., Larsson et al., 1986; Bryant et al., 1987a,b; Björkman et al., 1991) and herbivore damage (e.g., Baldwin, 1988; Karban, 1993; Julkunen-Tiitto et al., 1995) on plant chemistry and plant–herbivore associations. However, the relative importance of, and interactions among, genetic and environmental factors remains poorly understood (Karban, 1992; Stiling and Rossi, 1996).

We used an experimental system including quaking (=trembling) aspen (*Populus tremuloides*) and the gypsy moth (*Lymantria dispar*) to test the effects of plant genotype, two environmental factors (nutrient availability and foliar damage), and their interactions on phytochemistry and herbivore performance. Quaking aspen is adapted to a variety of habitats (Mitton and Grant, 1996) and is the most widely distributed tree species in North America (Dickmann and Stuart, 1983). Quaking aspen has high genetic variability; variation can be observed for leaf and bark morphology, leaf phenology, and growth rate (Barnes, 1969; Dickmann and Stuart, 1983; Perala, 1990; Mitton and Grant, 1996). Moreover, clones vary in susceptibility to disease and herbivores (Barnes, 1969; Dickmann and Stuart, 1983; Perala, 1990). Across its range, quaking aspen is attacked by over 100 species of insects, a number of which are prone to population outbreaks (Perala, 1990). One such insect is the gypsy moth, which causes significant, but not uniform, defoliation to aspen during outbreaks in the Great Lakes region of the United States.

This system is well suited to studies of genotype $\times$ environment interactions because variation in aspen phytochemistry is known to have both genetic and environmental components (Lindroth and Hwang, 1996) and because induced responses have been documented for *Populus* species (Nef, 1988; Mattson and Palmer, 1988; Havill and Raffa, 1999). Variation in phytochemistry among clones has consistently been found to impact herbivores, and high concentrations of phenolic glycosides have strong and typically negative effects on herbivore development (Hemming and Lindroth, 1995; Hwang and Lindroth, 1997, 1998) and

fecundity (Osier et al., 2000). Although less well studied than genotypic effects, environmental factors also alter phytochemistry and herbivore performance on aspen. For example, availability of resources (nutrients, CO_2, light) affects aspen phytochemistry and herbivore performance (Bryant et al., 1987b; Lindroth et al., 1993; Kinney et al., 1997; Hemming and Lindroth, 1999; Agrell et al., 2000). Induced chemical responses in aspen are less well understood. Previous research has documented rapidly induced responses in secondary chemistry as a result of foliar damage. Responses have included slight increases in phenolic glycoside content (Lindroth and Kinney, 1998) and increases in condensed tannin (Clausen et al., 1989; Roth et al., 1998) and total phenolic (Mattson and Palmer, 1988) concentrations. The demonstrated impact of such induced chemical changes on insect performance, however, has been small (Lindroth and Kinney, 1998) to nonexistent (Roth et al., 1998). Varied responses among studies with aspen suggest that genetic or environmental factors may modulate the induction response.

METHODS AND MATERIALS

Experimental Design. The experiment was a completely randomized design with four aspen genotypes, two levels of soil nutrients, and three levels of defoliation. Each combination of aspen genotype, nutrient availability, and defoliation (24 treatment combinations) was replicated with four independent saplings in a fully factorial design (a total of 96 saplings).

Aspen Clonal Material, Propagation and Growth Conditions. The four experimental genotypes represent a range of low to high levels of constitutive resistance (Hwang and Lindroth, 1997, 1998). Responses of the genotypes to nutrient availability and damage were unknown. The genotypes originated from root material collected from several sites in south-central Wisconsin (Hwang and Lindroth, 1997). Genotypes A, B, C, and D correspond to Wau 1 (Waushara County), Dan 1 and 2 (Dane County), and Sau 3 (Sauk County), respectively, of Hwang and Lindroth (1997). Root material for use in this study was obtained from saplings maintained for several years in a common garden on the University of Wisconsin–Madison campus.

The four aspen genotypes were propagated from root cuttings (as in Hwang and Lindroth, 1997) in summer 1996. Suckers were planted in 1-liter pots and grown outside until leaf drop in autumn 1996. Saplings were bare-rooted and overwintered in moist peat moss at 4°C. In the spring of 1997, saplings were potted individually in 16-liter pots containing a 7:3 mixture of sand and local field soil (silt loam). To manipulate nutrient availability, Osmocote 8–9 month slow release fertilizer (18:6:12 N-P-K + micronutrients) was added at a rate of 3.5 g/liter to high-nutrient pots; low nutrient pots received no fertilizer. Saplings were watered and maintained throughout the summer of 1997. In the spring of

1998, high-nutrient plants were treated (top dressed) for a second time with the same dose of fertilizer as used previously.

Artificial Damage. Saplings were defoliated May 14, 1998, to coincide with the period when attack by outbreaking herbivores, such as young gypsy moth or forest tent caterpillars, would be expected. The goals of our simulated herbivore feeding were to remove leaf area to produce carbon stress (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 goals, leaves were cut across the mid-rib near the base of the leaf. This procedure removed 90% of the area of each leaf damaged, yet produced a long cut edge. Herbivore damage was simulated using hair-thinning shears, which produce a more ragged cut than regular shears. The damage treatment had three levels: 0, 25, and 75% of the leaves on a plant damaged. Within each branch, leaf plastochron index 1, 2, 3, or 4 was chosen randomly as the starting point. In the 25% damage treatment, every fourth leaf from the starting point was damaged, and in the 75% damage treatment, every fourth leaf from the starting point was skipped.

Although studies have shown that mechanically damaged foliage is not always of identical quality to herbivore-damaged foliage (Baldwin, 1990; Hartley and Lawton, 1991), we chose to artificially damage our saplings for several reasons. First, artificial damage can be inflicted more uniformly across genotype and nutrient treatments than can damage by free-feeding insects. For example, we knew from previous studies that the well-defended genotype used in this study (genotype D) is extremely unpalatable to herbivores (Hwang and Lindroth, 1997, 1998) and that it would not be possible to inflict a uniform amount of insect damage among the replicates of this genotype. Second, free-feeding insects would likely feed selectively on the best foliage, leaving the poorer foliage for insect bioassays and chemistry collections. Such experimental artifacts would falsely indicate induction. Finally, a recent study with hybrid poplar (Havill and Raffa, 1999) showed that artificial damage and damage from feeding herbivores generated foliage of statistically similar quality for gypsy moths. Moreover, it showed that genotypes that responded to any induction stimulus (artificial damage or gypsy moth damage) were likely to respond similarly to others, that is, no genotype $\times$ elicitor type interaction was found.

