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Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency

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Abstract Plants defend themselves against herbivores via resistance, which reduces damage, and tolerance, which minimizes the negative effects of damage. Theory predicts the existence of tradeoffs between defense and growth, as well as between resistance and tolerance, that could maintain the genetic variation for resistance and tolerance often observed in plant populations. We examined resistance and tolerance among aspen (*Populus tremuloides*) trees grown under divergent soil nutrient regimes. This common garden experiment revealed substantial genetic variation for resistance and tolerance under both low- and high-nutrient conditions. Costs of resistance exist, particularly under high-nutrient conditions where allocation to resistance chemicals competes directly with growth for limited carbon resources. We found no significant costs of tolerance, however, under either nutrient condition. Despite genetic variation for both resistance and tolerance, we found no evidence for a tradeoff between these two defense traits suggesting that resistance and tolerance are complementary, rather than mutually exclusive, defenses in aspen.

Keywords Aspen · Compensation · Defoliation · Growth · Herbivory · Plant defense · Woody plant

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Introduction

Resistance and tolerance are two principal forms of plant defense against herbivores. Plant resistance reduces herbivore damage, while tolerance mitigates the negative effects of damage. These defenses may have attendant costs in the presence or absence of herbivores. In the presence of herbivores, the benefits of defense may outweigh the costs, as evidenced by greater plant fitness. In the absence of herbivores, however, only the costs and not the benefits of a defense are observed (Tiffin 2002). When costs outweigh benefits, more heavily defended plants are selected against. This cost–benefit dynamic may explain why genetic variation for defense (resistance and tolerance) commonly occurs in plant populations (Fritz and Simms 1992; Strauss and Agrawal 1999).

The costs of resistance and tolerance may also vary in response to differences in environmental conditions such as soil nutrient availability, herbivore damage, and intra- or interspecific plant competition (Prittinen et al. 2003; Fornoni et al. 2004b). Considerable experimental work has addressed how environmental conditions affect the costs of *resistance* (Bergelson and Purrington 1996; Koricheva 2002). Some theories on costs of resistance predict higher costs under more stressful conditions such as competition or nutrient-limitation (Rhoades 1979; Gulmon and Mooney 1986; Zangerl and Bazzaz 1992). Other theories, however, predict lower costs of resistance in resource-limited environments, where ''excess carbon'' can be allocated to defense (Bryant et al. 1983; Herms and Mattson 1992). Bergelson and Purrington's (1996) extensive review of costs of resistance in herbaceous plants revealed no consistent patterns in environmental effects on costs of resistance. In woody plants, costs of resistance have been reported to increase under both low (Mutikainen et al. 2002) and high soil nutrient conditions (Prittinen et al. 2003). Koricheva's (2002) meta-analysis of costs in both herbaceous and woody plants found that costs of resistance are more often present under high-nutrient conditions. This could reflect the greater variation in growth rates expected under favorable conditions, amplifying the statistical power for detecting costs.

In contrast to resistance, experiments examining how the environment affects the costs of *tolerance* are limited. Several studies have revealed costs of tolerance under stressful conditions, e.g., low-nutrient conditions in *Asclepias* (Hochwender et al. 2000), multiple types of herbivore damage in *Ipomoea* (Stinchcombe 2002), and hot and dry environments in *Datura* (Fornoni et al. 2004b). In contrast, Siemens et al. (2003) observed costs of tolerance in *Arabis* only under favorable (non-competitive) conditions.

Because resistance and tolerance both defend against herbivores, plants have been predicted to be either resistant or tolerant, but not both (van der Meijden et al. 1988). This prediction assumes that highly resistant plants are not likely to receive damage and thus will not benefit from tolerance. By the same token, highly tolerant plants suffer little from herbivore damage and thus do not benefit from resistance. Although this prediction is intuitively appealing, several studies report no detectable tradeoffs between resistance and tolerance (Mauricio et al. 1997; Shen and Bach 1997; Tiffin and Rausher 1999; Weinig et al. 2003; Puustinen et al. 2004).

Although resistance and tolerance can both benefit plants, they follow different evolutionary trajectories. In their analysis of pathogen systems, Roy and Kirchner (2000) explained that genes providing complete resistance cannot become fixed in a

population because as the frequency of resistance genes increases, the incidence of disease declines. In contrast, as the frequency of tolerance genes increases in a population, the incidence of disease increases, driving the population to fixation for tolerance. However, several models indicate that under certain conditions intermediate levels of both resistance and tolerance can be maintained (Tiffin 2000; Fornoni et al. 2004a).

The majority of resistance and tolerance studies conducted to date evaluate defenses in herbaceous annuals or short-lived perennials. Because these two groups of plants differ dramatically from trees in terms of both growth form and longevity, the findings of these studies may not apply to forest trees (Haukioja and Koricheva 2000). Here, we report research into the genetic basis for resistance and tolerance in aspen (*Populus tremuloides*), a long-lived woody species. Studies of resistance and tolerance are relevant for aspen as it is subject to attack by native outbreak folivores such as the forest tent caterpillar (FTC) (*Malacosoma disstria*) and large aspen tortrix (*Choristoneura conflictana*) (Mattson et al. 1991). These and other herbivores have surely shaped the expression of aspen defenses, including resistance and tolerance, over evolutionary time.

