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GENETICS, ENVIRONMENT, AND THEIR INTERACTION DETERMINE EFFICACY OF CHEMICAL DEFENSE IN TREMBLING ASPEN

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Abstract. Optimal defense theories suggest that a trade-off between defense costs and benefits maintains genetic variation within plant populations. This study assessed the independent and interactive effects of genetic- and environment-based variation in aspen leaf chemistry on insect performance, preference, and defoliation. Gypsy moth larvae were released into screenhouses containing eight aspen genotypes growing with high and low levels of nutrient availability. Plant chemistry, defoliation, and larval growth rates varied in response to genotype, nutrient availability, and their interaction. Total phenolic glycoside concentrations were inversely correlated with patterns of larval preference and were the best predictor of larval performance and defoliation among genotypes. Low-nutrient trees were less heavily defoliated and afforded decreased larval growth rates compared with high-nutrient trees. Nutrient availability mediated the defense benefits of phenolic glycosides, as plant chemistry explained significantly less variation in defoliation in low- compared with high-nutrient trees (7% vs. 44% of variation explained). These results suggest that spatial and temporal variation in resource availability may influence the relative magnitude of defense benefits in plants. Environmental mediation of the defense costs and benefits likely leads to diversifying selection and may maintain genetic polymorphisms in chemical defense traits in plant populations.

Key words: chemical defense; genetic variation; gypsy moth; Lymantria dispar; *mesocosms; plant defense;* Populus tremuloides; *preference.*

Introduction

Both primary and secondary plant metabolites strongly influence herbivore fitness, and considerable quantitative variation in allocation to these compounds occurs within plant species. A central issue in the discipline of plant–herbivore interactions has been characterization of the ecological and evolutionary mechanisms that allow for persistence of genetic variation in plant populations (Rhoades 1979, Coley et al. 1985, Herms and Mattson 1992, Hamilton et al. 2001). Differential selective pressures of herbivores on plants (Mauricio 2000), resource-mediated constraints (Coley et al. 1985), and trade-offs between growth and defense (Herms and Mattson 1992) have all been implicated as important factors in maintaining genetic variation in plant populations.

Numerous studies have analyzed physiological costs of chemical defense production (reviewed by Simms 1992, Bergelson and Purrington 1996, Koricheva 2002, Strauss et al. 2002), but few have critically examined the benefits (to plant fitness) of putative defenses in natural systems (Agrawal 1999). The anti-herbivore effects of plant chemical defenses have frequently been demonstrated; however, relatively few studies have shown that genetically variable levels of defensive chemicals lead to variable rates of damage in plant populations. Herbivore performance assays are an important tool for understanding the potential defensive effects of plant chemistry and are likely relevant to natural conditions much of the time, but not invariably so (e.g., Latta and Linhart 1997, Kause et al. 1999). Even when plant defenses slow herbivore growth, compensatory feeding responses may increase lifelong consumption (Augner 1995, Lindroth et al. 1995, Osier et al. 2000, Kondoh and Williams 2001, Parry et al. 2003), thereby decreasing plant fitness.

Phenolic glycosides have been implicated as important chemical defenses in trembling aspen (*Populus tremuloides*). These compounds affect the performance of defoliating lepidopterans, including gypsy moths and forest tent caterpillars, and concentrations can vary markedly among adjacent aspen clones (genotypes) in a stand (Hemming and Lindroth 1995, Lindroth and Hwang 1996*a*, Osier and Lindroth 2001). During forest tent caterpillar outbreaks of moderate intensity, defoliation differs markedly among clones. It is unclear, however, whether this variation is related to foliar chemistry or other factors such as budbreak phenology (Chilcote et al. 1992, Parry et al. 1998, Parry and Goyer 2004, Donaldson 2005) or oviposition preferences (Robison and Raffa 1994). Defoliation rarely causes

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mortality in healthy aspen trees, but significantly decreases growth (Churchill et al. 1964, Hogg et al. 2005) and delays flowering (Stevens 2005; C. Cole, *personal communication*).

This study addressed several key questions about the evolutionary ecology of plant defenses. First, does phenotypic variation in expression of secondary metabolites within a plant population lead to variable rates of herbivory (with potential fitness consequences) under outbreak conditions? If so, what is the underlying selectable (genetic) component of that variation, and can that variation be linked to a specific genetically based chemical mechanism? Finally, what are the roles of environment and gene × environment interactions in mediating expression of chemical defense traits and links between chemistry and resistance?

Trembling aspen provides an excellent system in which to address these questions. Aspen shows substantial genetically based variation in phytochemical traits (Lindroth and Hwang 1996*a*), and populations occurring in the Lake States region of the United States exhibit as much within-population variation as among-population variation (Lindroth and Hwang 1996*b*, Donaldson 2005, Donaldson et al. 2006*b*). We predicted that genotype, nutrient availability, and their interaction would influence levels of phenolic glycosides, condensed tannins, and nitrogen in aspen foliage, such that differential spatial distributions, food preferences, and performance of free-ranging gypsy moth larvae would produce variable defoliation rates. Specifically, we predicted that levels of defoliation would differ among genotypes in inverse proportion to levels of foliar phenolic glycosides. Previous work in this system suggests that condensed tannins do not affect gypsy moth performance, but high concentrations can increase relative consumption rates (Osier et al. 2000). Thus, we predicted that trees with high concentrations of tannins might experience increased defoliation. Genetically based variation in foliar nitrogen (protein) is usually subtle (Lindroth and Hwang 1996*a*, Osier and Lindroth 2001); thus we predicted that the effect of N on defoliation rates would vary little among genotypes. However, because nutrient availability can markedly alter foliar N (Osier 2001), we expected greater defoliation of fertilized trees, relative to nonfertilized trees (Price 1991).