Rationale for Magnitude of Treatments Applied. For the test of genotypic variation, we chose genotypes that span the known range of resistance against foliar-feeding Lepidoptera such as the gypsy moth, forest tent caterpillar, big poplar sphinx moth, and Canadian tiger swallowtail. Thus, our aspen genotypes represented a range of low (genotype A), moderate (genotypes B and C), and high (genotype D) levels of phenolic glycosides (Hwang and Lindroth, 1997). Likewise, for our nutrient treatment we applied levels that span the range from very poor growth (our low nutrient level) to optimal growth without overfertilization (our

high nutrient level) (Hemming and Lindroth, 1999). The nutrient levels we used produced 2.5-fold variation in size after one year (based on an index of height $\times$ basal diameter^2). Foliage of low-nutrient plants was more yellow, although not chlorotic, when compared to the deep green foliage of high-nutrient plants (Osier, personal observation). For the defoliation experiment, we used levels that span the range of defoliation experienced by aspen in the field (control, minimal damage without substantial carbon stress, and severe damage) as recommended by Neuvonen and Haukioja (1985). *Populus* species can withstand 25% defoliation (our low level) with no growth inhibition, whereas $\geq$75% defoliation (our high level) results in carbon stress and growth loss (Hodson, 1981; Bassman et al., 1982; Reichenbacker et al., 1996).

Insect Bioassays. To match the phenology of the larvae and foliage of the experimental saplings, bioassays began on May 24 (approximately four weeks after budbreak and 10 days after defoliation). At this time, the foliage was of appropriate age and toughness (fully expanded and nearly fully mature) for fourth stadium gypsy moths. Fourth instars were used in bioassays because responses to phytochemistry of this developmental stage are representative of much of the larval developmental period (Lindroth and Bloomer, 1991; Hwang and Lindroth, 1997; Hemming and Lindroth, 1999). 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 a 15L:9D photoperiod and 25/10°C to simulate early summer conditions in Madison, Wisconsin (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, St. Louis, Missouri) as a surfactant. All larvae were reared on aspen foliage known to contain low concentrations of plant secondary compounds until the end of the third stadium. Upon molting into the fourth stadium, larvae were assigned randomly among the treatments. To reduce the potential of bias due to loss of experimental cells, multiple larvae (subsamples) were reared on foliage from each of the four 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). Because the sapling served as the experimental unit, average performance of each group of larvae reared on foliage from a particular sapling was used for statistical analysis. To control for the effects of leaf age in insect bioassays and chemistry collections, 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. Foliage was removed in a haphazard manner from the remaining undamaged foliage located on the upper third of each sapling. To maintain leaf turgor and freshness, floral waterpiks were

used and foliage was changed at least every three days; treatment of foliage in this way has been shown to maintain foliar concentrations of aspen compounds important for insect feeding (Lindroth, unpublished data).

Larvae for use in the bioassays were restricted to females because with the experimental design employed (relatively low replication and minimal subsampling), there was a high probability of losing experimental cells due to nonuniform distribution of gender across replicates (i.e., all males or females on a sapling). Gender of newly molted fourth-instar larvae was determined by use of known weight distributions from previous studies. This approach was highly successful, as $>95\%$ of the larvae used in the experiment were females. At the conclusion of each bioassay, gender was definitively determined by inspection of the genital pores of the fifth stadium larvae. Males were removed from the study.

Final larval mass, stadium duration, and consumption were recorded for the fourth stadium. Herbivore relative growth rate (RGR) and efficiency of conversion of ingested food to biomass (ECI) were calculated as in Waldbauer (1968). Calculation of relative growth rate was modified to use initial biomass rather than average biomass as the relative term (Farrar et al., 1989).

Chemical Analyses. Foliage was collected from experimental saplings on May 29, 1998, two weeks after defoliation and approximately midway through the insect bioassays. Fifteen leaves were collected per sapling by snipping leaves cleanly at the petioles; removing leaves in this way has been shown not to induce a response from the ramet (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 40 mesh in a Wiley mill, and stored at -20°C until analysis. Treatment of aspen leaf material in this manner preserves labile compounds such as phenolic glycosides (Lindroth and Koss, 1996).

Percent water was determined gravimetrically, by the ratio of leaf dry mass to fresh mass. To measure nitrogen levels, Kjeldahl acid digestions were conducted using the method of Parkinson and Allen (1975), followed by nitrogen quantification using the micro-Nesslerization method of Lang (1958). Glycine *p*-toluene-sulfonic acid (5.665% nitrogen) was similarly digested and served as the standard. Condensed tannins were exhaustively extracted from leaf tissue in 70% acetone at 4°C (with 10 mM ascorbic acid as an antioxidant). To quantify condensed tannins in the extract, we used the butanol–HCl method of Porter et al. (1986). As the standard, we used condensed tannins purified from aspen by the method of Hagerman and Butler (1980). Concentrations of phenolic glycosides were determined by high performance thin-layer chromatography as described by Lindroth et al. (1993). Salicortin and tremulacin purified from aspen leaves served as standards. Starch was determined by the enzymatic method of Schoeneberger et al. (unpublished method).

Statistical Analyses. The effects of aspen genotype, soil nutrient availability, defoliation, and their interactions on aspen phytochemistry and insect performance data were analyzed using fixed-effects analysis of variance [PROC MIXED, Version 8 (SAS Institute, 1989)] with a completely randomized design. Critical α was 5% for this study. When subsampling was used (i.e., multiple insects reared per sapling), a mean was generated so that the unit of replication was the sapling ($N = 4$). We did not use analysis of covariance for the analysis of herbivore performance parameters, contrary to the recommendation of Raubenheimer and Simpson (1992). Because of the subsampled nature of this design, variation in initial larval weights among saplings was very low after generating means within saplings. Therefore, ANCOVA was not used, as specified by the model fitting guidelines of Littell et al. (1996).

To relate gypsy moth performance to quantitative variation in aspen phytochemistry, we used stepwise multiple regression [PROC REG, Version 8 (SAS Institute, 1989)]. Stepwise regression in SAS uses a combination of forward selection ($\alpha = 0.10$) and backward elimination ($\alpha = 0.10$) to fit a model. We used group means for each defoliation × genotype × nutrient combination ($N = 24$). To illustrate the relative proportion of variance explained by individual independent variables (phytochemicals), the contributions of individual coefficients of determination (partial R^2) to the total (R^2) are described when applicable. This is a valid approach in multiple regression when independent variables (phytochemical concentrations) in the model are not intercorrelated (Sokal and Rohlf, 1995). This was the case for the phytochemicals related with insect relative growth rate and final mass in this study (Table 1 and Table 3). To investigate relationships among phytochemicals and between constitutive and induced levels of plant secondary compounds, we used correlation analyses [PROC CORR, Version 8 (SAS Institute, 1989)].