With regard to potential resistance traits, aspen produces large quantities of allelochemicals, particularly phenolic glycosides and condensed tannins. Aspen phenolic glycosides reduce the performance of numerous insect species (Hemming and Lindroth 1995, 2000; Hwang and Lindroth 1997, 1998; Osier and Lindroth 2001). Fewer studies support the role of condensed tannins as anti-insect compounds (Ayres et al. 1997), but they do reduce the preference of mammalian herbivores for *Populus* (Bailey et al. 2004). We therefore refer to both condensed tannins and phenolic glycosides as resistance traits, or simply as resistance.

Aspen should tolerate as well as resist herbivory for several reasons. First, its rapid growth rate facilitates compensatory growth after damage. Second, the clonal nature of aspen may enhance tolerance by providing large amounts of stored resources in stems and roots that could be translocated to allow regrowth after damage (Stevens 2005). Finally, aspen clones experience periodic insect outbreaks that often result in nearly 100% defoliation. Under such conditions, strong selection for tolerance is likely (Mattson et al. 1991).

Our goals were first to assess levels of genetic variation for resistance and tolerance in this long-lived woody species. To understand how variation in these defense traits is maintained, we also evaluated the costs of both resistance and tolerance as well as tradeoffs between the two traits. Finally, we assessed how soil nutrient availability affects the expression of genetic variation for, and costs of, resistance and tolerance.

Materials and methods

Experimental design

We established a common garden experiment on the campus of University of Wisconsin-Madison that provided two contrasting soil nutrient environments and two levels of defoliation (0 and 75%—details below). We planted clonal replicates of 12 aspen genotypes into this split-plot design. The soil nutrient and defoliation

treatments were crossed at the whole plot level with genotype as a sub-plot. The 48 treatment combinations were replicated across nine blocks for a total of 432 trees.

Genotypes

The aspen genotypes were micropropagated from root material field-collected from source trees growing in south-central Wisconsin. These genotypes represent a broad range of variation in growth and resistance. Micropropagation allowed us to replicate genotypes and minimize non-genetic effects from source individuals (analogous to maternal effects) (Wright 1976). Microsatellite analyses (C.T. Cole et al., unpublished data) confirmed that each genotype was unique.

Propagation

In the spring of 2001, the micropropagates were planted outside in 5-L pots containing a 40:40:20 mix of sand, silt-loam field soil, and perlite as well as Osmocote 3–4 month slow release fertilizer (14:14:14, N–P–K + micronutrients; 4.5 g/L of soil). In spring 2002, we transplanted the trees (average height = 1.1 m) into 80-L pots that were arranged in experimental blocks. Each pot contained a mixture of 70% sand and 30% silt-loam field soil. To the high-nutrient pots we added Osmocote 8–9 month slow release fertilizer (18:6:12, N–P–K + micronutrients) at a rate of 4.5 g/L in the spring of 2002 and 2003; low-nutrient pots received no fertilizer.

Defoliation

To mimic the intensity and duration of an insect outbreak (Mattson et al. 1991; Parry et al. 2003), we severely defoliated experimental trees in two successive years. The defoliation treatment employed both FTCs and scissors. We used insects to elicit more natural responses to defoliation (Karban and Baldwin 1997; Havill and Raffa 1999), and scissors to ensure that each individual (and thus each genotype) received the same level of damage regardless of its genetic level of resistance (Stowe et al. 2000; Siemens et al. 2003). FTCs were selected as the insect defoliator because they are outbreak, generalist herbivores that often consume aspen.

    In both 2002 and 2003, a subset of branches on each tree in the defoliation treatment was bagged along with 5–20 third-instar FTC that had been reared in the laboratory on an aspen leaf diet. The actual number of caterpillars used was based on the number of branches (2002) or tree size (d^2h) [(diameter^2) × (height)] (2003) to scale the application of caterpillars proportionately to each tree. In both years, the FTCs were allowed to feed for 10 days and then were removed. The FTC provided the saliva, frass, and other signals that may be necessary to trigger herbivore-induced chemical resistance and compensatory growth (Karban and Baldwin 1997; Havill and Raffa 1999). We used scissors to remove 75% of each leaf by cutting near each leaf base. Scissor defoliation in 2002 began on June 10th and was completed in 4 days. In 2003, the process began on June 2nd and took 9 days to complete. Background levels of natural herbivory at our common garden were very low (<2% leaf area loss).

Growth and fitness

We assessed growth by measuring increments in the diameter (d) and height (h) of each tree. Diameter was measured 1 cm above the soil surface, and height was measured from the soil surface to the apical meristem. All nine replicate blocks of trees were measured initially in June 2002 and finally in August 2003 after the two summers of defoliation. We used relative growth as the response variable for assessments of tolerance. Relative growth was calculated as [ln(final d^2h) - ln(initial d^2h)]. Three respective harvests, initial (June 2002), interim (June 2003), and final (August 2003), of four replicate trees per treatment combination revealed that d^2h is highly correlated with total biomass in both low- ($r^2 = 0.717, 0.508, 0.706$; all $P < 0.001$) and high- ($r^2 = 0.633, 0.560, 0.489$; all $P < 0.001$) nutrient environments. Such measurements of d^2h have been widely used as an index of growth in other studies of *Populus* (Abrahamson et al. 1990; Robison and Raffa 1994).

Growth is a major component of fitness in long-lived plants (Simms 1992; Strauss and Agrawal 1999). This is especially true for aspen, which exhibits clonal growth. Because increasing plant size usually increases both survival, especially during early stages of growth, and reproduction, biomass is a good index of fitness (Arendt 1997). In addition, long-lived plants often delay reproduction until they have compensated for herbivore damage. Thus, we can more easily observe tolerance in woody plants in terms of growth rather than reproductive output (Dangerfield and Modukanele 1996; Haukioja and Koricheva 2000).