Methods

Plant materials

Aspen genotypes used in this study were originally collected as root cuttings from clones growing in Dane (D1, D2), Columbia (P3, P12), Sauk (S1, S3), and Waushara (W1, W2) counties in south-central Wisconsin. Aspen populations at each of these sites consist of numerous genotypes, and neutral genetic variation is as great within populations as among populations (Cole 2005). The aspen clones used in this study were identified as unique genotypes on the basis of microsatellite marker analysis (16 loci evaluated; C. Cole and R. L. Lindroth, *unpublished data*). Replicate individuals of each genotype were generated for this study via a micropropagation system developed at the University of Wisconsin, Madison (B. McCown, Department of Horticulture). Micropropagation methods are detailed by Donaldson (2005).

In winter 2001, 2–5 cm tall micro-cuttings were rooted in 96-cell planting trays containing standard potting medium. Cuttings were gradually acclimated to the greenhouse and then transplanted to 650-mL D40 Conetainers (Stuewe and Sons, Corvallis, Oregon, USA). In May 2001, when trees were 25–35 cm tall, they were transferred outside into 5-L pots in a 40:40:20 mix of torpedo sand, silt-loam soil, and perlite. All trees were given 4.5 g/L 14-14-14 (N-P-K and micronutrients) Osmocote 3–4 month slow-release fertilizer (Scotts, Marysville, Ohio, USA; rate determined from Hemming and Lindroth [1999] and Osier and Lindroth [2001]).

In spring 2002 trees of similar size were transplanted to 40-L pots. Growth medium was a 70:30 mix of torpedo sand and silt-loam soil. At time of transplanting, trees were beginning their second growth season and were 0.8–1.2 m tall. Low- and high-nutrient treatments were initiated at this time to support growth rates that ranged from low to very good (but less than maximum), based on previous fertilization studies with young aspen (Hemming and Lindroth 1999). Trees in the low-nutrient treatment received 0.5 g/L 18-6-12 (N-P-K and micronutrients) Osmocote 8–9 month slow-release fertilizer while trees in the high-nutrient treatment received 4.5 g/L. In spring 2003 trees in the low-nutrient treatment received no fertilizer and the high-nutrient treatment was repeated.

Defoliation experiment

In spring 2003, trees were arranged in 4 × 3 m screenhouse "mesocosms" covered with loose-knit fiberglass screen cloth (to exclude natural enemies and contain larvae). The screen reduced incident radiation by 30%, which has negligible impact on phenolic glycoside levels (Hemming and Lindroth 1999). The experimental design was an 8 × 2 split-plot factorial, with eight genotypes and two soil nutrient levels randomized within eight screenhouses ($n = 8$ true replicates). Trees were grouped within screenhouses by nutrient treatment (whole plot) to increase our statistical power to resolve defoliation differences among genotypes (subplot). For each genotype × nutrient treatment combination, four additional protected "defoliation control" trees were randomly distributed among the eight screenhouses to be used in assessments of defoliation.

Gypsy moth neonates were hatched in the laboratory at 25°C (egg masses provided by USDA-APHIS, Otis Air National Guard Base, Massachusetts, USA), precounted into vials, and added to each tree at densities calculated to effect ~80% defoliation on the most

susceptible trees. Prior to budbreak, the number of vegetative buds per tree were counted to estimate the number of leaves per tree. Larvae were added at a rate of one per estimated 20 leaves (Valentine and Talerico 1980, Lindroth et al. 1997). Vials containing precounted neonate larvae were attached to the lowest branch of each tree and allowed to emerge in aggregate masses.

Genetic- and environment-based differences in budbreak phenology can have marked effects on larval growth and plant damage in aspen (Chilcote et al. 1992, Parry et al. 1998, Donaldson 2005). To avoid this potentially confounding factor, insects were added to trees within each treatment combination as emerging leaves began to unfold. Eight days elapsed between the first and last deployment (i.e., timing of leaf-out varied by approximately one week).

Defoliation was allowed to progress with larvae freely dispersing among trees. When the most heavily defoliated trees had sustained ~80% defoliation and larvae had begun to pupate, the study was terminated. Pupae and remaining larvae were removed by hand and counted and weighed collectively for each tree. Final larval distributions were standardized to leaf area by dividing the total number of larvae per tree by the number of leaves initiated per tree.

At the end of the defoliation treatment, every third branch on the main stem and every third short shoot on the terminal shoot were removed from each tree for estimation of percentage of defoliation (mass basis) as follows:

$$\%\ \text{defoliation} = \left[\frac{\text{final leaf mass}}{\text{no. leaves} \times (\text{mass/leaf})}\right] \times 100.$$

The original number of leaves per tree was obtained by counting the total number of leaves, petioles, and current year's leaf scars on excised branches. Final leaf mass was measured after drying remaining leaves and petioles at 60°C to a constant mass, and mean mass per leaf was estimated from 25 haphazardly selected leaves from each of the four protected control trees per genotype × nutrient treatment.

