Variation in tolerance to herbivory is mediated by differences in biomass allocation in aspen

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Summary

1. Herbivore damage can select for tolerance in plant populations where genetic variation for tolerance exists. The causes underlying variation in tolerance are not fully resolved. We assessed the importance of two potential mechanisms for tolerance by examining its relationship with leaf photosynthetic rate and relative biomass allocation across plant organs.

2. We monitored responses of 12 aspen (*Populus tremuloides*) genotypes, grown in a common garden under two levels of nutrient availability, to defoliation in two successive seasons. Tolerance of each genotype was calculated as the difference in growth between defoliated and undefoliated trees grown under the same nutrient conditions.

3. Although light-saturated leaf photosynthesis increased in response to nutrient addition and defoliation, it did not vary among genotypes and was not correlated with tolerance.

4. Tolerance was, however, correlated with patterns of biomass allocation. Under low-nutrient conditions it was positively correlated with the proportion of biomass in stems just prior to defoliation, while under high-nutrient conditions it was correlated with greater allocation to stems in response to damage.

5. Herbivores may select for specific patterns of biomass allocation in trees, and do so differently in different environments. The positive correlation between tolerance and relative allocation to stems, as opposed to roots, runs counter to reports from studies of herbaceous species and underscores the need for further exploration of mechanisms of tolerance in woody plants.

Key-words: defoliation, photosynthesis, plant defence, plant–insect interactions, *Populus tremuloides*

Introduction

As herbivory typically has a negative effect on plant fitness, it can function as a selective force to increase levels of plant defence. Plant defence strategies include both resistance and tolerance. A resistant plant deters herbivores via chemical or physical means, while a tolerant plant counteracts the negative effects of herbivory by compensating for tissues lost. While much research has focused on plant resistance, plant tolerance has only recently been explored by ecologists (Strauss & Agrawal 1999; Stowe *et al.* 2000) and mechanisms of tolerance have received limited attention (Tiffin 2000; Fornoni, Núñez-Farfán & Valverde 2003).

The mechanisms underlying tolerance are potentially complex and can involve numerous plant traits. Additionally, the expression of tolerance may be influenced by genetic and environmental factors, and their interactions (Strauss & Agrawal 1999; Wise & Abrahamson 2005). Intraspecific comparisons allow for assessments of genetic variation for tolerance and help elucidate how species may evolve higher levels of tolerance (Strauss & Agrawal 1999). With regard to environmental influences on tolerance, the compensatory continuum hypothesis (CCH) predicts that a plant's ability to tolerate herbivore damage is greater in resource-rich environments (Maschinski & Whitham 1989). Although this hypothesis may seem intuitive, the meta-analysis of Hawkes & Sullivan (2001), and further assessment by Wise & Abrahamson (2007), did not generally support the CCH. Because of the potential complexity of tolerance and the interactive effects of genes and the environment on its expression, mechanisms for tolerance remain poorly understood (Fornoni *et al.* 2003).

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We were particularly interested in examining how potential mechanisms of tolerance are influenced by genetic and environmental factors in a woody species, since the majority of tolerance studies to date have utilized herbaceous species and their conclusions may not pertain to trees (Haukioja & Koricheva 2000; Tiffin 2000). Here, we report research on mechanisms of tolerance to herbivory in aspen (*Populus tremuloides*). Trees in general may be more tolerant than herbs due to their larger size and capacity for storage of energy reserves and nutrients (Marquis 1984; Byington, Gottschalk & McGraw 1994). Size and storage factors may be further enhanced in clonally integrated trees such as aspen (Rosenthal & Welter 1995).

Aspen's relatively large size and capacity for storage may facilitate tolerance to herbivory. Additionally, extant aspen genotypes have likely been selected for tolerance over time. Outbreaks of native folivores such as the forest tent caterpillar (*Malacosoma disstria*) and large aspen tortrix (*Choristoneura conflictana*) regularly subject aspen populations to multiple years of nearly complete defoliation, regardless of their levels of resistance (Mattson *et al.* 1991; Donaldson 2005). Aspen genotypes with effective mechanisms for tolerance are likely favoured under such outbreak conditions. If the benefits of these mechanisms outweigh their costs, then tolerance-conferring traits should increase in plant populations.