Chemical analyses

To assess levels of chemical resistance (i.e., phenolic glycosides and condensed tannins), and nutritional quality (i.e., nitrogen, an index of protein), we collected leaves in mid-June 2002 and mid-June 2003, 1 week after defoliating the trees in 2002 and 2003. From control trees we haphazardly collected 10–15 undamaged leaves from throughout the crown. We followed the same protocol for the damaged trees except we collected 10–15 leaf remnants. To preserve leaf chemistry, samples were kept under ice in the field and then flash-frozen in liquid N_2 and freeze-dried in the laboratory (Lindroth and Koss 1996). We used high-performance thin layer chromatography and purified aspen phenolic glycoside standards to quantify levels of the phenolic glycosides salicin, salicortin, tremuloidin, and tremulacin (Lindroth et al. 1993). Initial analyses across genotypes revealed very low levels of salicin and tremuloidin, so we report levels of only salicortin and tremulacin. These two phenolic glycosides are typically present at higher concentrations and are more biologically active than are salicin and tremuloidin (Lindroth and Hemming 1990). Condensed tannins were extracted from leaf tissue with 70% acetone at 4°C and quantified using the acid butanol method of Porter et al. (1986) and purified aspen tannin standards. We used a LECO elemental analyzer (St Joseph, MI, USA) to assess nitrogen levels; glycine *p*-toluenesulfonic acid (N = 5.665%) served as a standard.

In 2003, we collected leaves from four of the nine experimental blocks, as four replicates per treatment combination provided sufficient power to reveal effects of nutrients, defoliation, genotype, and their interactions, on levels of leaf chemicals examined in 2002 (Stevens and Lindroth 2005). Levels of allelochemicals were generally consistent between 2002 and 2003. We used an additional eight saplings

per treatment (four destructively harvested in each of 2002 and 2003) to assess total crown allocation to leaves. Because Koricheva (1999) argued for using absolute content per plant or plant part in studies of defense allocation, we determined the total foliar crown content of allelochemicals by multiplying mean leaf concentration by mean foliar crown biomass for each treatment combination, using data from both 2002 and 2003. Using the means of data collected in 2002 and 2003 allowed for the most complete assessment of allocation to resistance across the duration of our multi-year defoliation study. Finally, we used genotype means within treatment combinations to assess tradeoffs between resistance and other plant traits.

Statistical analyses

Genetic variation for foliar resistance and nutritional quality

To focus on genetic differences in phytochemicals and variable genotypic responses to defoliation within environments, we analyzed data from low- and high-nutrient environments separately. We assessed the effects of defoliation, genotype, and their interaction, on concentrations of each phytochemical with a mixed-model, two-factor split-plot ANOVA using JMP IN Version 4.0.4 (SAS Institute Inc. 2001). We considered defoliation a fixed effect while genotype and block were considered random effects. We analyzed defoliation treatment as a whole plot effect and incorporated genotype as a sub-plot factor within the whole plot treatment. In the split-plot analysis, a whole plot error term (replicates within whole plot error) was used to test the whole plot effect (defoliation) while the random effect of genotype was tested over the interaction of defoliation and genotype. A split-plot error term (residual error) was used to test the defoliation × genotype interaction. We arcsin-square-root transformed phenolic glycoside data to reduce heteroscedasticity. The condensed tannin and nitrogen data displayed normality and uniform variances and were not transformed. Additionally, we ran a mixed-model, three-factor split-plot ANOVA (SAS Institute Inc. 2001) to examine possible three-way interactions between nutrients, defoliation, and genotype. In this analysis, nutrient treatment was added as a fixed effect. We also estimated broad-sense heritability by determining the proportion of total phenotypic variance explained by genotypic variance within each treatment combination (Falconer 1985; Stevens and Lindroth 2005).

Genetic variation for growth and tolerance

We used the same two-factor model described above to determine the effects of defoliation, genotype, and their interaction, on relative growth. We were particularly interested in how genotypes differ in their growth response to defoliation, as such an interaction reveals genetic variation for tolerance. For both low- and high-nutrient treatments, we used relative growth values. The growth data displayed normality and equal variances. Initial size (d^2h in June 2002) was used as a covariate for both nutrient conditions.

Costs of resistance

Resistance is any plant trait that reduces the preference or performance of herbivores. Because resistance confers a benefit only in the presence of herbivores, costs

(reductions in growth) will be most evident in the absence of herbivores. We therefore assessed costs of resistance by evaluating the correlation between constitutive resistance and growth in undamaged trees in both low- and high-nutrient environments. We determined levels of constitutive resistance in two ways, using (1) leaf concentrations and (2) total foliar crown contents of phenolic glycosides, condensed tannins, and total allelochemicals (phenolic glycosides + condensed tannins). To provide the best assessment of resistance over the course of the study, we averaged leaf concentrations from 2002 to 2003. Total foliar crown contents were determined by the product of mean leaf concentrations and mean foliar crown biomasses, also averaged from 2002 to 2003. Additionally, we used analysis of covariance (ANCOVA) (SAS Institute Inc. 2001) to assess costs and compare the slopes of correlations in the two nutrient environments.

Costs of tolerance

Tolerance is assessed by comparing the growth or fitness of damaged plants (D) to that of undamaged plants (U) of the same genotype (Strauss and Agrawal 1999; Stowe et al. 2000). Tolerance can be defined either as a ratio (D/U) or as a difference (D - U) (Strauss and Agrawal 1999). In studies such as ours, where damage is experimentally manipulated and is a categorical rather than a continuous variable, tolerance is typically defined as a difference (D - U) (Tiffin and Rausher 1999; Hochwender et al. 2000; Weinig et al. 2003).