Gypsy moth performance assays

One of the objectives of this study was to link the effects of variable phytochemistry on insect performance to variation in levels of defoliation. Therefore, a no-choice assessment of larval performance was conducted on trees within six of the eight screenhouses coincident with the defoliation experiment (8 genotypes × 2 nutrient levels × 6 replicates = 96 trees). Larvae were hatched and reared in environmental chambers at 25°C and a 15:9 h (light : dark) photoperiod. Larvae were fed a standard wheat germ diet until they began to molt into the fourth stadium. Females were sorted from males based on mass distributions (95% accurate; Osier 2001) and starved during and after the molt. When sufficient fourth-instar females had been collected (within 24 h of molting), groups of four individuals were weighed collectively and transferred into 25 × 60 cm mesh bags and placed on experimental trees (at the same time that free-ranging larvae were in the fourth stadium). Larvae were allowed to feed for 6 d and then were collected and reweighed. Mean larval relative growth rate (RGR) was calculated as suggested by Farrar et al. (1989): (final − initial larval mass)/(initial larval mass × number of days).

Gypsy moth preference assays

At the same time as performance was measured in mesocosms, pairwise comparisons of fourth-stadium larval feeding preferences were conducted to determine whether preferred genotypes sustained more defoliation and supported higher rates of larval growth. These assays were simply intended to provide additional support for patterns that emerged during the defoliation experiment, so only a subset of genotypes from the high nutrient availability treatment was evaluated. High-nutrient trees were selected because of the clearly distinguishable rates of defoliation among genotypes within this treatment. Three "preferred" genotypes (W1, W2, P3), one "intermediate" genotype (D1), and two "nonpreferred" genotypes (D2, S3) were selected based on preliminary (visual) estimates of defoliation. Pairwise combinations included: D1 vs. S3, D1 vs. P3, W1 vs. D2, and W2 vs. S3. For each tree ($n = 8$) from the respective genotype pair, two leaves with petioles were collected, inserted into water piks, and brought to the laboratory in an ice chest. Leaf areas were determined by digital scans, using Scion Image software (Scion, Frederick, Maryland, USA).

Preference assays were conducted in 150 × 30 mm rearing dishes containing a moistened filter paper disk to prevent leaf desiccation. Water piks containing leaves from the appropriate paired genotypes were placed at opposite ends of the rearing dishes and two newly emerged and starved fourth-stadium larvae were preweighed collectively and placed in the center of each rearing dish. After 24 h, larvae were removed and remaining leaf areas were measured. Leaf area consumed was calculated (initial area − final area) and converted to mass consumed on the basis of final leaf area and oven-dry mass measurements. Relative mass consumed was calculated by dividing by initial mass of the two larvae.

Phytochemical analyses

Leaf samples were haphazardly selected and excised (~8 leaves/tree) from throughout the canopy of both experimental (defoliated) and protected trees at two times during the study. "Initial" samples were collected within days of the respective times when neonate larvae were released on treatments (i.e., at a consistent phenological stage) and before notable feeding had occurred. "Final" chemistry samples were collected on the same day that defoliation estimates were made. This timing allowed us to account for the overall effects of

foliar chemistry during critical times for larvae, both as neonates became established (gypsy moth neonates disperse from nonpreferred hosts) and during the period of maximal feeding (fifth larval stadium).

Leaves were transferred to the laboratory in an ice chest, flash-frozen in liquid nitrogen, and freeze-dried. Dried leaves (laminas only) were ground in a Wiley mill (40-mesh screen). Aspen phenolic glycosides, including salicortin and tremulacin, were quantified using high-performance thin-layer chromatography methods described by Lindroth et al. (1993), with purified aspen phenolic glycosides as standards. Total phenolic glycoside concentrations were calculated as the sum of salicortin and tremulacin concentrations. Condensed tannins were quantified by the acid butanol assay described by Porter et al. (1986), using purified aspen condensed tannins as a standard. Nitrogen concentrations were determined with a LECO elemental analyzer (LECO, St. Joseph, Michigan, USA) using glycine *p*-toluenesulfonate as a standard.

Statistical analyses

The independent and interactive effects of genotype and nutrient availability on gypsy moth distribution, defoliation, larval RGR, and leaf chemistry were assessed using a mixed-model ANOVA in JMP IN version 4.0.4 (SAS Institute 2001). The design was a split-plot with fixed nutrient (whole plot), genotype (subplot), and nutrient × genotype (subplot) effects. The error for the random term, replicate nested within nutrient treatment (whole plot error), was used to test whole-plot (nutrient treatment) effects, while the split-plot error term (residual error) was used to test subplot (genotype and genotype × nutrient) effects. Fourth-instar gypsy moth preferences were assessed using paired *t* tests in JMP IN.

To assess among-treatment variation in concentrations of salicortin, tremulacin, condensed tannins, and nitrogen, we used the mean of initial and final phytochemical concentrations for each tree. Treatment effects were analyzed in a split-plot model identical to that described above. Using mean values accounts for changes that occurred in leaf chemistry as leaves expanded, and as suggested by Osier et al. (2000), likely provides the best model to describe the overall effects of plant chemistry on gypsy moth distributions and plant protection (defoliation).