Two likely mechanisms of tolerance are increased leaf photosynthetic rates and altered allocation of photoassimilate across plant organs in response to damage (Gassmann 2004). For each of these mechanisms, we examined both its initial state (prior to damage) and its responsiveness to damage, because both may influence the expression of tolerance (Stowe *et al.* 2000). A high photosynthetic rate prior to damage may afford tolerance, and photosynthetic rates often increase in damaged leaves and in undamaged leaves adjacent to damage (Danckwerts 1993; Meyer 1998; Thomson *et al.* 2003). In terms of biomass, tolerance has been shown to be conferred both by prior distribution to storage organs (Hochwender, Marquis & Stowe 2000) and by post-herbivory shifts in allocation from roots to shoots (McNaughton 1983; Trumble, Kolodny-Hirsch & Ting 1993; Houle & Simard 1996; Mabry & Wayne 1997).

Materials and methods

EXPERIMENTAL DESIGN

We established a common garden of potted aspen trees on the campus of University of Wisconsin in Madison, WI, USA. The trees were used in a $2 \times 2 \times 12$ experiment incorporating two levels of soil nutrients, two levels of defoliation (0% and 75%) and 12 aspen genotypes (six male, six female). The nutrient and defoliation treatments were crossed at the whole-plot level (2×2 factorial), with genotype as a sub-plot. The garden was spatially arranged in a split-plot design for ease of treatment application and to minimize asymmetric competition for light. The 48 treatment combinations were replicated across 15 blocks for a total of 720 trees. Each tree was planted in its own pot. Potted trees were placed adjacent to each other in paired rows (two pairs per block), with 0·5 m aisles between

pairs. Three destructive harvests were conducted over the course of the experiment: two blocks were harvested in June 2002, four blocks in June 2003, and four blocks in August 2003. The remaining five blocks were not harvested, but non-destructive measurements from these five blocks were used, in concert with pre-harvest measurements from the four-block harvest in August 2003, to determine relative growth and tolerance ($n = 9$).

GENOTYPES AND PROPAGATION

The 12 experimental genotypes were derived from root material field-collected from aspen growing in south-central Wisconsin. Microsatellites verified that each aspen genotype was distinct (C. Cole, D. Waller and R. Lindroth, unpublished data). We used micropropagation to replicate the genotypes because such techniques reduce the retention of non-genetic factors from source individuals, analogous to maternal effects (Wright 1976). In spring 2001, we established the micropropagates outdoors in 5-L pots containing a 40 : 40 : 20 mix (by volume) of sand : silt-loam field soil : perlite. We added Osmocote 3–4 month slow release fertilizer (14 : 14 : 14, N : P : K + micronutrients) at a rate of 4·5 g L^{-1} of soil. The following spring (2002), the trees (mean height ± SE: 109·8 cm ± 0·7 cm) were transplanted into the experimental array of 80-L pots containing sand (70%) and silt-loam field soil (30%). To the soil of 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^{-1} of soil. The soil of low-nutrient pots received no fertilizer. Throughout the experiment, trees were watered as needed.

DEFOLIATION

From half of the experimental trees, we removed 75% of each leaf present at initial flush in June of 2002 and 2003. The severity and duration of the defoliation treatment was designed to approximate an insect outbreak (Mattson *et al.* 1991; Parry, Herms & Mattson 2003). In both years, we exposed defoliated trees to 10 days of forest tent caterpillar herbivory by introducing several third-instar larvae into bags placed on a subset of branches on each tree. The actual number of caterpillars used (5–20) was scaled based on tree branch number (in 2002) or tree size (basal diameter and height) (in 2003). The majority of the defoliation, however, was conducted using scissors, cutting each leaf perpendicular to the midrib near its base. In 2002, scissor defoliation began on June 10 and took 4 days to complete. In 2003, the process began on June 2 and was completed in 9 days. Manual defoliation ensured that each genotype received the same level of damage regardless of its level of resistance (Stowe *et al.* 2000; Wise & Abrahamson 2005), while the presence of caterpillars provided a more natural elicitation of plant response to damage (Karban & Baldwin 1997; Havill & Raffa 1999).