Similar to resistance, tolerance confers a benefit only in the presence of damage, so in the absence of damage only costs will be seen. We assessed costs of tolerance by evaluating genetic correlations between tolerance and relative growth of undamaged trees in both low- and high-nutrient environments. We compared the slopes of the correlations in the two nutrient environments via ANCOVA (SAS Institute Inc. 2001). Because tolerance is a function of growth in both damaged and undamaged trees of the same genotype, a simple correlation between tolerance and growth of undamaged trees is biased by the artifactual covariance introduced by using the same plants to estimate tolerance and growth in the undamaged state (Tiffin and Rausher 1999; Weinig et al. 2003). Using methods described by Tiffin and Rausher (1999), and a SAS macro provided by John Stinchcombe (University of Toronto; Stinchcombe 2005), we calculated this artifactual covariance and subtracted it from the estimated covariance between tolerance and growth of undamaged trees to obtain a bias-free estimate of their covariance in the two nutrient environments. We then determined if the corrected covariance was significantly negative (indicating costs) using standard jackknifing techniques to generate a 95% confidence limit (Stinchcombe 2005). Significantly negative covariances have confidence limits that do not include 0 (Stinchcombe 2002; Weinig et al. 2003).

Resistance–tolerance tradeoffs

Pearson's correlation coefficients were calculated for the relationship between constitutive resistance and tolerance using levels of phenolic glycosides, condensed tannins, and their sum (total allelochemicals) assessed in undefoliated trees. Resistance was characterized with leaf concentrations and also with total foliar crown contents.

Results

Environmental and genetic effects on leaf chemistry: resistance and nutritional quality

Soil nutrient addition affected levels of phenolic glycosides, condensed tannins, and nitrogen in different ways. Nutrient addition had little effect on phenolic glycoside concentrations, while it decreased condensed tannin levels and increased nitrogen levels (Fig. 1; Table 1).

Defoliation decreased levels of phenolic glycosides and increased levels of condensed tannins (Fig. 1). The declines in phenolic glycosides (37 and 23%, in low- and high-nutrient environments, respectively) were similar to, or smaller than, the increases in condensed tannins (33 and 134%, respectively). For nitrogen, the direction of the response to defoliation depended on the soil nutrient environment. Defoliation increased levels of nitrogen under low-nutrient conditions (21%) and decreased levels of nitrogen under high-nutrient conditions (6%). The 12 genotypes differed greatly in their concentrations of phenolic glycosides and condensed

Fig. 1 Environmental and genetic effects on leaf chemistry and tree growth. Norm of reaction plots for foliar concentrations of phenolic glycosides, condensed tannins, nitrogen, and relative growth after two successive seasons of 75% defoliation. Relative growth was calculated as [ln(final d^2h) - ln(initial d^2h)] where d = diameter at base and h = height, for each tree. Each *line* represents the mean response (n = 4 replicates for leaf chemicals and n = 9 replicates for growth) of a single aspen genotype in the undefoliated versus defoliated condition under low and high soil nutrient conditions. Results from statistical analyses are provided in Table 1
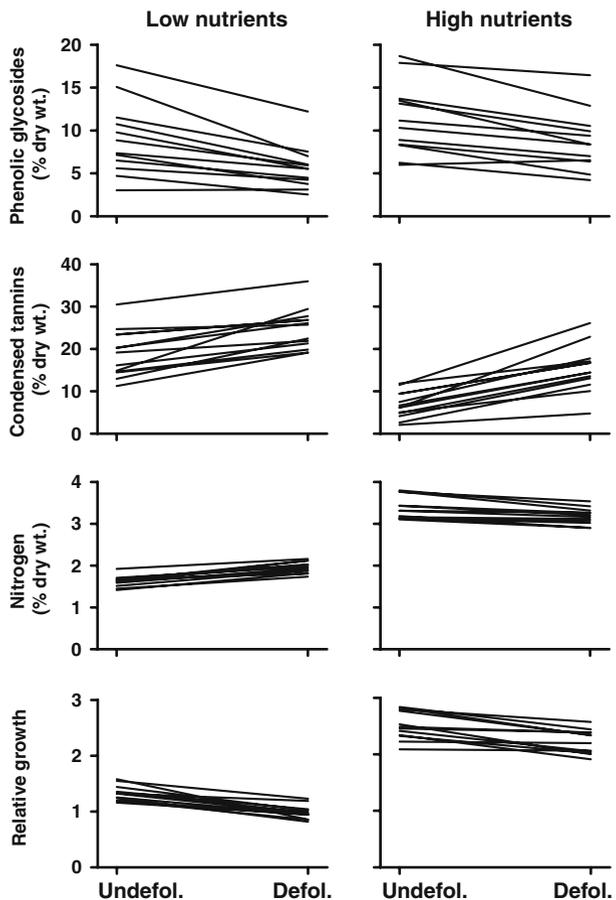
Table 1 Results of ANOVA used to test the effects of block, defoliation (D), genotype (G), and D × G interactions on foliar concentrations of phenolic glycosides, condensed tannins, and nitrogen in low and high soil nutrient environments