The relationships between host quality (salicortin, tremulacin, condensed tannin, and nitrogen concentrations) and final gypsy moth distribution, defoliation levels, and larval RGR were assessed using stepwise multiple regressions in JMP IN. Separate analyses were conducted for fertilized and unfertilized trees in order to more clearly resolve genotypic differences. For gypsy moth distributions and defoliation, means of initial and final phytochemistry concentrations were used as explanatory variables. Because larval RGR was assessed near the time of final chemistry measurements, final chemistry concentrations were the most appropriate explanatory variables to use in those regressions. Salicortin and tremulacin concentrations were highly correlated, so concentrations of these compounds were summed and total phenolic glycoside concentration was included as an explanatory variable. Partial correlation coefficients (R^2) were calculated using JMP IN. Because of the multicollinearity among condensed tannin and phenolic glycoside concentrations, we used backward elimination at $\alpha = 0.10$ to select the best-fit model. Using backward elimination decreases the likelihood of inappropriately estimating the most influential partial regression components (Zar 1999).

Estimates of the proportion of total phenotypic variance explained by genotypic variance were calculated for defoliation rates, larval RGR, and phytochemical variables. This proportion is typically known as "broad-sense heritability," but in the case of clonal plants, "clonal repeatability" is the preferred term (Falconer 1989). Variance components were derived from one-way ANOVAs, and separate calculations were made for low- and high-nutrient trees, according to Falconer (1989). As for broad-sense heritability, clonal repeatability values should be interpreted cautiously, as they include additive, dominance, and maternal genetic variation as well as their interactions.

Results

Gypsy moth distributions, defoliation rates, and larval performance

Free-ranging gypsy moth dispersal behaviors were consistent with descriptions given by Doane and McManus (1981). For example, larvae dispersed via "ballooning" (wind dispersal) following initial release and again as third and older instars crawled among trees within screenhouses. Approximately 30% of the larvae released survived to the end of the defoliation experiment (based on final counts). Survival was fairly consistent among replicate screenhouses, ranging from 22% to 32%. Larvae showed clear patterns of preference among aspen genotypes, and they favored high-nutrient trees (Fig. 1A). Differences between nutrient treatments were particularly pronounced on genotypes P3, S1, W1, and W2 (genotype × nutrient interaction).

In spite of the relatively high rate of larval mortality, trees suffered considerable, and highly variable, damage. Defoliation varied more than twofold among genotypes at low nutrient availability and threefold among genotypes under high nutrient availability. On average, trees grown under high nutrient availability were nearly 1.7 times more heavily defoliated than those with low nutrient availability (Fig. 1B). For genotypes D1, S1, and S3, however, nutrient availability had no effect on defoliation (genotype × nutrient interaction).

In no-choice feeding trials, plant genotype accounted for 40- and 5-fold differences in larval relative growth rates (RGR) at low and high nutrient availability, respectively (Fig. 1C). These differences were in large

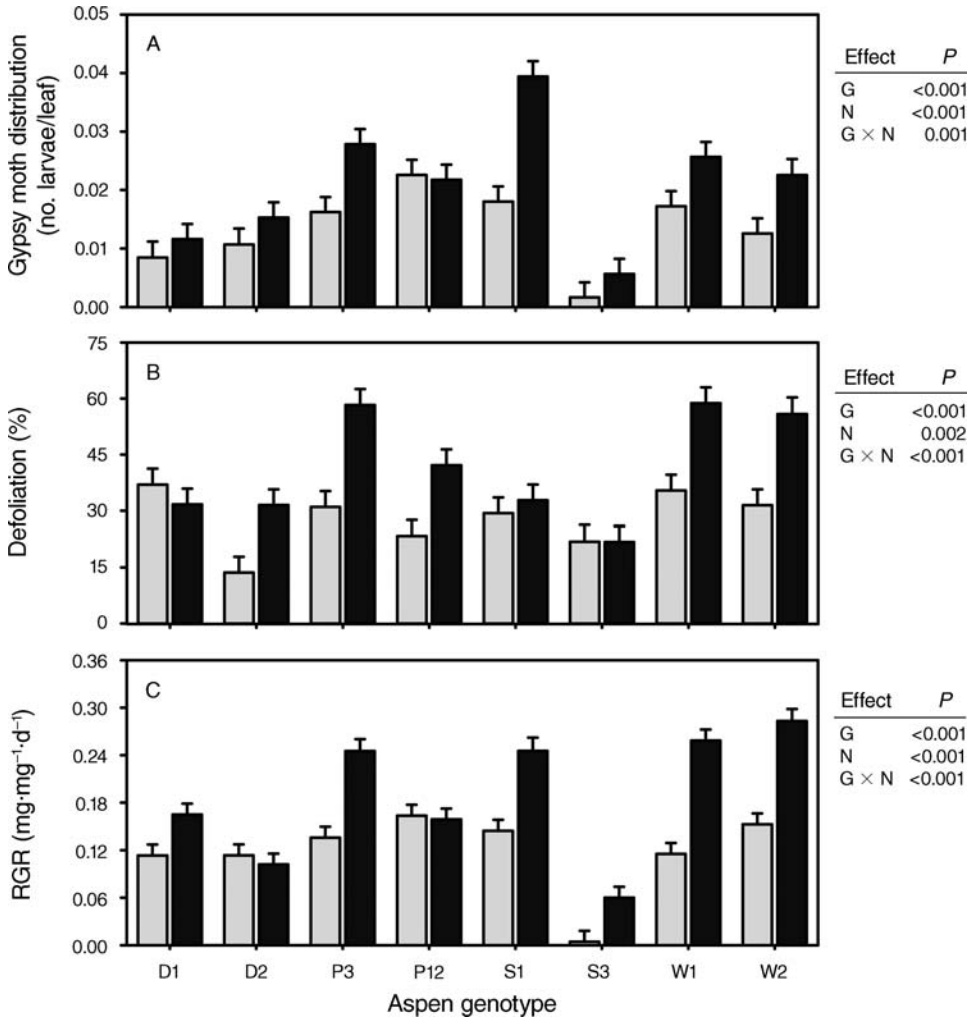

FIG. 1. (A) Final number of gypsy moth larvae per leaf, (B) percentage of defoliation, and (C) larval relative growth rates (RGR) among genotypes (G) and nutrient treatments (N). Bar heights are least-squares means + 1 SE (pooled); $n = 8$ except for RGR where $n = 6$. The gray bars represent the low-nutrient treatment; black bars represent the high-nutrient treatment.

part due to exceptionally poor larval growth on genotype S3. Overall, larvae grew more quickly on trees with high compared with low nutrient availability. Again, this was particularly true for genotypes P3, S1, W1, and W2.