GROWTH AND TOLERANCE MEASUREMENTS

We measured basal diameter (d) and height (h) of each tree at the beginning (June 2002) and end (August 2003) of the experiment. We used calipers to measure diameter 1 cm above the soil surface, and measured height from the soil surface to the apical meristem. Tree relative growth was calculated using [ln(final $d^2 h$) – ln(initial $d^2 h$)]. Destructive harvests in June 2002 (two blocks), June 2003 (four blocks) and August 2003 (four blocks) showed that $d^2 h$ was highly and linearly correlated with total mass in aspen (Stevens, Waller & Lindroth 2007). Diameter and height measurements have been widely used by other researchers to assess growth in *Populus* (Abrahamson

et al. 1990; Robison & Raffa 1994; McDonald *et al.* 2002). Growth is a key component of fitness in perennial plants (Simms 1992; Strauss & Agrawal 1999) and is especially important in aspen because of the species' propensity to reproduce clonally (Stevens *et al.* 2007).

The tolerance of each genotype was assessed under low- and high-nutrients separately by comparing the mean relative growth of the defoliated trees of a given genotype to that of the undefoliated trees of the same genotype (Strauss & Agrawal 1999; Stowe *et al.* 2000; Stevens *et al.* 2007). Tolerance was defined as the difference between damaged and undamaged relative growth (Tiffin & Rausher 1999; Hochwender *et al.* 2000; Weinig, Stinchcombe & Schmitt 2003).

PHOTOSYNTHESIS

We assessed light-saturated rates of leaf gas exchange on an area basis ($\mu mol\ m^{-2}\ s^{-1}$), using a LI-COR 6400 photosynthesis system (LI-COR Biosciences, Lincoln, NE), after defoliation in both 2002 and 2003. Assessments were conducted between 0800 and 1100 h. during a several-day period in mid-August of 2002 and mid-July of 2003. We measured an arbitrarily selected, mature, sunlit, undamaged leaf produced after defoliation from each of four replicate trees in each treatment combination. The leaves we collected were the same age in both defoliation treatments. The leaves produced subsequent to defoliation by undamaged trees were distinguished from the initial flush of leaves based on phenology. Measurements were taken at a cuvette temperature of 25 °C, a cuvette partial pressure of 37 Pa CO_2, and a photosynthetic photon flux density of 1800 $\mu mol\ m^{-2}\ s^{-1}$ (using a red–blue LED array). We quantified the responsiveness of each genotype to defoliation by subtracting the mean undamaged photosynthetic rate from the mean damaged photosynthetic rate for each genotype, using methods similar to our assessments of tolerance.

BIOMASS DISTRIBUTION AND ALLOCATION

In the three whole-tree harvests conducted during this study, we first lopped shoots at the base and then separated them into leaf and stem. Roots were then harvested and rinsed of soil. Plant material was dried to a constant weight at 60 °C. Throughout the remainder of this paper, we use the term *distribution* to refer to initial percentages of tree mass in each organ and the term *allocation* to refer to the amount of biomass apportioned to each organ over the course of the experiment. To estimate the relationship between total biomass and $d^2\,h$, and to determine the initial distribution of biomass, we destructively harvested two blocks of trees in June 2002. Because we conducted our harvest just prior to defoliation, the harvest of two blocks actually provided four replicates of each nutrient–genotype treatment combination. To non-destructively quantify the amount of leaf material produced after defoliation in the first season of growth, we bagged the entire crown of four replicates of each nutrient–defoliation–genotype combination and collected all the leaves from each tree after senescence. These data were used with $d^2\,h$ data to allometrically estimate post-defoliation leaf growth for all trees. Similarly, a destructive harvest in June 2003 of the same four replicates allowed us to estimate the amount of leaf mass removed from the remaining trees in the second defoliation.