Source	Phenolic glycosides				Condensed tannins				Nitrogen				Relative growth			
	df	MS	F	P	df	MS	F	P	df	MS	F	P	df	MS	F	P
Low nutrients																
Block	3	0.010	10.6	<0.001	3	334.286	29.8	<0.001	3	5.388	177.4	<0.001	8	0.203	5.2	<0.001
Defoliation	1	0.095	41.7	<0.001	1	889.384	35.8	<0.001	1	2.698	119.5	<0.001	1	6.843	90.8	<0.001
Genotype	11	0.030	13.4	<0.001	11	201.603	8.1	<0.001	11	0.121	5.4	0.005	11	0.177	2.4	0.081
D × G	11	0.002	2.4	0.015	11	24.816	2.2	0.023	11	0.023	0.7	0.693	11	0.076	1.9	0.037
Initial size													1	1.089	27.8	<0.001
Error	69	0.001			69	11.207			69	0.030			175	0.039		
High nutrients																
Block	3	0.007	5.8	0.001	3	87.339	17.6	<0.001	3	2.080	63.1	<0.001	8	0.220	6.8	<0.001
Defoliation	1	0.045	33.1	<0.001	1	1,820.040	60.0	<0.001	1	0.914	26.9	<0.001	1	5.207	75.6	<0.001
Genotype	11	0.030	22.3	<0.001	11	135.760	4.5	0.010	11	0.399	11.7	<0.001	11	0.406	6.0	0.003
D × G	11	0.001	1.1	0.354	11	30.314	6.1	<0.001	11	0.034	1.0	0.430	11	0.069	2.1	0.020
Initial size													1	5.474	170.1	<0.001
Error	69	0.001			69	4.950			69	0.033			177	0.032		

ANCOVA was employed for analysis of relative growth data over the June 2002 to August 2003 interval, using the same statistical model but with initial size (diameter^2 × height; June 2002) added as a covariate (see Fig. 1)

tannins when grown under both low and high soil nutrient conditions (Fig. 1). Genotypes also varied significantly in nitrogen concentrations, although over a narrow range. When averaged over the four nutrient-defoliation treatment combinations, broad-sense heritability estimates for phenolic glycosides, condensed tannins, and nitrogen were 0.718, 0.584, and 0.168, respectively. We observed a genotype by defoliation interaction for phenolic glycosides under low-nutrient conditions (Table 1). Defoliation reduced levels proportionately more in genotypes with high initial concentrations than in genotypes with low initial concentrations. Such interactions for phenolic glycosides are best described as genetic variation in induced susceptibility (Karban and Baldwin 1997), as phenolic glycoside levels were actually lower among defoliated trees. Genotype by defoliation interactions for condensed tannins under both low- and high-nutrient conditions show that some genotypes responded more strongly to defoliation than did others. Such interactions reveal genetic variation in induced resistance for condensed tannins. Genotypes responded consistently to defoliation in terms of nitrogen concentrations ($D \times G$, Table 1).

We found no significant three-way interactions ($N \times D \times G$) for any of the phytochemicals when the effects of nutrients, defoliation, genotype, and their interactions were analyzed simultaneously. This finding indicates that patterns of genetic variation for induced susceptibility and resistance are consistent across this range of soil nutrient conditions.

Environmental and genetic effects on growth and tolerance

As expected, soil nutrient addition greatly increased growth, whereas defoliation decreased growth, in the 12 aspen genotypes (Fig. 1). Genotypic growth differences were evident in both low- and high-nutrient environments. Growth differences were limited under low-nutrient conditions, but more pronounced under higher nutrient conditions (Fig. 1). Broad-sense heritability estimates for growth also followed this pattern and were lower under low-nutrient conditions (0.197) and higher under high-nutrient conditions (0.367). Genotypes responded differently to defoliation under both nutrient conditions (significant defoliation by genotype interactions) (Table 1), indicating that aspen expresses genetic variation for tolerance to defoliation. The absence of significant three-way interactions for growth among nutrients, defoliation, and genotype demonstrates that patterns of genetic variation for tolerance are consistent across soil nutrient environments (as with genetic variation for resistance). Similarly, we found that the expression of tolerance did not differ across nutrient environments ($P = 0.369$).

Table 2 Results of ANCOVA used to test the effects of nutrient environment (N), allelochemistry (A), and their interaction on relative growth of undamaged trees using genotype means (see Fig. 2)

Source	df	MS	F	P
Nutrient	1	3.578	113.6	<0.001
Allelochemistry	1	0.210	6.7	0.018
N × A	1	0.079	2.5	0.130
Error	20	0.032		

Fig. 2 Costs of resistance under low- and high-nutrient conditions. Correlations between relative growth in undamaged trees and constitutive (undamaged) total allelochemical concentrations (phenolic glycosides + condensed tannins) averaged over 2 years for each aspen genotype. Each *point* represents the mean response (*n* = 9 replicates for growth and *n* = 4 replicates for total allelochemicals) of a single genotype. Results from statistical analyses are provided in Table 2
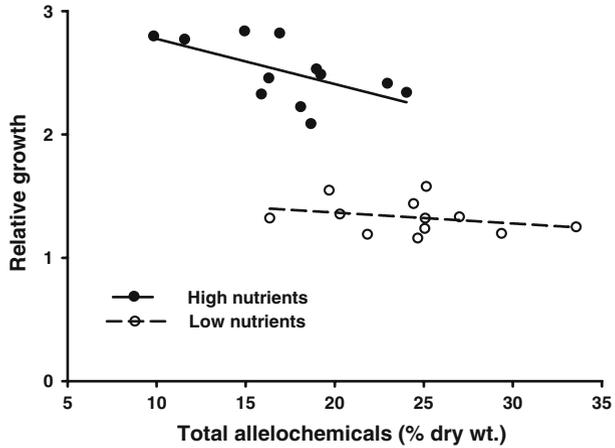