Larval host preferences

In each of the four pairwise preference comparisons, fourth-stadium larvae showed striking preferences (at least 8:1) between aspen genotypes (Fig. 2). Larvae offered a choice between genotypes D1 and S3 consumed almost 40% less combined leaf mass than those in other experimental genotype combinations ($P = 0.009$). Assuming that the lower combined consumption in those comparisons can be interpreted as poorer food quality of genotype D1 relative to other preferred genotypes, we infer relative preferences as follows: P3, W1, W2 > D1 > D2, S3.

Genotype and environment effects on foliar chemistry

Mean concentrations of secondary metabolites and nitrogen varied by plant genotype and nutrient availability (Fig. 3A–D). Salicortin and tremulacin concentrations varied fourfold and sevenfold, respectively, among genotypes and were highly correlated with each other across treatments ($r = 0.96$). Concentrations of phenolic glycosides responded differently to nutrient availability depending on aspen genotype (genotype × nutrient interaction) and, overall, were much less variable across nutrient levels than were concentrations of tannins. Tannin levels varied almost fourfold among genotypes. Concentrations decreased with increased nutrient availability, and the magnitude of response differed among genotypes (genotype × nutrient interaction). Nitrogen concentrations were much less variable among genotypes than were phenolic glycosides and tannins and increased significantly in fertilized trees.

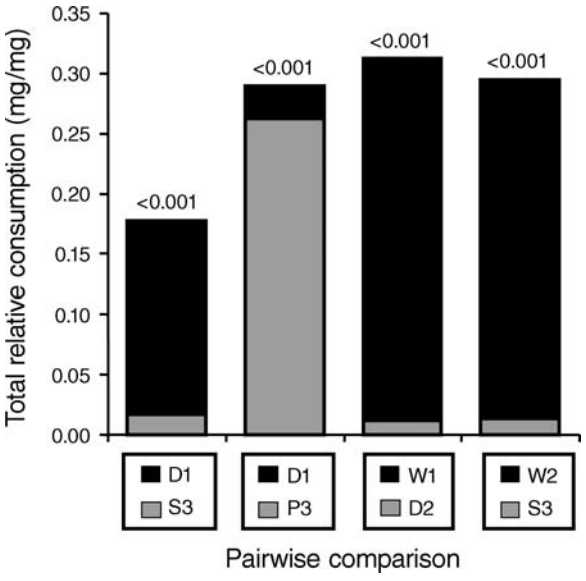

FIG. 2. Fourth-stadium larvae showed strong feeding preferences in laboratory pairwise comparisons. Only high-nutrient treatment trees were assayed. Bar heights are mean total mass consumed relative to initial larval mass in each of the four preference tests. Gray and black portions of bars are mean masses consumed of individual aspen genotypes within the respective preference tests. Numbers above bars are P values from paired t tests ($n = 8$ replicates). Genotypes are from clones growing in Dane (D1, D2), Columbia (P3), Sauk (S3), and Waushara (W1, W2) counties in south-central Wisconsin, USA.

Clonal repeatability

From an evolutionary perspective, an assessment of chemical defense benefits requires that the underlying genetic basis for variation in these traits be identified and correlated with levels of damage. The effect of genotype was highly significant in ANOVAs, and estimates of clonal repeatability (i.e., broad-sense heritability) were high for all response variables (Table 1). The genetic component of variation in larval distributions and defoliation rates and foliar nitrogen levels was greater under high than under low nutrient availability. This pattern was reversed and slightly less pronounced for tannins. Plant genotype explained >90% of phenotypic variance in levels of salicortin and tremulacin in both low- and high-nutrient treatments.

Regression analyses

Aspen phytochemistry explained a significant portion of the variation in gypsy moth distributions, defoliation levels, and larval RGR among treatments (Table 2). Gypsy moth final distributions were positively related to condensed tannins and nitrogen concentrations in both low- and high-fertility treatments and negatively related to phenolic glycoside concentrations in the high-fertility treatment. At low fertility, none of the phytochemical variables was more important than others in determining larval distributions. At high fertility, condensed tannins and phenolic glycosides explained significantly more of the variation than did nitrogen. Phenolic glycosides accounted for all of the explained variation in defoliation. However, the relationship was much stronger at high compared with low nutrient availability (41% vs. 9%, respectively; Table 2). For trees growing under low nutrient availability, quantitative variation in phytochemicals explained <10% of the variation in defoliation. Larval RGRs were strongly and negatively related to concentrations of phenolic glycosides (Fig. 4). Phenolic glycoside concentration (together with nitrogen in the low-nutrient treatment) explained 52% and 64% of the variation in RGRs, at low and high nutrient availability, respectively (Fig. 4; high-nutrient treatment shown).