At the end of the experiment (August 2003), we destructively harvested four of the remaining nine blocks (five blocks were saved for other studies) to determine total biomass gain and the portion of that gain allocated to leaves, stems and roots during the entirety of the study. The calculations were based on the actual mass of each tree harvested in August 2003 and the allometric estimate of its initial

mass in June 2002. We also accounted for leaf mass that was produced and lost, through senescence or defoliation, during the study. To assess the responsiveness of each genotype to defoliation, we used methods similar to our assessments of tolerance and subtracted undamaged mass allocation (%) from damaged mass allocation (%) for leaves, stems and roots. A genotype with a positive change for a given plant organ allocated relatively more to that organ in response to defoliation in a given nutrient environment.

The research reported here is a component of a large study that addressed tolerance and resistance in aspen (Stevens 2005). A companion study (Stevens *et al.* 2007) examined genetic variation for, and costs of, tolerance, while this study focused on mechanisms of tolerance. One notable finding from the companion study was that although defoliation reduced growth of all aspen genotypes, there was no significant relationship between tolerance and relative growth rate of undamaged trees. Thus the latter was not pursued as a potential mechanism underlying tolerance in the present study.

STATISTICAL ANALYSES

We analysed the effects of soil nutrient availability, defoliation, genotype, and their interactions, on leaf photosynthetic rates and mass allocation among plant organs using a mixed-model, three factor split-plot ANOVA with JMP IN version 4·0·4 (SAS Institute Inc. 2001). Nutrient level and defoliation were considered fixed effects, fully crossed and analysed at the whole-plot level. Genotype was considered a random effect and incorporated as a sub-plot factor within whole-plot treatments. All interactions between genotype and whole-plot treatments were included as sub-plot interactions. Block was incorporated as a random effect and retained when significant. In the split-plot analysis, we used a whole-plot error term (replicates within whole-plot error) to test the fixed effects of nutrient level and defoliation, while the random effect of genotype was tested over the interaction of genotype and the fixed effects. Residual error was used to test the nutrient × defoliation × genotype interaction. Photosynthesis data were normally distributed and had similar variances. Relative mass allocation percentages were arcsin-square-root transformed to improve homoscedasticity (Sokal & Rohlf 1995).

Pearson correlation coefficients were calculated to assess the relationship between tolerance and both photosynthetic rates of undamaged trees and the photosynthetic response to defoliation. Additionally, we calculated the correlation coefficients between tolerance and both initial biomass distribution and relative biomass allocation changes in response to defoliation. Because distribution across plant organs often varies with plant size (McConnaughay & Coleman 1999), when an organ mass percentage was significantly related to $d^2\,h$ within a nutrient level, genotype means for that percentage were adjusted for variation in $d^2\,h$ with analysis of covariance (SAS Institute 2001).

Results

PHOTOSYNTHESIS: EFFECTS OF NUTRIENTS, DEFOLIATION, GENOTYPE, AND THEIR INTERACTIONS

Leaf photosynthesis responded to treatments similarly in August 2002 (after one defoliation event) and July 2003 (after two successive defoliations) (Table 1, Fig. 1). Nutrient addition increased photosynthetic rates by 32% in 2002 and 22% in 2003. Defoliation also increased photosynthetic rates by 21% in 2002 and 36% in 2003. The photosynthetic response

Table 1. *F*-ratios and *P*-values from a 3-factor, split-plot ANOVA assessing the effect of soil nutrients (N) (df = 1), defoliation (D) (df = 1), genotype (G) (df = 11), and their interactions, on photosynthetic rates in August 2002 and July 2003. Corresponding data are shown in Fig. 1

	2002		2003	
	F-ratio	*P*-value	*F*-ratio	*P*-value
Nutrients	41·9	< 0·001	26·5	< 0·001
Defoliation	24·0	< 0·001	59·6	< 0·001
Genotype	4·4	0·110	1·2	0·365
N × D	0·7	0·415	1·9	0·191
N × G	1·3	0·350	0·1	0·999
D × G	0·7	0·724	0·2	0·991
N × D × G	1·5	0·138	1·2	0·314
Block	3·3	< 0·001	4·4	< 0·001