Fig. 3 Costs of tolerance under low- and high-nutrient conditions. Correlations between relative growth in undamaged trees and tolerance. Tolerance was calculated using relative growth (damaged–undamaged) of trees for each aspen genotype. Each *point* represents the mean growth response (*n* = 9 replicates) of a single genotype. Results from statistical analyses are provided in Table 3
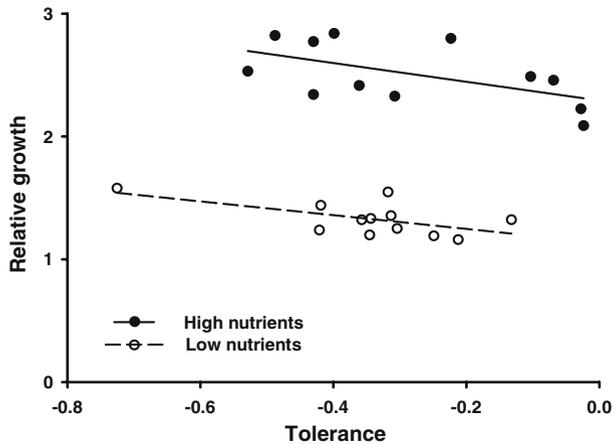
Costs of resistance

Analysis of covariance revealed that genotypes with higher concentrations of total allelochemicals grew more slowly in the absence of defoliation in both nutrient environments, reflecting costs of resistance (Table 2). ANCOVA also indicated that costs did not differ significantly in the two nutrient environments (no

Table 3 The relationship between relative growth in undamaged trees and tolerance was assessed separately in low and high soil nutrient environments using correlation analysis and adjusted covariances

Soil nutrient environment	Correlation coefficient	Adjusted covariance	95% confidence limits	Slope
Low	−0.599	−0.005	(−0.029, 0.010)	−0.560
High	−0.565	−0.017	(−0.054, 0.021)	−0.762

The adjusted covariances were jackknifed to determine their 95% confidence limits (see Fig. 3)

nutrient × allelochemistry interaction) (Table 2; Fig. 2). However, when we assessed costs in the two nutrient environments separately, we found significant costs under only high-nutrient conditions (low nutrient, $r = -0.296$, $P = 0.351$; high nutrient, $r = -0.597$, $P = 0.041$). We found comparable relationships when growth was related to total foliar crown contents rather than concentrations (low nutrient, $r = -0.464$, $P = 0.129$; high nutrient, $r = -0.567$, $P = 0.055$). The higher apparent costs under high-nutrient conditions could reflect the fact that favorable growing conditions increase both the range of growth and the selective pressures of herbivores. Alternatively, the greater genotypic variance that accompanies higher growth in the nutrient-rich environment could simply make it statistically easier to detect the costs of resistance.

Costs of tolerance

More tolerant genotypes tended to grow more slowly in the absence of damage under both low- and high-nutrient conditions (Fig. 3). However, these relationships appear to reflect the artifactual covariance between tolerance and growth of undamaged trees as the 95% confidence limits for the corrected covariances include 0 (Table 3). An ANCOVA showed that the relationship between tolerance and growth of undamaged trees is similar in the two nutrient environments as the slopes of the two lines do not differ ($P = 0.662$).

Resistance–tolerance tradeoffs

We examined tradeoffs between constitutive (undamaged) levels of resistance and tolerance in both nutrient environments. Resistance values were considered in terms of both the sum total (''total allelochemicals'') as well as separate levels of phenolic glycosides and condensed tannins. We evaluated tradeoffs using both concentrations and total foliar crown contents as metrics for resistance. We found no evidence for resistance–tolerance tradeoffs for constitutive levels of total allelochemicals (assessed as either concentrations or contents) under either soil nutrient treatment (low nutrient concentration, $r = -0.296$, $P = 0.350$; low nutrient content, $r = -0.196$, $P = 0.542$; high nutrient concentration, $r = -0.045$, $P = 0.890$; high nutrient content, $r = -0.006$, $P = 0.984$). Similarly, we found no resistance–tolerance tradeoffs when resistance was classified as either concentration or total foliar crown contents of phenolic glycosides and condensed tannins or when we assessed resistance using the product of concentration and leaf mass ratio. Although our ability to detect resistance–tolerance tradeoffs with 12 genotypes may be limited, retrospective power analyses revealed that negative correlations between these two defense traits in aspen are subtle, and if they truly existed, would require >100 genotypes to achieve statistical significance.

Discussion

Resistance

This study demonstrates that aspen genotypes differ substantially in their constitutive levels of resistance and nutritional quality across a range of environmental

conditions. This variation is substantially genetic, as revealed by the high broad-sense heritabilities, but also responsive to environmental conditions. Theory predicts that if costs (and benefits) of resistance vary over time and space, plant populations should maintain genetic variation for resistance as observed in many plant species (Strauss and Agrawal 1999).