DISCUSSION

One of the primary objectives of this study was to identify benefits of putative chemical defenses subject to selection in a genetically variable plant species. The results provide convincing evidence for such a benefit by explicitly linking a genetically based chemical trait (phenolic glycoside concentrations) to genetically based variation in levels of herbivory. Previous work (e.g., Hemming and Lindroth 1995, Osier et al. 2000) has shown that aspen allelochemicals strongly affect insect performance, but until now, their defensive role has been largely assumed. In this study, free-ranging gypsy moth larvae accepted hosts selectively and defoliated at markedly different rates among aspen genotypes and between nutrient treatments. Quantitative variation in plant chemistry explained ~50% of the variation in final larval distributions and levels of defoliation among genotypes growing with high nutrient availability. While plant chemistry also had a statistically significant effect in low-nutrient trees, the correlation with larval distributions and defoliation was considerably weaker.

As in previous work with aspen (Hemming and Lindroth 1995, Osier et al. 2000, Osier and Lindroth 2001, Hale et al. 2005), phenolic glycosides were the most influential phytochemical variable explaining larval performance (RGR). Moreover, multivariate regression analyses as well as the results from complementary preference assays provided strong evidence that high concentrations of phenolic glycosides protect trees from defoliation. Among-genotype differences in concentrations of these compounds are strongly genetically based. Although clonal repeatability values may be inflated by nongenetic factors (maternal environmental effects), they nonetheless indicate a high selection potential for phenolic glycoside levels. Combined with the marked biological effects of these compounds (both for the plant and against herbivores), these data suggest that phenolic glycosides are an evolutionarily relevant defense in aspen.

Condensed tannin concentrations were also highly variable among genotypes. Accumulating evidence, however, suggests that these compounds have limited

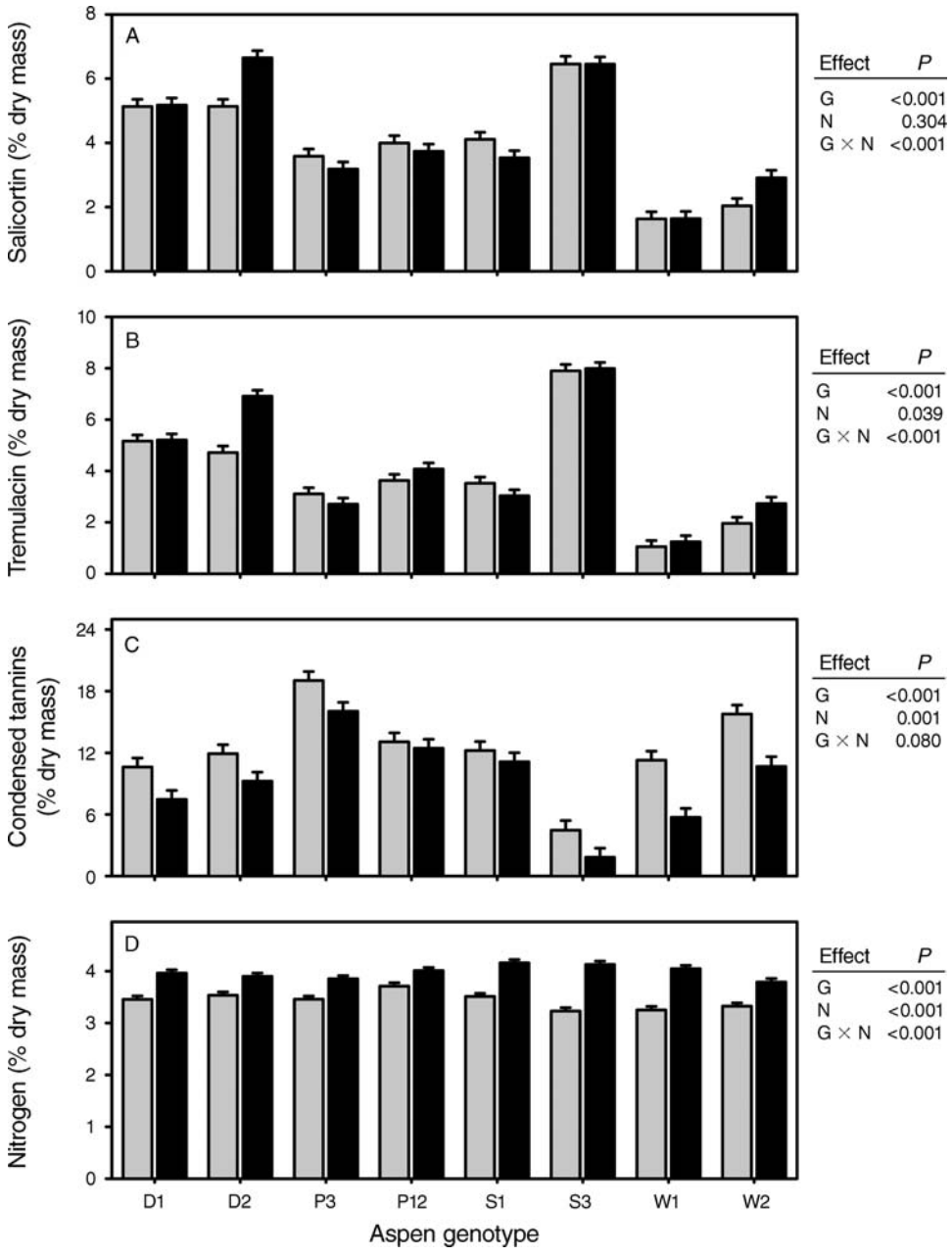

FIG. 3. Aspen leaf chemistry varied among genotypes and nutrient treatments. Bar heights are means of initial and final phytochemical concentrations (taken during the first and fifth larval stadia, respectively) + 1 SE (least-squares means, pooled error); $n = 8$ replicate trees. The gray bars represent the low-nutrient treatment; black bars represent the high-nutrient treatment.