Table 2. *F*-ratios and *P*-values from a 3-factor, split-plot ANOVA assessing the effect of soil nutrients (N) (df = 1), defoliation (D) (df = 1), genotype (G) (df = 11), and their interactions, on percent of total biomass gain that was allocated to leaves, stems and roots from June 2002 to August 2003. The effect of block was initially included in the model for leaves, but was removed when block effects were not significant. Corresponding data are shown in Fig. 2

	Leaves		Stems		Roots	
	F-ratio	*P*-value	*F*-ratio	*P*-value	*F*-ratio	*P*-value
Nutrients	38·7	< 0·001	76·5	< 0·001	86·3	< 0·001
Defoliation	2152·0	< 0·001	13·4	0·002	120·7	< 0·001
Genotype	8·5	0·488	3·2	0·066	3·1	0·057
N × D	280·8	< 0·001	0·6	0·469	32·4	< 0·001
N × G	0·6	0·794	1·3	0·319	2·3	0·096
D × G	0·7	0·746	1·4	0·302	1·1	0·454
N × D × G	1·9	0·049	2·4	0·009	1·1	0·333
Block			7·4	< 0·001	5·5	< 0·001

Fig. 1. Norm of reaction plots for photosynthetic rates (leaf area basis) in August 2002 and July 2003. Each line represents the mean response (*n* = 4 replicates) of a single aspen genotype in the undefoliated vs. defoliated condition under low and high soil nutrient conditions. Results from statistical analyses are provided in Table 1.

to defoliation was consistent across nutrient environments and photosynthetic rates did not vary significantly among aspen genotypes under any combination of nutrient and defoliation treatments.

BIOMASS ALLOCATION: EFFECTS OF NUTRIENTS, DEFOLIATION, GENOTYPE, AND THEIR INTERACTIONS

Relative biomass allocation across plant organs between the initial and final harvest was affected by nutrients, defoliation, genotype, and their interactions (Table 2, Fig. 2). Nutrient addition increased relative allocation to leaves (by 5%) and stems (by 47%) and decreased relative allocation to roots (by 27%). Defoliation substantially increased relative allocation to leaves and the effect was four-fold greater under low-nutrient conditions than under high-nutrient conditions (239% vs. 60%). Defoliation decreased relative allocation to stems and the decrease (15%) was similar in the two nutrient environments.
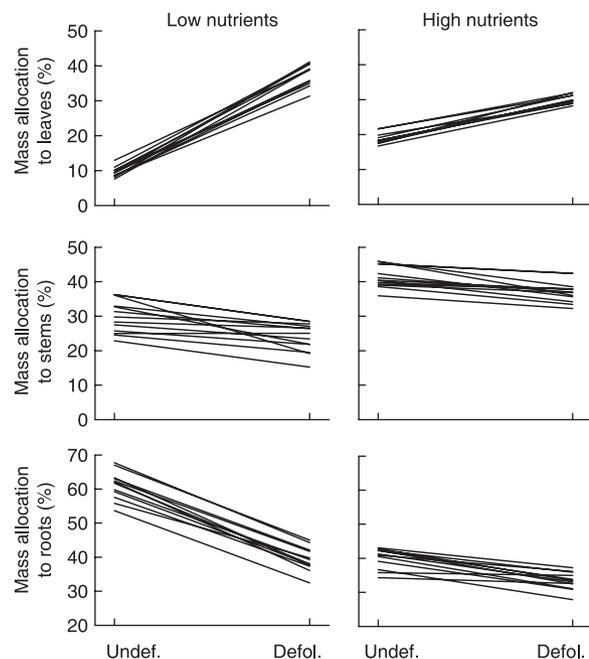

Fig. 2. Norm of reaction plots for percent of total biomass gain that was allocated to leaves, stems and roots from June 2002 to August 2003. Each line represents the mean response (*n* = 4 replicates) of a single aspen genotype in the undefoliated vs. defoliated condition under low and high soil nutrient conditions. Results from statistical analyses are provided in Table 2.