TABLE 1. CORRELATION MATRIX OF RELATIONSHIPS AMONG ASPEN PHYTOCHEMICALS FROM 24 COMBINATIONS OF PLANT GENOTYPE, NUTRIENT AVAILABILITY, AND DEFOLIATION[a]

	Phenolic glycosides		Condensed tannins		Nitrogen		Starch	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Condensed tannins	−0.283	0.180						
Nitrogen	−0.105	0.625	−0.770	<0.001				
Starch	−0.374	0.072	0.165	0.440	−0.268	0.203		
Water	−0.649	<0.001	−0.410	0.046	0.700	<0.001	0.164	0.442

[a] Pearson product–moment correlations are based on the mean values for each treatment.

RESULTS

Phytochemistry. Concentrations of both secondary and primary metabolites were universally affected by plant genotype and soil nutrient availability and in some cases were influenced by defoliation (Figures 1 and 2). Significant interactions among the factors were generally uncommon. Foliar concentrations of plant secondary metabolites (phenolic glycosides and condensed tannins) responded differently to the treatments (Figure 1). Phenolic glycoside concentrations were highly variable among genotypes and were slightly higher under conditions of high nutrient availability, but were unaffected by defoliation (Figure 1). Phenolic

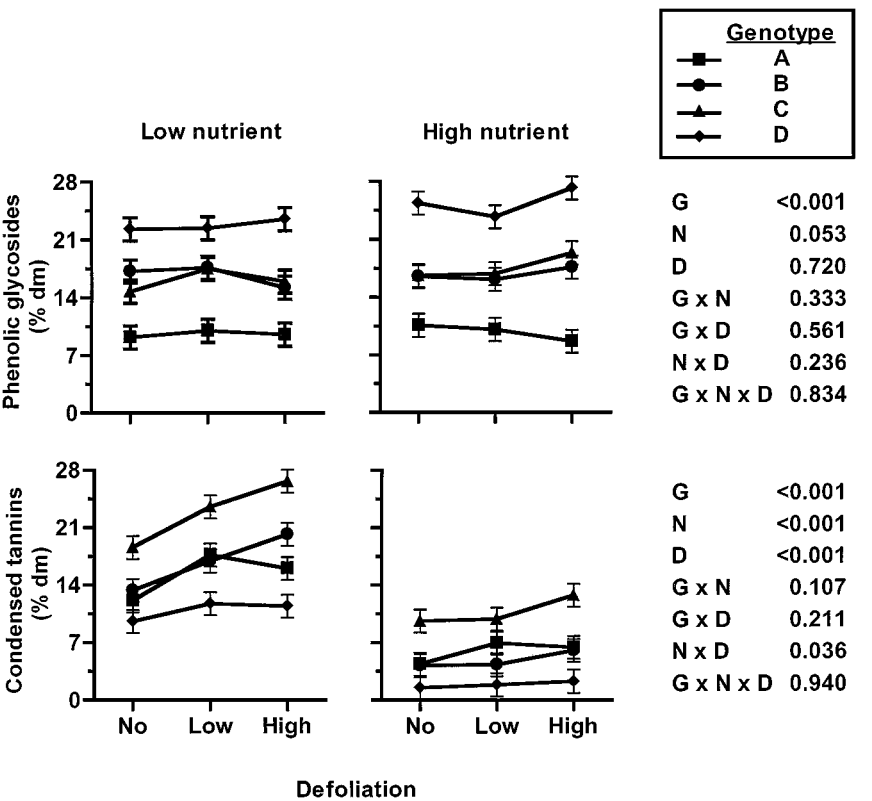

FIG. 1. Norm of reaction plots for phenolic glycoside and condensed tannin concentrations of aspen foliage in relation to nutrient availability and defoliation. *P* values indicate the results of three-way ANOVA: genotype (G) $df = 3$; nutrient availability (N) $df = 1$; defoliation (D) $df = 2$; G × N $df = 3$; G × D $df = 6$; N × D $df = 2$; G × N D $df = 6$. Each line represents a single aspen genotype. Vertical lines represent 1 standard error (calculated based on the pooled variance) of the mean.

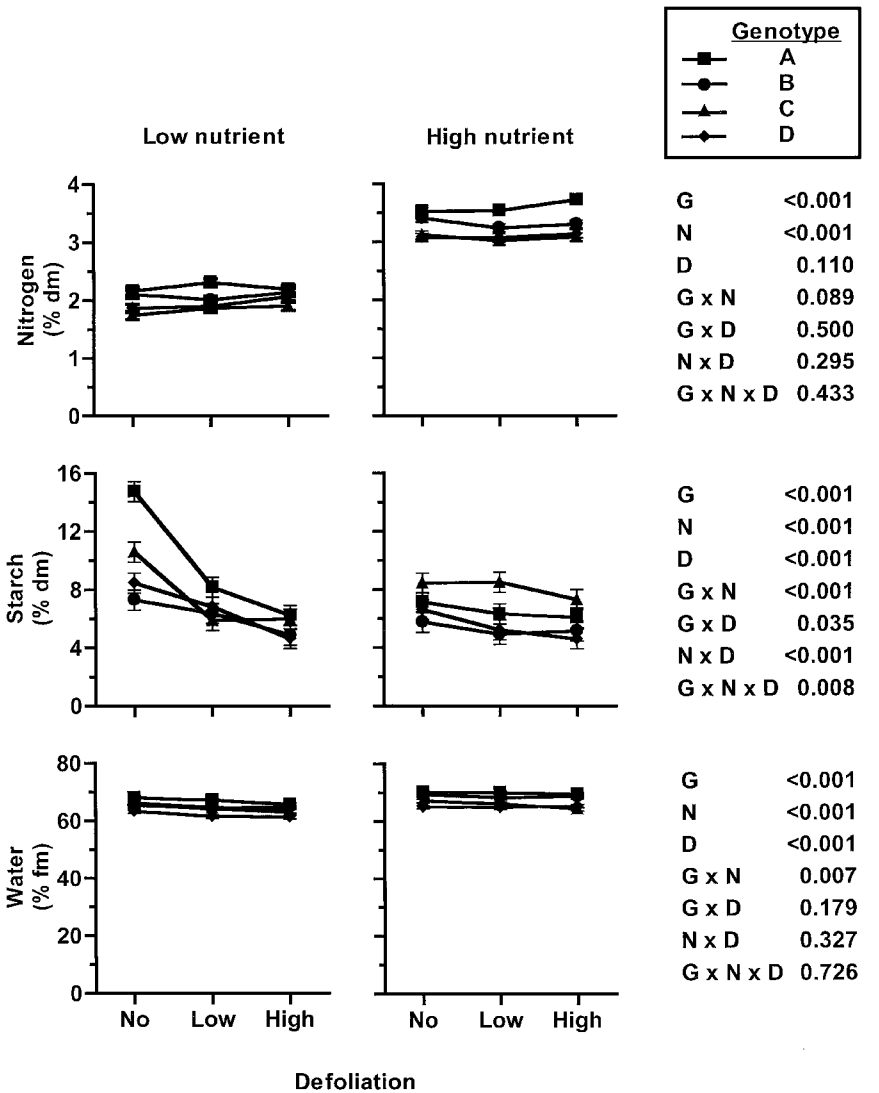

FIG. 2. Norm of reaction plots for foliar nitrogen, starch, and water concentrations of aspen foliage in relation to nutrient availability and defoliation. Format as in Figure 1.