activity against herbivorous insects (Schultz 1989, Lindroth and Hwang 1996*a*, Ayres et al. 1997, Osier et al. 2000, Osier and Lindroth 2001, Close and McArthur 2002). Further, several studies have found that gypsy moth larvae show a compensatory feeding response when reared on aspen containing high concentrations of tannins (Osier et al. 2000, Osier and Lindroth 2001). Here, condensed tannin levels were positively correlated with larval distributions and were not related to defoliation rates and larval RGR. However, given what we know about the biological activity of tannins in this system, the positive correlation of tannin levels with larval distributions was more likely a consequence of a strong negative correlation between tannin and phenolic glycoside concentrations ($r = -0.60$ and -0.34 at low and high nutrient availability, respectively) than a positive effect of tannins.

Nutrient availability had substantial direct and indirect effects on plant chemistry and plant–insect interactions in this study. The plant vigor hypothesis

TABLE 1. The genetic component of phenotypic variance (clonal repeatability) under low- and high-nutrient treatments.

Variable†	Nutrient treatment: Low			Nutrient treatment: High		
	Clonal repeatability‡	*F*	*P*	Clonal repeatability‡	*F*	*P*
Larval distribution	0.303	6.23	<0.001	0.470	11.62	<0.001
Defoliation	0.152	3.15	0.011	0.427	9.93	<0.001
Relative growth rate	0.542	15.19	<0.001	0.621	20.68	<0.001
Salicortin	0.850	69.14	<0.001	0.832	60.31	<0.001
Tremulacin	0.900	109.10	<0.001	0.839	63.57	<0.001
Condensed tannins	0.641	22.45	<0.001	0.509	13.42	<0.001
Nitrogen	0.121	2.65	0.025	0.302	6.20	<0.001

Note: *F* and *P* values are from one-way ANOVAs with df = 7, 35.

† Phytochemical variables (salicortin, tremulacin, condensed tannins, nitrogen) refer to means of initial and final chemistry collections.

‡ Calculated as in Falconer (1989).

(Price 1991) suggests that herbivores tend to favor more vigorous individuals in a population. Our data indicate that such is the case for gypsy moths feeding on aspen. Changing soil nutrient availability resulted in corresponding changes in leaf nitrogen concentrations, which had a marked effect on larval RGR and defoliation rates. On average, high-nutrient trees suffered 1.7 times the defoliation rate of low-nutrient trees. Previous work has shown that artificial diets high in nitrogen improve larval performance (Lindroth et al. 1997, Hemming and Lindroth 1999). Our data indicate that larvae both grow faster and prefer high-nutrient trees, leading to increased rates of defoliation relative to nutrient-limited trees.

Plant nutrient status mediated the ecological effect of chemical defense. The explanatory power of phenolic glycosides for defoliation levels was more than four times greater in high- compared with low-nutrient trees. Nevertheless, the effect of these compounds on larval growth rates was considerable in both treatments. Thus, larval performance was a reliable predictor of plant protection (i.e., decreased defoliation) only when resources were not limiting. This pattern is likely due to the fact that low levels of foliar N in low-nutrient trees led to overall depressed larval growth, and, subsequently, to small variance in defoliation rates. In contrast, high-nutrient trees afforded the potential for optimal insect growth. On more heavily defended genotypes this potential was not met, and, hence, defoliation was more variable in the high-nutrient treatment. These patterns suggest that nutrient availability may set the general boundaries of insect performance, within which secondary compounds exert their effects (Haukioja 2003).

To quantify fitness benefits of defense, measurements of plant growth and/or reproduction are necessary. For a clonal species such as aspen, for which vegetative propagation is important, growth is an appropriate measure of plant fitness (Perala 1990). Defoliation markedly reduces aspen growth both in the field

TABLE 2. Phytochemical variables accounting for differences in larval distribution, defoliation, and larval relative growth rate.

Parameter and nutrient level	Multiple regression: Model	R^2	*P*	Partial regression components†: Variable	Partial R^2	*P*
Larval distribution						
Low	$Y = -0.027 + 0.011$(mean N)	0.355	<0.001	mean CT	0.122	0.010
	$+ 0.001$(mean CT) $- 0.001$(mean PG)			mean N	0.115	0.012
				mean PG	0.068	0.068
High	$Y = -0.033 + 0.013$(mean N)	0.450	<0.001	mean CT	0.223	<0.001
	$+ 0.001$(mean CT) $- 0.001$(mean PG)			mean PG	0.214	<0.001
				mean N	0.089	0.019
Defoliation						
Low	$Y = 36.56 - 1.10$(mean PG)	0.094	0.015	mean PG	0.094	0.015
High	$Y = 68.41 - 3.08$(mean PG)	0.414	<0.001	mean PG	0.414	<0.001
Larval RGR						
Low	$Y = 0.064 + 0.057$(N) $- 0.007$(PG)	0.518	<0.001	PG	0.513	<0.001
				N	0.072	0.072
High	$Y = 0.301 - 0.012$(PG)	0.639	<0.001	PG	0.639	<0.001

Notes: Stepwise multiple regressions, backward elimination with $\alpha = 0.10$ as the criterion for inclusion in the model. Abbreviations are: mean, mean of initial and final chemistry (see *Methods*); N, final total leaf nitrogen; CT, final condensed tannins; PG, final total phenolic glycosides.