Defoliation also decreased relative allocation to roots, but the effect was greater under low-nutrient conditions than under high-nutrient conditions (36% vs. 17%, respectively). Genetic differences (main effects) in relative mass allocation were not evident for leaves, but were marginally significant for stems and roots. Genotypic variation was more pronounced in the interactive effects: genotypes responded differently to defoliation between the two nutrient environments for both leaf and stem allocation. For an analysis of whole-tree growth
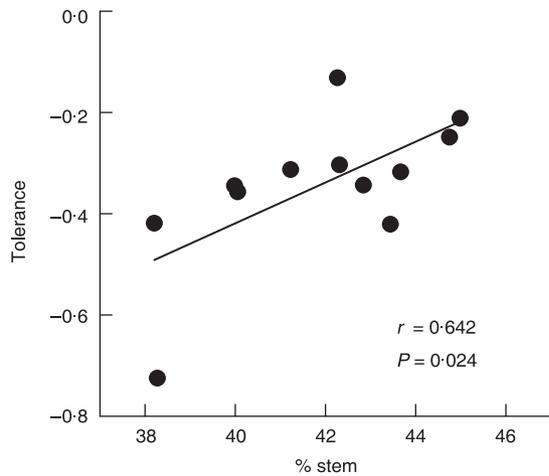
Fig. 3. Correlations between tolerance (difference between relative growth of damaged and undamaged trees) and percent of tree mass in stems under low-nutrient conditions, prior to damage (June 2002). Each point represents the mean response of tolerance (*n* = 9 replicates) and percent distribution to stems (*n* = 4 replicates) of a single aspen genotype.

responses to nutrients, defoliation and genotype, and their interactions, see Stevens *et al.* (2007).

CORRELATIONS BETWEEN TOLERANCE AND PHOTOSYNTHESIS

Tolerance was not correlated with photosynthetic rates of undamaged trees in either 2002 or 2003. Moreover, although photosynthetic rates increased in response to defoliation in both years, tolerance was not correlated with the responsiveness of photosynthetic rates to defoliation (data not shown).

CORRELATIONS BETWEEN TOLERANCE AND BIOMASS

Under low-nutrient conditions, tolerance was positively correlated with the initial percentage of mass in stems (Fig. 3), but not leaves or roots, just prior to defoliation. The relationship between tolerance and stem mass did not differ when male and female genotypes were analysed separately. Under high-nutrient conditions, no correlations of this sort were found.

Under low-nutrient conditions, we found no significant correlations between tolerance and change in mass allocation to any plant organ following defoliation. Under high-nutrient conditions, we found no correlations between tolerance and change in leaf mass allocation, but striking and opposite relationships were observed between tolerance and change in stem mass allocation vs. root mass allocation (Fig. 4). Comparatively tolerant genotypes allocated a greater proportion of new biomass to stems and a smaller proportion to roots than did less tolerant genotypes. Additional analyses revealed that these relationships did not differ between male and female genotypes (data not shown).
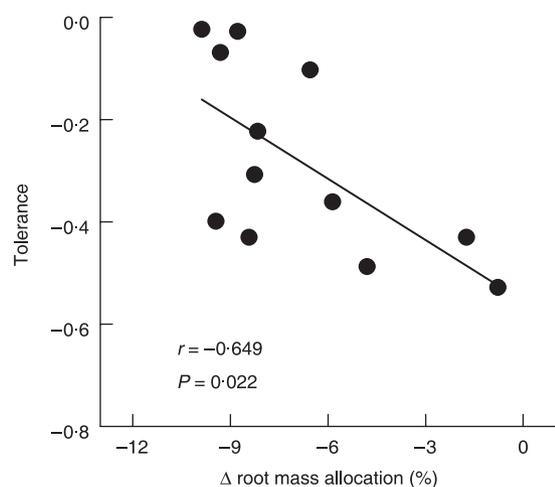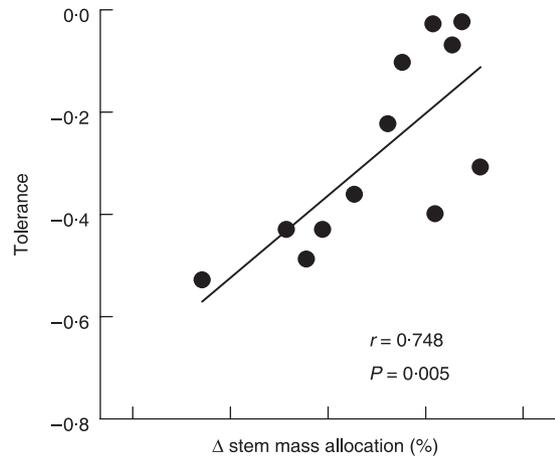