glycoside concentrations were strongly dependent upon aspen genotype, which accounted for 93% of the variation explained (Figure 3). In contrast, concentrations of condensed tannins were strongly affected by all three treatments (Figure 1). Tannin concentrations averaged 1.8-fold higher under low nutrient availability and

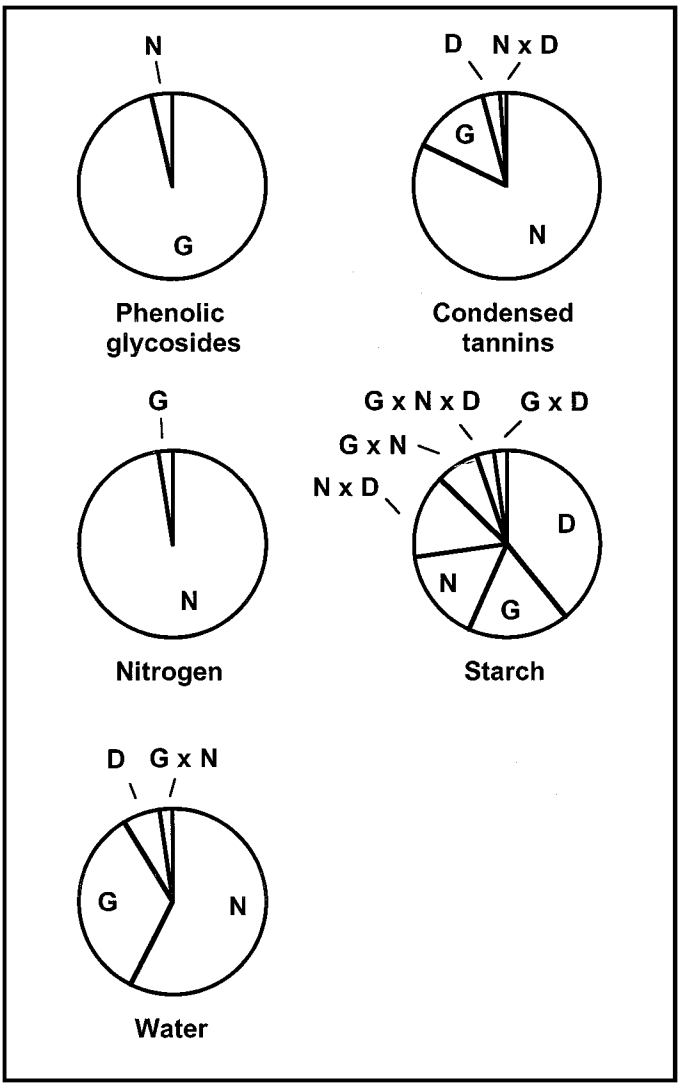

FIG. 3. Proportion of "explained variation" in relation to aspen genotype (G), nutrient availability (N), defoliation (D), and their interactions for phytochemical variables. For each variable, experimental treatments and interactions are ranked in decreasing order (clockwise from 12 o'clock) of the proportion of variation explained. Proportion of "explained variation" was calculated as: mean square for each treatment/total mean square explained. For the sake of clarity, only variation due to significant parameters is shown (in all cases variation due to nonsignificant factors was $<1\%$ of the total).

TABLE 2. CORRELATIONS BETWEEN INDUCED LEVELS OF CONDENSED TANNINS AND CONSTITUTIVE RESISTANCE (CONDENSED TANNINS, PHENOLIC GLYCOSIDES, OR THEIR COMBINATION) UNDER LOW AND HIGH NUTRIENT AVAILABILITY[a]

	Induced levels of tannins under the experimental treatments							
	Low damage, Low nutrient		Low damage, High nutrient		High damage, Low nutrient		High damage, High nutrient	
Constitutive levels	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Condensed tannins	0.566	0.435	−0.132	0.867	0.914	0.086	0.973	0.027
Phenolic glycosides	0.968	0.032	−0.682	0.318	−0.311	0.689	−0.621	0.379
Combined	0.572	0.428	−0.826	0.174	0.328	0.672	−0.087	0.913

[a] Pearson product–moment correlations are based on a mean value per treatment ($N = 4$ clones).

were induced by defoliation. In addition, the effect of defoliation was influenced by nutrient availability; condensed tannins were more strongly induced under conditions of low nutrient availability. Soil nutrient availability accounted for the greatest amount of the variation (82%) among treatments, whereas plant genotype, defoliation, and the nutrient × defoliation interaction accounted for only 13, 3, and 1% of the variation, respectively (Figure 3). Under conditions of low damage, induced levels of condensed tannins related positively to phenolic glycosides (low nutrient availability) (Table 2). Under conditions of severe damage, however, induced levels of tannins related positively to constitutive levels of tannins (Table 2).

Similar to the secondary metabolites, patterns of responses of primary metabolites varied among the treatments (Figure 2). Foliar nitrogen was minimally variable among the genotypes and increased 1.4-fold with nutrient addition, but was not affected by defoliation (Figures 2 and 3). Foliar concentrations of starch were variable among genotypes and reduced by nutrient addition and defoliation. Although all factors interacted significantly, the magnitude of the defoliation effect was reduced dramatically by high nutrient availability for some genotypes (Figures 2 and 3). Concentrations of foliar water were significantly affected by plant genotype, soil nutrient availability, and defoliation, but the magnitude of variation in response to these treatments was small compared to that of the other phytochemicals measured (Figure 1).