† Partial R^2 should be interpreted with caution as CT and PG concentrations were intercorrelated (see *Statistical analyses*).

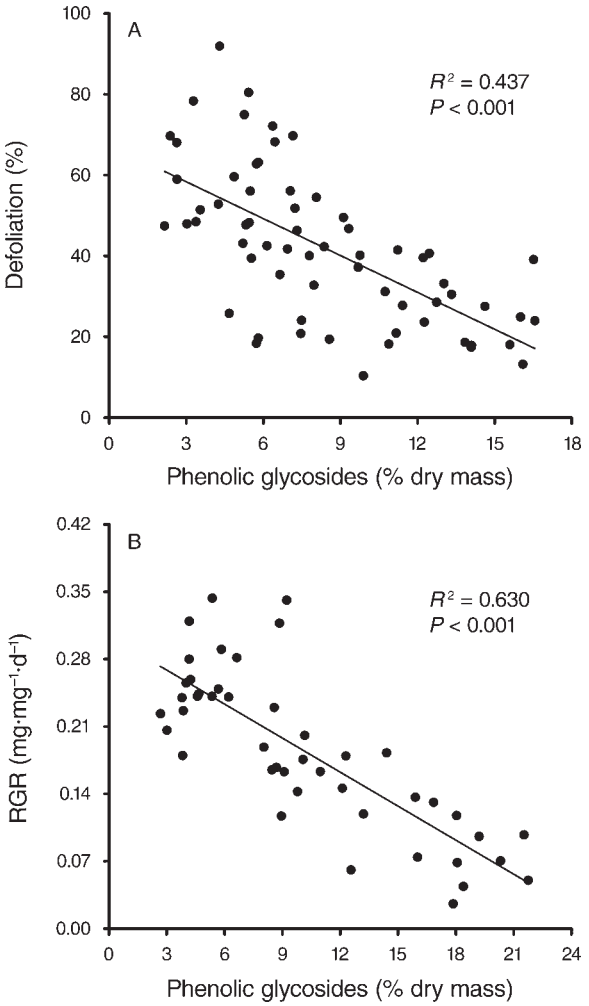

FIG. 4. Concentrations of phenolic glycosides explained much of the variability in (A) defoliation and (B) larval relative growth rate (RGR). Phenolic glycoside concentrations are means of initial and final concentrations in plot A and final concentrations in plot B (see *Methods*). Only high-nutrient trees are included. Points represent individual trees.

(Hodson 1941, Duncan and Hodson 1958) and in common gardens (Stevens 2005). Although growth and reproduction were not measured here, considerable evidence suggests that the influence of phenolic glycosides on defoliation was sufficient to have significant fitness consequences. In fact, in a parallel study using trees of the same genotypes and age as used in this study, Stevens (2005) demonstrated that defoliation (75%) not only markedly decreases growth, but also leads to significantly delayed flowering in aspen saplings.

The highly variable and genetically based allocation to phenolic glycosides in aspen (Lindroth and Hwang 1996*a*) may reflect trade-offs between defense and growth. Many studies have assessed the role of costs in maintaining genetic variation in chemical defenses (Koricheva 2002, Strauss et al. 2002), and recent studies (Donaldson et al. 2006*a*, Osier and Lindroth 2006) reveal a significant cost of production of phenolic glycosides in aspen. However, for genetic variation to be maintained in a plant population, costs and benefits must vary spatially or temporally (Louda 1982). Costs may be exacerbated when plants are stressed by resource limitation or competition (Weis and Hochberg 2000, Marak et al. 2003, Prittinen et al. 2003, Siemens et al. 2003, Donaldson et al. 2006*a*). Similarly, benefits of defense may occur only when the risk of herbivory is high (e.g., during outbreaks). Thus, the selective advantage of genotypes with high concentrations of defense chemicals will likely be realized infrequently. In such a scenario the long-term effects of natural selection favor genetic diversity of defense traits within a population (Louda 1982, Adler and Kittelson 2004). Edaphic heterogeneity can significantly affect herbivory pressure (Boege and Dirzo 2004), but very little is known about how environment or genotype × environment interactions influence the relative benefits of phytochemical defenses in plants. As discussed previously, resource availability was important in this study and suggests that the selective potential (i.e., benefits) of plant chemical defense may vary across spatially and temporally heterogeneous environments.

Field research shows that, during outbreaks of defoliating lepidopterans, populations can overwhelm aspen chemical defenses, leading to complete defoliation irrespective of phenolic glycoside levels (Donaldson 2005). During such outbreaks, trees may resort to tolerance as a defense mechanism (Mattson et al. 1991, Stevens 2005). Thus, aspen phenolic glycosides may be most important against aspen-adapted insects during incipient stages of outbreaks.

In conclusion, phenolic glycosides appear to be both ecologically and evolutionarily relevant defenses in aspen. Genetically based variation in phenolic glycoside concentrations explains genetically based variation in levels of defoliation. In addition, our results suggest that environmental heterogeneity (nutrient availability) can markedly influence the realized benefits of defense, thereby demonstrating an additional mechanism by which genetic polymorphisms in chemical defenses may be maintained in plant populations.

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