Fig. 4. Correlations between tolerance (difference between relative growth of damaged and undamaged trees) and change in mass allocation to stems and roots from June 2002 to August 2003 in response to two successive years of defoliation under high-nutrient conditions. Each point represents the mean response of tolerance (*n* = 9 replicates) and change in percent mass allocation to each plant organ (*n* = 4 replicates) of a single aspen genotype.

Discussion

Few studies have examined plant characteristics associated with tolerance in species wherein genetic variation for tolerance has been demonstrated (Strauss & Agrawal 1999). Because our companion study (Stevens *et al.* 2007) revealed genetic variation for tolerance in aspen under both low- and high-nutrient conditions, we were particularly interested in identifying the mechanisms associated with tolerance in this woody species.

TOLERANCE AS A FUNCTION OF PHOTOSYNTHESIS

Although defoliation induced substantial increases in photosynthetic rates, tolerance was not related to variation in this response or to baseline photosynthetic rates in undamaged trees. Other studies have also reported that tolerance was not

related to compensatory photosynthesis (Nowak & Caldwell 1984; Gassmann 2004). Research on domesticated plants has shown that photosynthetic rates are less responsive to selection and may be more genetically-constrained than are changes in relative biomass allocation (Gifford & Evans 1981; Bloom 1985; Welter & Steggall 1993). Indeed, genetic constraints on selection for photosynthetic rates may have been exhibited by our 12 aspen genotypes, as we found no genetic variation for photosynthetic rates among undamaged trees or in rates of defoliation-induced photosynthesis. Moreover, direct correlations between tolerance and photosynthesis may be obscured by the way photosynthate is utilized by a plant (Gassmann 2004). For example, in their comparison of tolerance to herbivory in wild vs. domesticated tomatoes, Welter & Steggall (1993) found that wild plants were more tolerant of herbivory due to their higher relative allocation to storage structures, including stems and roots. Our data indicate likewise for aspen.

TOLERANCE AS A FUNCTION OF BIOMASS

Tolerance was related to initial (pre-defoliation) differences in biomass distribution among genotypes under only low-nutrient conditions. Under those conditions, trees with a high percentage of mass in stem were more tolerant, while initial percentages in leaves or roots were not related to tolerance.

Although low-nutrient trees responded to damage by allocating more to leaves and less to stems and roots, variation in their responsiveness did not correlate with the expression of tolerance. Under high-nutrient conditions, however, genotypes that allocated a larger proportion of new growth to stem tissue in response to damage were more tolerant of herbivory, while those that allocated more to roots were less tolerant. Genetic variation for stem allocation (and associated leaf allocation) was expressed in terms of differential responses to defoliation across nutrient environments. Such genetic variation indicates that patterns of relative biomass allocation can respond to selection from herbivores as mechanisms of tolerance and that the magnitude of such responses differs across environments. Our data suggest that integral plant characteristics can be shaped by the interactive forces of herbivores and soil nutrient availability over time.