In aspen, costs of resistance have been documented under a variety of conditions. Osier and Lindroth (2006) reported tradeoffs between growth and phenolic glycoside concentrations among genotypes in light- and nutrient-limited environments but not in resource-rich environments. In contrast, this study found costs of resistance to be most apparent under favorable nutrient conditions. These divergent findings may partly reflect differences in plant age. Osier and Lindroth (2006) used younger saplings than those evaluated in the present study. Donaldson et al. (2006) found levels of phenolic glycosides to decrease sharply with ramet age in wild populations, while levels of condensed tannins increased with age. Similar but less extreme patterns have been observed in experiments with single ramets (R.L. Lindroth, unpublished data). Costs of phenolic glycosides may be greatest in very young trees when phenolic glycoside concentrations are high. As trees age, however, costs of resistance may be better represented by both phenolic glycosides and tannins. Likewise, ontogenetic shifts in growth rates and nutrient-use efficiency may explain why very young aspen express the strongest costs under low-nutrient conditions and older trees show the greatest costs under high-nutrient conditions. Hwang and Lindroth (1997) also observed a tradeoff between growth and total allelochemical concentrations under favorable soil nutrient conditions in aspen.

Costs of resistance are often modulated by the environment (Purrington 2000; Koricheva 2002), but theories offer conflicting predictions regarding how environmental differences should affect costs. Some theories predict higher costs under more stressful conditions (Rhoades 1979; Gulmon and Mooney 1986; Zangerl and Bazzaz 1992), while others predict lower costs of resistance in certain stressful environments (Bryant et al. 1983; Herms and Mattson 1992). Experimental evidence is mixed as well, with some supporting the defense-stress cost hypothesis and others refuting it (Bergelson and Purrington 1996; Koricheva 2002). However, a meta-analysis of 70 studies (Koricheva 2002) revealed that, contrary to many predictions, costs are more frequently expressed under high-nutrient conditions.

Costs may be more apparent under resource-rich conditions for physiological, evolutionary, and statistical reasons. Physiologically, resource limitations that reduce plant growth more than photosynthesis can result in "excess carbon" that may be used to construct carbon-based resistance chemicals (e.g., phenolic glycosides and condensed tannins) at little cost to growth (Bryant et al. 1983; Herms and Mattson 1992). However, under favorable conditions, maximum growth may require all available carbon, so genetically determined allocation to constitutive levels of resistance may compete directly with growth. Evolutionarily, costs are predicted to reflect the intensity of selection (Bergelson and Purrington 1996). Selection pressures (and costs) may increase under high-nutrient conditions for several reasons. In this study, nutrient addition increased growth in undamaged trees by 89%, so the potential gains and losses in growth are compounded under favorable conditions. Likewise, Donaldson (2005) found that the overall damage by gypsy moth larvae was much greater in high-nutrient versus low-nutrient trees and may reflect the important interplay between resistance and the nutritional quality of leaves. In terms of reproduction, we found that defoliation greatly decreased catkin production among

high-nutrient trees, while low-nutrient trees produced no catkins regardless of their defoliation status (M.T. Stevens et al., unpublished data). Thus, selection for resistance may increase under favorable growing conditions, which could make costs easier to detect. Finally, we also observed that high-nutrient conditions increased growth in a way that also increased genotypic variability in growth. Such effects make it statistically easier to detect costs as well.

In addition to finding genetic variation for resistance, we observed genetic variation for how aspen responds chemically to defoliation (defoliation by genotype interactions). Phenolic glycoside concentrations decreased in defoliated, low-nutrient trees, revealing genetic variation for induced susceptibility. In contrast, condensed tannin levels increased in defoliated trees under both nutrient conditions, showing genetic variation for induced resistance. Other researchers have also found genetic variation in induced susceptibility (Agrawal et al. 1999a). Genetic variation in induced resistance has been reported previously in the Salicaceae, including aspen (Stevens and Lindroth 2005), hybrid poplar (Robison and Raffa 1997), and willow (Ruuhola et al. 2001). The fact that plant populations maintain genetic variation for inducibility under a range of environmental conditions may reflect the costs of inducibility as the ability to respond chemically to damage may restrict the commitment of resources to growth (Agrawal et al. 2002; Relyea 2002; Stevens and Lindroth 2005).

Tolerance

Tolerance was expressed similarly in both nutrient environments. Under the compensatory continuum hypothesis, we expect resource-rich environments to facilitate plant tolerance (Maschinski and Whitham 1989). This prediction, however, has not been widely supported by data (Hawkes and Sullivan 2001; Wise and Abrahamson 2005). Tolerance was similar across three nutrient levels in *Salix*, a member of the same family as *Populus* (Houle and Simard 1996). We also found genetic variation for tolerance to herbivory in aspen under both low- and high-nutrient conditions. Although genetic variation in tolerance has been reported widely among herbaceous plants (reviewed by Strauss and Agrawal 1999), it has been reported in only a few other studies of woody plants, including red oak (*Quercus rubra*—Byington et al. 1994), hybrid poplar (*Populus* spp.—Robison and Raffa 1994), and sand-dune willow (*Salix cordata*—Shen and Bach 1997). Earlier studies with *S. cordata* (Bach 1994) and *P. tremuloides* (Osier and Lindroth 2004) failed to detect genetic variation for tolerance, perhaps due to small sample sizes and limited number of genotypes, respectively. Other studies of woody plants, including *Betula pendula* (Anttonen et al. 2002; Prittinen et al. 2003) and *Terminalia sericea* (Katjiua and Ward 2006), have failed to detect genetic variation for tolerance.