Insect Performance. Plant genotype and nutrient availability affected all gypsy moth performance parameters, whereas defoliation affected only herbivore relative growth rate (Figures 4 and 5). Insect relative growth rate was strongly affected by aspen genotype, which accounted for the greatest proportion (78%) of the variation (Figures 4 and 6). Nutrient availability, although highly significant, had less of an effect on growth rate than did plant genotype (Figures 4 and 6). Larvae grew faster feeding on foliage from the high-nutrient treatment, and this effect depended upon plant genotype (Figure 4). Relative growth rates were weakly

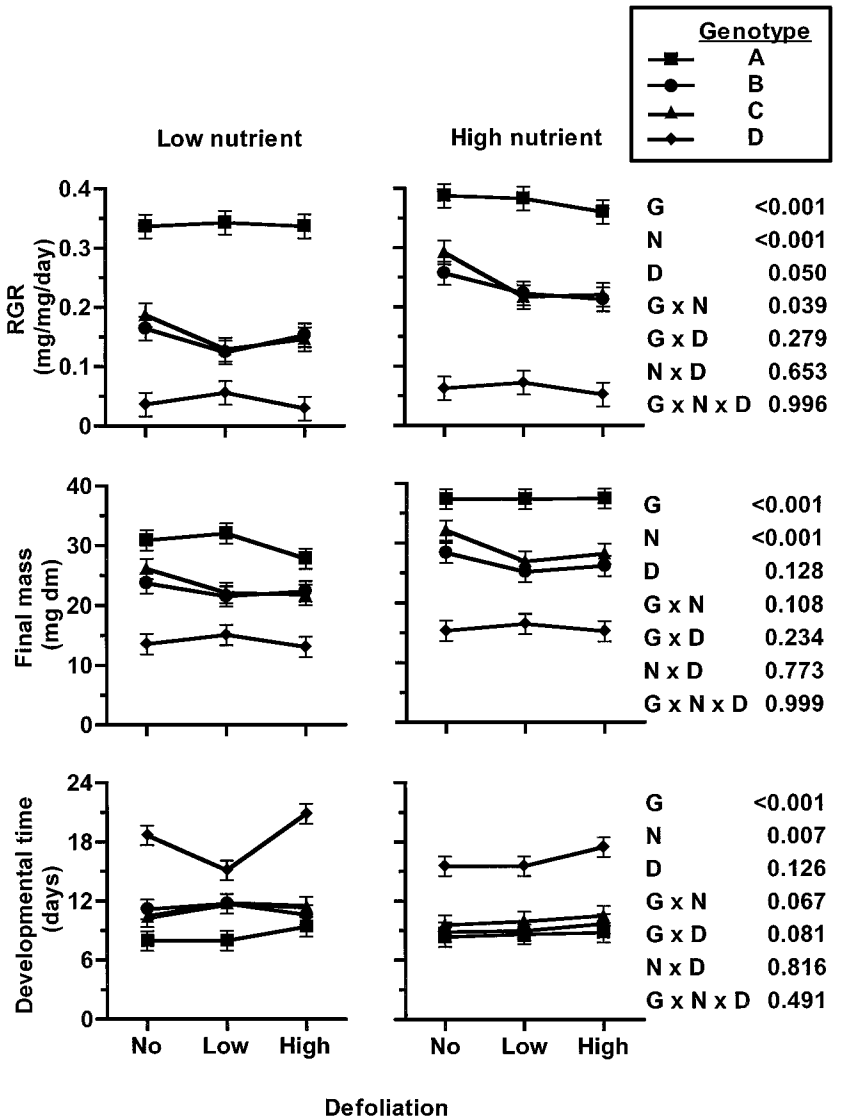

FIG. 4. Norm of reaction plots for gypsy moth relative growth rate, final mass, and developmental time in relation to nutrient availability and defoliation. Format as in Figure 1.

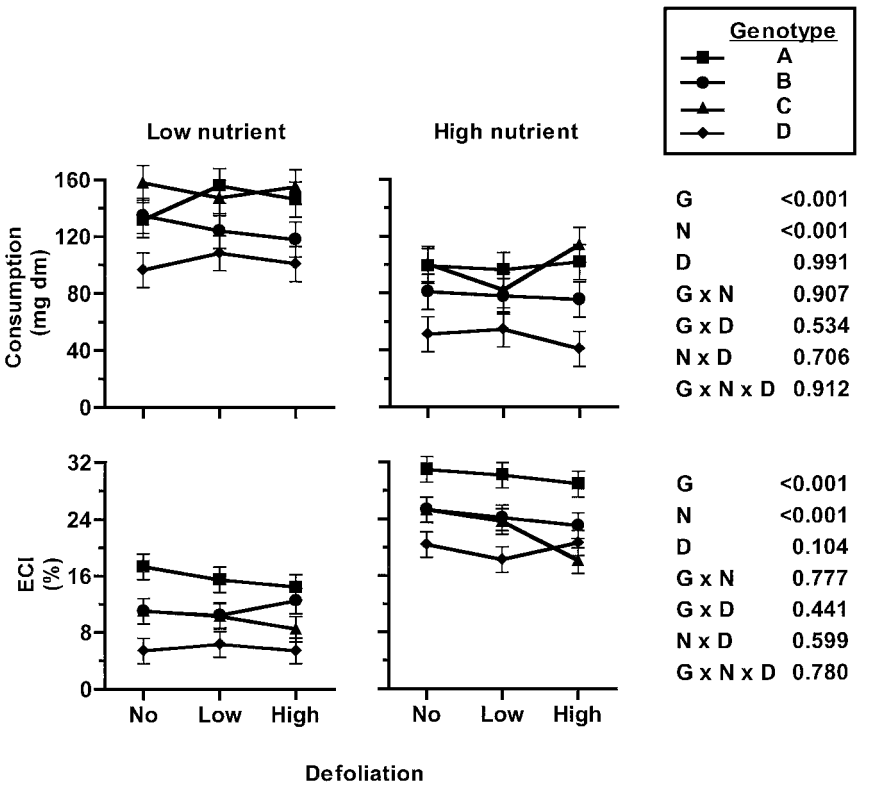

FIG. 5. Norm of reaction plots for gypsy moth consumption and utilization efficiency in relation to nutrient availability and defoliation. Format as in Figure 1.

affected by the defoliation treatments; insects feeding on defoliated plants grew more slowly than did those feeding on undamaged plants (Figure 4). Treatment effects on the dry mass of newly molted fifth-stadium larvae and on developmental time paralleled those of relative growth rate, except that a defoliation effect was not observed (Figures 4 and 6). Food consumption by larvae varied among the aspen genotypes and increased an average of 1.6-fold under low nutrient availability. Consumption was not, however, affected by defoliation treatment (Figures 5 and 6). Similarly, the efficiency of conversion of ingested food to biomass (ECI) was moderately affected by aspen genotype, enhanced by high nutrient availability, and unaffected by defoliation (Figures 5 and 6).