In summary, under low-nutrient conditions tolerance was positively correlated with allocation to stems prior to damage, while under high-nutrient conditions tolerance was positively correlated with allocation to stems and negatively correlated with allocation to roots in response to damage. Under growth-limiting conditions, genetically-determined differences in allocation prior to damage may be particularly important for tolerance since low-nutrient conditions may slow compensation. Hochwender *et al.* (2000) also found that patterns of biomass distribution prior to damage were predictive of tolerance under only low-nutrient conditions. In contrast, high-nutrient conditions provide for more rapid compensatory growth, so the genetically-determined *response* to defoliation is more important for tolerance than is biomass distribution prior to damage.

Our finding of higher tolerance among genotypes with greater commitment to stems before damage runs counter to many studies of herbaceous species that report instead that tolerance is conferred by prior commitment to roots (van der Meijden, Wijn & Verkaar 1988; Welter & Steggall 1993; Mabry & Wayne 1997; Hochwender *et al.* 2000). The contrasting finding in a woody species underpins the importance of examining tolerance in trees as well as herbs. Recent work on the tree *Melaleuca quinquenervia* corroborates our finding that woody plant tolerance is a function of stem allocation (Pratt *et al.* 2005).

Based on research on herbaceous *Asclepias*, Hochwender *et al.* (2000) proposed that folivores may select for plants that store more resources below-ground, especially under low-nutrient conditions. This may apply to herbs, but our results for a woody plant suggest that heavy allocation to roots does not always confer a greater degree of tolerance. In fact, in the competitive environments where early successional trees such as aspen grow, substantial allocation to roots may be disadvantageous. Conservative (i.e., rooty) aspen genotypes may have higher levels of underground storage and protect those resources from above-ground herbivores, but such genotypes would likely be out-competed for light by both intra- and inter-specific competitors with less conservative growth forms. van der Meijden *et al.* (1988) indicate that leaf herbivory should select for plants with a greater percentage of their mass in roots, but in aggressively growing plants such as aspen, leaf herbivory may select for genotypes that instead increase allocation to leaves and stems at the expense of roots.

Although roots are generally recognized as important organs for storage, recent work on aspen has shown that the stem and branches are a major source of carbohydrates for both initial leaf flush and re-flush after defoliation (Landhäusser & Lieffers 2003). This may help explain why undamaged genotypes with relatively large stems are more tolerant of defoliation under low-nutrient conditions and why genotypes that allocate a larger proportion of new growth to stems are more tolerant under high-nutrient conditions. In the present study, under high-nutrient conditions, a trade-off in allocation appeared to occur between stems and roots, as leaf allocation patterns following defoliation varied only modestly among genotypes. Thus, there presumably were no major opportunity costs related to increased stem allocation, as long as trees were supplied with ample below-ground resources. In any case, the specific causes underlying the benefit of increased allocation to stems remain unclear. Perhaps the benefit was manifested only after repeated defoliation. For example, if more biomass was allocated to stems after the first defoliation, benefits in the form of increases in stored reserves and numbers of apical meristems for compensatory foliage production would be realized after the second defoliation.

Investing heavily in stems may be a particularly advantageous strategy for early-successional trees because in addition to providing storage, stems provide the vertical and horizontal expansion necessary for capturing light in competitive environments. Allocation to stems may be especially beneficial

to aspen as the bark of aspen stems is photosynthetic (Foote & Schaedle 1978).

Conclusions

We found that tolerance was related to both biomass distribution across plant organs prior to damage and to changes in allocation patterns across plant organs in response to damage, but not in the same environment. Initial distribution was more predictive of tolerance under low-nutrient conditions while responsiveness to damage was more closely aligned with tolerance under high-nutrient conditions. The different findings in the two soil nutrient environments underscore the importance of assessing tolerance mechanisms in multiple environments (Hochwender *et al*. 2000). That we found greater tolerance among genotypes with higher allocation to stems (rather than roots) indicates that patterns described for herbs may not apply to trees. Tolerance expressed as a change in allocation patterns across plant organs is likely to be a very important defence strategy for other woody plant species that have proportionately large storage reserves. An improved understanding of the relationship between plant characteristics and tolerance helps clarify how the selective forces of herbivores have shaped key woody plant traits over time.

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