Costs of tolerance (reduced fitness in the absence of damage) could help maintain variable levels of tolerance in populations. More tolerant aspen genotypes displayed a tendency to grow more slowly when undamaged (Fig. 3), but those relationships appear to reflect the artifactual covariance between tolerance and growth of undamaged trees (Table 3). Although costs of tolerance have been examined by several researchers (Lennartsson et al. 1997; Mauricio et al. 1997; Agrawal et al. 1999b, Weinig et al. 2003; Honkanen and Jormalainen 2005), physiological costs of tolerance to herbivory have been reported in only a handful of species, including *Ipomoea purpurea* (Tiffin and Rausher 1999), *Ipomoea hederacea* (Stinchcombe

2002), *Asclepias syriaca* (Hochwender et al. 2000), *Arabis perennans* (Siemens et al. 2003), and *Datura stramonium* (Fornoni et al. 2004b). These species are all herbaceous, and, with the exception of *Asclepias*, annual or short-lived. Although costs of tolerance were recently evaluated in woody *T. sericea* (Katjiua and Ward 2006), such costs have yet to be reported for a woody species.

We expect woody plant tolerance to differ from herbaceous plant tolerance for several reasons. First, because of their longevity and size, woody plants are more apparent (sensu Feeny 1976) than herbs. The greater apparency of woody plants means that even with strong resistance, some level of herbivore damage is likely (Haukioja and Koricheva 2000), favoring the evolution of tolerance mechanisms. Second, the longevity of woody plants allows the expression of tolerance to change ontogenetically as a plant ages and develops. Del-Val and Dirzo (2003) suggested that *Cecropia* trees are more tolerant when young and become more resistant with age. Similarly, Boege (2005) found that juvenile saplings of *Casearia nitida* are better able to compensate for high levels of defoliation than are reproductive trees. In clonally integrated plants such as aspen where ramets vary in age, young ramets may derive higher degrees of tolerance from their ability to access photosynthate from other ramets. Third, because woody plants allocate proportionately less biomass to leaves (Körner 1994; Poorter and Nagel 2000), they are less sensitive to losses due to folivory and have proportionately more resources in stems and roots available for compensatory growth (Stevens 2005).

Woody plant tolerance may also respond differently than herbaceous plant tolerance to environmental conditions. Little work, however, has evaluated environmental effects on costs of tolerance. Fornoni et al. (2004b) found costs of tolerance in an herbaceous plant population growing in a tropical dry forest but not in a population growing in a pine-oak forest. Hochwender et al. (2000) and Stinchcombe (2002) found costs in herbaceous plants only under sub-optimal conditions (low soil nutrients and additional herbivores, respectively). In contrast, Siemens et al. (2003) observed costs of tolerance only in favorable (non-competitive) environments. In aspen, we found that the tradeoffs between growth and tolerance do not differ between low- and high-nutrient environments. In certain environments, the physiological costs required to retain mechanisms for tolerance may be high enough to favor less tolerant genotypes (Strauss and Agrawal 1999). Genetic variation for tolerance could then be maintained due to varying costs under different environmental conditions (Fornoni et al. 2004b). Environmental conditions that are patchily distributed and variable over time would further favor the maintenance of genetic variation.

Variable levels of selection resulting from fluctuating herbivore loads in both space and time could also maintain genetic variation for tolerance (Tiffin and Rausher 1999). Aspen is favored by outbreak folivores such as FTCs, large aspen tortrix, and gypsy moths. Such herbivores introduce large, multi-annual fluctuations in damage and may thus contribute to the maintenance of genetic variation for tolerance. Tolerance is certainly selected for during outbreak years when herbivory can result in nearly 100% defoliation, but costs of maintaining mechanisms of tolerance in the years between outbreaks (when damage is light) could tend to favor less tolerant genotypes.

Mechanisms of tolerance include plant characteristics maintained prior to damage, as well as the ability to respond positively to damage (Strauss and Agrawal 1999; Stowe et al. 2000; Stevens 2005). In a companion study (Stevens 2005), we provide

insight into the mechanisms underlying tolerance assessed in this study. In short, tolerance is positively correlated with stem mass before damage under low-nutrient conditions and with increased allocation to stems after damage under high-nutrient conditions.

Resistance–tolerance tradeoffs

Negative genetic correlations between resistance and tolerance have been predicted by several authors (van der Meijden et al. 1988; Herms and Mattson 1992; Belsky et al. 1993). Since both resistance and tolerance offer fitness benefits in the presence of herbivores, a negative genetic correlation between the two traits could preclude the fixation of alleles for either trait (Strauss and Agrawal 1999). Although assessed in several studies, such tradeoffs have been reported in only a few genera, including *Ipomoea* (Fineblum and Rausher 1995), *Brassica* (Stowe 1998), and *Raphanus* (Strauss et al. 2003). In a recent review, Leimu and Koricheva (2006) reported that resistance–tolerance correlation coefficients tend to be negative, but not significantly different from 0, in wild plant populations. Two studies in the woody plant genera *Salix* (Shen and Bach 1997) and *Betula* (Prittinen et al. 2003) found no tradeoff. Our inability to detect a resistance–tolerance tradeoff in aspen is concordant with results from other studies of woody plants and supports predictions that such tradeoffs should be uncommon (de Jong and van der Meijden 2000). The model of de Jong and van der Meijden (2000) involved plants with resistance factors effective against background levels of generalist herbivores but also occasionally damaged regardless of commitments to resistance. Such conditions favor plant populations that maintain intermediate levels of resistance *and* tolerance. This scenario fits well with the aspen—outbreak folivore system where resistance factors may effectively deter herbivores at background population levels, but fail during outbreaks when genotypes are often completely defoliated regardless of their level of resistance (Donaldson 2005). That is, outbreak conditions favor tolerant genotypes while the intervening years favor resistant genotypes. Such variable selective forces could maintain genetic variation for both resistance and tolerance, allowing the two defense strategies to coexist as complementary, rather than competing, alternatives.

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