Relationship of Herbivore Performance to Phytochemistry. Stepwise regressions indicate that gypsy moth performance was related to concentrations of a number of phytochemicals; these correlations accounted for a large proportion of

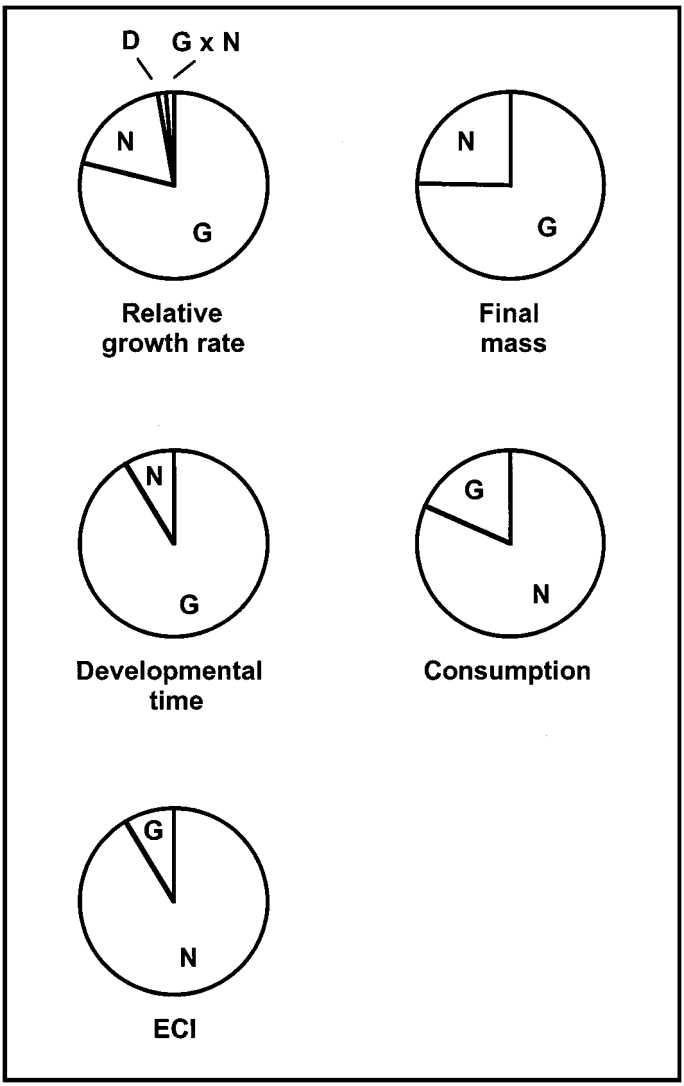

FIG. 6. Proportion of "explained variation" in relation to aspen genotype (G), nutrient availability (N), defoliation (D), and their interactions for insect performance variables. For each variable, experimental treatments and interactions are ranked in decreasing order (clockwise from 12 o'clock) of the proportion of variation explained. Proportion of "explained variation" was calculated as: mean square for each treatment/total mean square explained. For the sake of clarity, only variation due to significant parameters is shown (in all cases variation due to non-significant factors was $<1\%$ of the total).

TABLE 3. PHYTOCHEMICAL COMPONENTS ACCOUNTING FOR VARIATION IN GYPSY MOTH PERFORMANCE[a]

	Regression model			Partial regression components		
Parameter	Equation	R^2	P	Variable	R^2	P
Relative growth rate	$Y = 0.27 - 0.02(PG) + 0.07(N) + 0.01(S)$	0.957	<0.001	PG	0.818	<0.001
				N	0.128	<0.001
				S	0.012	0.030
Final dry mass	$Y = 33.34 - 1.19(PG) + 4.39(N)$	0.931	<0.001	PG	0.782	<0.001
				N	0.149	<0.001
Developmental time	$Y = 74.18 + 0.19(PG) - 0.97(W) - 0.18(CT)$	0.834	<0.001	PG	0.731	<0.001
				W	0.063	<0.020
				CT	0.040	<0.039
Consumption	$Y = 177.31 + 2.28(CT) - 2.70(PG) - 18.79(N)$	0.905	<0.001	CT	0.781	<0.001
				PG	0.083	0.002
				N	0.041	0.008
ECI	$Y = -92.10 + 7.59(N) + 1.35(W)$	0.964	<0.001	N	0.872	<0.001
				W	0.092	<0.001

Partial regression components for developmental time, consumption and ECI should be interpreted with caution due to intercorrelation of independent variables (see *Statistical Analyses* section).

[a] Stepwise multiple regressions, $\alpha = 0.10$ 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.

the total variation in herbivore performance (Table 3). Relative growth rates were related negatively to foliar phenolic glycoside concentrations and positively to foliar nitrogen and starch concentrations (Table 3, Figure 7). Quantitative variation in these three types of compounds explained 96% of the among-treatment variation in relative growth rate (Table 3). Variation in phenolic glycoside concentrations explained a much greater proportion of the total variation in relative growth rate (82%) than did nitrogen (13%) or starch (1%). As was the case for relative growth rate, insect final mass was related negatively to phenolic glycoside concentrations and positively to nitrogen concentrations, explaining a total of 93% of variation (Table 3). Developmental time of gypsy moths was positively related to phenolic glycosides and negatively related to water and condensed tannin concentrations. These three foliar constituents accounted for a total of 83% of the variation in developmental time, and phenolic glycosides appeared most important (Table 3). Insect consumption was related positively to concentrations of condensed tannins and negatively to concentrations of phenolic glycosides and nitrogen. These three variables explained a total of 90% of the variation in insect consumption (Table 3). Two variables, nitrogen and water, explained 96% of the variation in insect growth efficiency (ECI; Table 3).

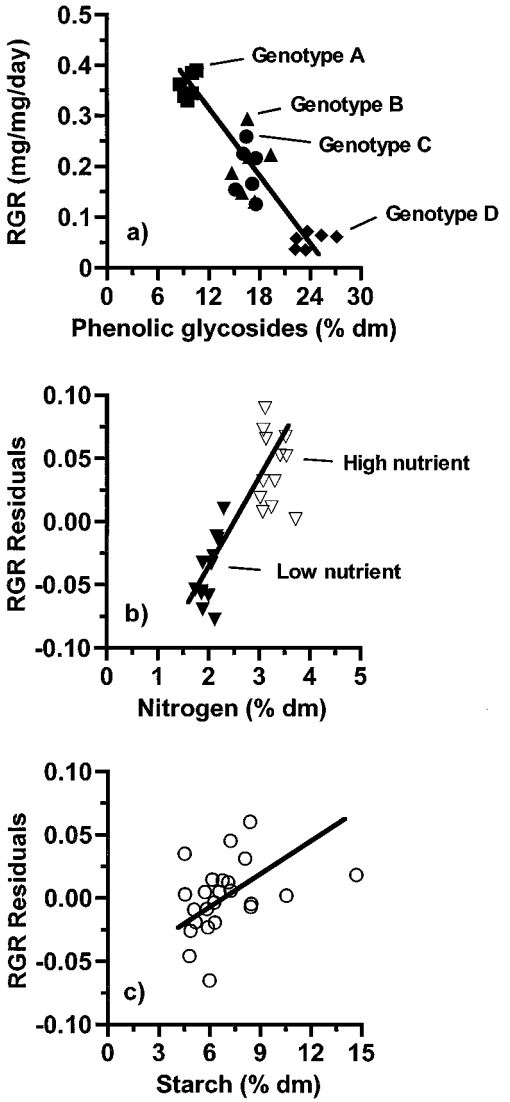

FIG. 7. Relationship of gypsy moth growth to concentrations of phytochemicals implicated as important by stepwise regression analyses. (a) The relationship of relative growth rate to phenolic glycoside concentrations; (b) the variation in relative growth rate unexplained by phenolic glycosides (residuals) plotted against foliar nitrogen concentrations; and (c) the variation in relative growth rate unexplained by both phenolic glycosides and nitrogen plotted against foliar starch concentrations.

To highlight how variation in phytochemical concentrations related to experimental treatments and likely determined patterns of herbivore performance, we present a series of figures illustrating results of the regression analyses for a single performance parameter (Figure 7). Insect relative growth rate was strongly and negatively related to phenolic glycoside concentrations. Variation in phenolic glycoside concentrations (along the *x* axis) is largely associated with aspen genotype (Figure 7a). A plot of the residuals (unexplained variation) from the first regression analysis versus nitrogen concentrations reveals a strong positive relationship (Figure 7b). Data points along the *x* axis are no longer grouped by genotype, but by nutrient availability, suggesting that soil nutrient availability is driving much of the variation in nitrogen concentrations. Finally, the remaining residuals are weakly and positively related to starch concentrations (Figure 7c). Variation in starch was affected by all treatments and their interactions, so position along the *x* axis is not clearly dominated by one or a combination of these.

DISCUSSION

Phytochemistry. Plant genotype, soil nutrient availability, and defoliation treatments affected phytochemical concentrations, although the magnitude and direction of responses to these treatments differed among chemical constituents. Aspen genotype was responsible for most of the variation in phenolic glycoside concentrations, while nutrient availability had a secondary effect. Nutrient addition resulted in a slight, but significant, increase in concentrations of phenolic glycosides. Such a response is not consistent with the predictions of the carbon–nutrient balance hypothesis (Bryant et al., 1983), growth–differentiation balance hypothesis (Herms and Mattson, 1992), or the protein competition model of phenolic allocation (Jones and Hartley, 1999). That phenolic glycosides responded positively to nutrient addition was unexpected given that in previous studies concentrations either responded as would be predicted (Hemming and Lindroth, 1999) or not at all (Kinney et al., 1997).

Condensed tannins responded most strongly to the nutrient treatment and were less influenced by plant genotype, defoliation, and the defoliation by nutrient interaction. As 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), concentrations of condensed tannins were markedly lower under conditions of high nutrient availability. The short-term induction response of condensed tannins in our saplings is consistent with that of other studies using quaking aspen (Roth et al., 1998; Lindroth and Kinney, 1998). Furthermore, the induction response was ameliorated by the addition of nutrients. Such a pattern suggests a passive response due to altered carbon–nutrient balance, as demonstrated by Hunter and Schultz (1995)

for *Quercus prinus*, rather than an active defensive response on the part of the plant. Although the responses of tannins to nutrient addition accord with the predictions of the carbon–nutrient balance hypothesis, other phytochemical evidence suggests that the induction response is indeed an active response by the plant to defoliation, rather than a passive response driven by nutrient loss due to defoliation. The defoliated saplings appear to be under carbon (rather than nutrient) stress, indicated by the very low starch concentrations in these plants (as compared to undefoliated plants). The low carbon–nutrient ratios in the defoliated plants would predict low levels of tannins, rather than the dramatic increases observed.

The overall weak response of phenolic glycosides to environmental factors (both nutrient availability and defoliation) is consistent with other studies of aspen and is a striking contrast to the strong response of condensed tannins (Lindroth et al., 1993; Kinney and Lindroth, 1997; Roth et al., 1998; Hemming and Lindroth, 1999; Agrell et al., 2000). Although differences in environmental plasticity between phenolic glycosides and condensed tannins were striking, different suites of compounds within a plant can behave differently in response to environmental variability (Koricheva et al., 1998; Keinänen et al., 1999).

Although physiological responses to environmental conditions are important in determining allocation to phenolic glycosides and condensed tannins, allocation was found be largely genetically determined. The question arises as to what factors contribute to the maintenance, at the population level, of polymorphisms in concentrations of these secondary compounds. Polymorphisms in defensive allocation are likely driven, in part, by costs of production and storage (sensu Herms and Mattson, 1992). This appears to be the case with phenolic glycosides in quaking aspen, which are produced at a cost to growth (Osier and Lindroth, unpublished data). For condensed tannins, however, no ecological cost of production has been observed.

Trade-offs between constitutive defense and inducibility are considered as evidence that defenses are costly, although few such relationships have been demonstrated (Karban and Baldwin, 1997). In contrast to predictions, we found positive relationships between damage-induced changes in condensed tannins and constitutive levels of secondary metabolites under several nutrient–defoliation regimes. This is especially true for plants that were severely defoliated, as plants that had the highest constitutive levels of tannins also exhibited the strongest induced response. Our results differ from two other studies that reported negative correlations between induced and constitutive levels of phenolics in poplars. Mattson and Palmer (1988) reported a negative relationship between induced and constitutive levels of total phenolics in aspen clones with high levels of total phenolics, although a strong negative relationship was not observed across the entire range of genotypes used in that study. Nef (1988) found a negative relationship between induced and constitutive levels of total phenolics for three poplar hybrids. Both of these studies suggest that a negative relationship between induced and constitutive resistance

should be expected in aspen, but our results are strongly contradictory. One difference between our work and that of other researchers is that previous studies relied upon the method of Folin and Denis (1912), which measures not only "total phenolics," but also other compounds (e.g., reducing sugars) not quantified in our study.

Foliar nitrogen varied primarily in response to nutrient availability. Responses accorded well with predictions of the carbon–nutrient balance hypothesis (Bryant et al., 1983), as has been shown previously for aspen (Kinney and Lindroth, 1997; Hemming and Lindroth, 1999). Also similar to previous work (Hwang and Lindroth, 1997, 1998; Osier et al., 2000), foliar nitrogen varied among genotypes, although not dramatically so. Foliar starch concentrations decreased as a result of both nutrient addition and defoliation, as predicted by the carbon–nutrient balance hypothesis (Bryant et al., 1983; Tuomi et al., 1988). Moreover, the effect of defoliation was dependent upon nutrient availability; plants stressed by low nutrient availability experienced the greatest decrease in starch concentrations. Foliar water concentration was affected by plant genotype, soil nutrient availability, defoliation, and the interaction of genotype and nutrient availability. Although foliar water is important for herbivores (Scriber and Slansky, 1981), the variation in this study was small and unlikely to affect herbivore performance.

Herbivore Performance. Gypsy moth relative growth rates were most strongly affected by aspen genotype, less so by nutrient availability, and only minimally by defoliation and the interaction of genotype and nutrient availability. That plant genotype had a large effect on relative growth rates was not surprising, given that differences among aspen genotypes have been found to have similar effects in previous studies (Hemming and Lindroth, 1995; Hwang and Lindroth, 1997, 1998; Osier et al., 2000). Although the effect of genotype was expected to be strong, its magnitude far exceeded that of the environmental treatments. As expected, insects performed better on fertilized plants. The pattern for herbivore final dry mass and developmental time closely followed the pattern for herbivore relative growth rate, except there was no defoliation main effect and the effect of soil nutrient availability did not depend upon genotype.

Phenolic glycoside concentrations were implicated as the primary determinant of host quality; insects feeding on foliage from saplings containing high concentrations of phenolic glycosides took longer to develop and attained a lower final mass, as has been found previously (Hemming and Lindroth, 1995; Hwang and Lindroth, 1997, 1998; Osier et al., 2000). Studies in which purified phenolic glycosides were added to either foliage or artificial diet confirm the role of phenolic glycosides in altering host quality (Lindroth and Hemming, 1990; Hemming and Lindroth, 1995). In addition to phenolic glycosides, variation in nitrogen concentrations (due primarily to nutrient treatment) provided explanatory power for herbivore growth rate and final mass. This result is not surprising given the variation in nitrogen levels observed and that such variation is an important

determinant of insect performance (Mattson, 1980; Scriber and Slansky, 1981; Slansky, 1993).

Food consumption by gypsy moths was most dramatically influenced by nutrient availability. As is commonly observed (Mattson, 1980), larvae fed in a compensatory manner on the poorer quality foliage in the low-nutrient treatment. Although little evidence exists for aspen condensed tannins functioning as feeding deterrents or toxins to insects (Lindroth and Hwang, 1996), high concentrations of these compounds may have diluted nutrients and led to compensatory feeding. This response has been found previously (Osier et al., 2000) for condensed tannins in aspen and is similar to the response of insects to indigestible dietary components such as cellulose (Slansky, 1993). Larvae fed less on foliage containing high levels of phenolic glycosides and nitrogen, likely due to their feeding deterrent and nutritive properties, respectively. Although less important than nutrient availability, the magnitude of the genotype effect on consumption was similar to that observed previously for insects reared on aspen (Hwang and Lindroth, 1997, 1998). Insects fed foliage grown under low nutrient availability were much less efficient in converting food to body mass than were insects fed foliage from high-nutrient plants. As expected, the larvae were most efficient at converting ingested food to biomass when foliage contained high concentrations of nitrogen and water (Mattson, 1980; Scriber and Slansky, 1981; Slansky, 1993). Surprisingly, and in contrast to earlier research (Hwang and Lindroth, 1997), phenolic glycosides were not implicated as determinants of gypsy moth growth efficiency in this study. The absence of relationships may, in part, be a statistical artifact. Foliar water concentrations related strongly and negatively to those of phenolic glycosides (Table 1), and water may have served as a proxy for phenolic glycosides in the regression model.

The lack of a substantial damage-induced change in food quality was surprising given the importance of induced responses in many other systems (Karban and Baldwin, 1997). Absence of an effective induction response may explain why aspen forests are susceptible to outbreaks of spring-feeding insects such as gypsy moths, forest tent caterpillars (*Malacosoma disstria*), and large aspen tortrix (*Choristoneura conflictana*). Even though the defoliation treatment did not appear to markedly affect gypsy moth performance, we note that the aspen saplings did respond with a rapid induction of condensed tannins. This response may confer resistance against other types of enemies such as browsing mammals or pathogens at the site of wounding. Additionally, the possibility exists that extended duration of defoliation or repeated defoliation may alter the induction response observed (Karban and Baldwin, 1997). Other unpublished work with aspen, however, suggests that repeated defoliation (D. Parry, personal communication) or defoliation in the previous year (Osier and Lindroth, unpublished data) produces responses similar to those in this study.

Relative Importance of Plant Genotype and Environment. Results from other studies that compare the relative roles of plant genotype and environmental

conditions have implicated either genotype (Abrahamson et al., 1988; Hakulinen et al., 1995; Horner and Abrahamson, 1999) or environment (Orians and Fritz, 1996; Rossi and Stiling, 1998) as primary causes of variation important for herbivores. Studies with *Salix sericea* (another member of the Salicaceae) found results very different than ours; genotype was of relatively little importance compared to soil nutrient availability in determining the abundance of a suite of herbivores in the field (Orians and Fritz, 1996). Employing an experimental design similar to ours, a study with *Betula pendula* determined that fertilization explained most of the variation in insect performance and plant genotype explained less (Mutikainen et al., 2000). As with our study, however, defoliation (a delayed induced response) was relatively unimportant for insect feeding.

In addition to highlighting the importance of plant genotypic variation, our study revealed few interactions between genotype and environment for phytochemicals important for insect herbivores. The paucity of interactions between genotype and environment in our study is not surprising, given that such interactions have rarely been found to play a major role in other systems (Houle and Simard, 1996; Stiling and Rossi, 1996; but see also Horner and Abrahamson, 1999).

Because the relative strength of each treatment applied is important when attempting to rank treatment types, we endeavored to apply levels of each treatment that were biologically realistic and of comparable strength to the other treatments (see Methods and Materials). Even when environmental treatments were pushed nearly to extremes, environment and genotype $\times$ environment interactions were markedly less important than genotype in this study. Our results suggest that patterns of insect performance among aspen clones in the field are likely due to genetic variability rather than to environmental heterogeneity in nutrient availability or defoliation.

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