Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*)

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Summary

• Costs of defense are thought to maintain genetic variations in the expression of defense within plant populations. As with many plant species, aspen exhibits considerable variation in allocation to secondary metabolites. This study examined the independent and interactive effects of genotype, soil fertility and belowground competition on defensive chemistry and growth in trembling aspen (*Populus tremuloides*).

• Four aspen genotypes were grown with high and low soil fertility, and with and without root competition. Physiological, morphological and allocational determinants of growth were measured to identify growth–defense tradeoffs.

• Nutrient limitation and competition decreased growth, leaf mass ratio, leaf nitrogen concentration and photosynthesis, and increased root : shoot ratio and leaf condensed tannin concentrations. The competition treatment also resulted in increased leaf phenolic glycoside (PG) concentrations.

• Aspen growth was negatively correlated with PG concentrations under low fertility with competition. The relationship between growth and its major determinants was also negatively related to foliar condensed tannins expressed as a proportion of tree mass, indicating an additional indirect cost of allocation to secondary metabolites.

Key words: chemical defenses, condensed tannins, costs of defense, marsh reed grass (*Calamagrostis canadensis*), phenolic glycosides, plant growth, plant physiology, trembling aspen (*Populus tremuloides*).

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Introduction

Despite the significant selective pressure that herbivores and pathogens exert on plants, marked, genetically based, quantitative variations in chemical defenses (genetic polymorphisms) persist in plant populations (Simms, 1992; Karban & Baldwin, 1997; Mauricio, 2000). Defense costs, in the form of tradeoffs between growth, reproduction and defense, provide the most widely proposed explanation for why defensive traits remain variable (Simms & Rausher, 1987; Herms & Mattson, 1992).

Several prominent theories of plant defense against herbivores, such as optimal defense (McKey, 1974; Rhoades, 1979) and growth–differentiation balance (Herms & Mattson, 1992), suggest that optimization of costs and benefits leads to genetically variable levels of defense production within plant populations. In essence, these theories suggest that when herbivores are present and benefits outweigh costs, plant defenses increase fitness. If, however, defenses are costly and herbivores are absent, then high levels of defense decrease fitness. Defense costs are generally defined as a decrease in growth or reproduction in defended individuals in the absence of herbivores (Simms & Rausher, 1992).

According to several reviews, the number of studies that have identified costs associated with allocation to defense roughly equals the number that failed to do so (Simms, 1992; Bergelson & Purrington, 1996; Koricheva, 2002; Strauss *et al.*,

2002). Although these equivocal results have led to some doubt about the importance of costs, it is more likely that they reflect an incomplete understanding of the mechanisms by which costs are realized, and the importance of ecological factors in mediating costs (Berenbaum, 1995; Purrington, 2000; Strauss *et al.*, 2002). Costs of defense are probably manifest via a plant's biotic and abiotic interactions (e.g. competition, herbivore complexes, abiotic stress: Koricheva, 2002; Strauss *et al.*, 2002). As noted by Purrington (2000), hundreds of studies have identified costs, and recent literature is focusing not on whether defense is costly, but rather under what conditions and by what mechanisms costs are or are not realized.

Resource availability may have a marked influence on the realization of defense costs. For example, high resource availability may diminish allocation costs, thereby allowing for both growth and defense (Siemens *et al.*, 2002; Osier & Lindroth, 2006). Correspondingly, environmental stress may significantly increase the magnitude of defense costs (Weis & Hochberg, 2000; Marak *et al.*, 2003; Siemens *et al.*, 2003; Osier & Lindroth, 2006). According to the growth–differentiation balance hypothesis (Herms & Mattson, 1992), if levels of defense remain stable, but resources such as light or nutrients decrease, then fewer resources will be available for growth or reproduction. Further, ecological stress (water, nutrients, competition) can induce plants to produce increased concentrations of secondary metabolites (Gershenzon, 1984; Inbar *et al.*, 2001; Osier & Lindroth, 2006), thereby compounding costs. Theoretically, however, environmental stress may not always compound the costs of defense (Bergelson & Purrington, 1996). Bryant *et al.* (1983) predicted that nitrogen limitation constrains growth more than photosynthesis, leading to a relative increase in the fixed carbon pool. 'Excess' C is predicted to result in increased allocation to 'C-based' secondary metabolites (tannins; phenolic glycosides, PG) at little cost to growth.

Aspen (*Populus tremuloides*) experiences considerable intra- and interspecific competition, particularly when it is young (Landhäusser & Lieffers, 1998). Marsh reed grass (blue joint grass, *Calamagrostis canadensis*) is an aggressive native species in mixed boreal forests that quickly colonizes and dominates disturbed areas (Dyrness & Norum, 1983). Marsh reed grass and other herbaceous cover can suppress both sexual and asexual aspen regeneration due to shading, thermal inhibition (Landhäusser & Lieffers, 1998; Ball *et al.*, 2002), and root competition (Scholes & Archer, 1997; Landhäusser & Lieffers, 1998; Powell & Bork, 2004). Although light limitation is typically assumed to be the most important stress, competition for water and nutrients is often equally, if not more important for plants (Wilson, 1988; Coomes & Grubb, 2000; Powell & Bork, 2004). Belowground competition is a significant stress for regenerating aspen, and competitive interactions in the first season of growth are very important for seedling survival (Powell & Bork, 2004). As suggested by Purrington (2000), plant competition may increase the magnitude of growth–defense tradeoffs.

A primary objective of this study was to assess the independent and interactive effects of plant genotype, nutrient availability and belowground competition on allocation to secondary metabolites and growth. We were interested in the extent to which environmental stress (nutrient stress and belowground competition) changes patterns of biomass allocation (e.g. root : shoot; leaf mass ratio; specific leaf area; condensed tannins; PG concentrations) in young trembling aspen. A second objective was to identify potential tradeoffs between growth and allocation to secondary metabolites and to assess the degree to which environmental stress mediates defense costs. Tradeoffs between growth and allocation to secondary metabolites were assessed via negative phenotypic correlations. Finally, we examined growth determinants (specific leaf area; photosynthesis; leaf mass ratio) to identify potential mechanisms by which costs may be realized.

Materials and Methods

The overall experimental design was a completely randomized $4 \times 2 \times 2$ factorial with four aspen (*Populus tremuloides* Michx.) genotypes; two levels of nutrient availability (low, high); and two levels of competition (grown with and without grass). Treatments were replicated 12 times using individual pots as experimental units (192 total plants). At the end of the study, a subset ($n = 5$) of these 12 replicates was harvested for biomass growth determinations and used in assessments of photosynthesis, biomass distribution and foliar chemistry.

Plant materials

The four aspen genotypes included in this study (Dan 1, PG 1, PG 2, Wau 1) are maintained in a common garden at the University of Wisconsin–Madison and were originally collected from field populations occurring in south-central Wisconsin (Hwang & Lindroth, 1997, 1998). Genotypes were selected to span the range in foliar concentrations of condensed tannins and PGs observed among natural aspen populations in south-central Wisconsin (Hwang & Lindroth, 1997), and the range of growth rates observed in experimental trees (Donaldson, 2005; Osier & Lindroth, 2006). Replicates of genotypes were generated using a tissue-culture micropropagation technique (Sellmer *et al.*, 1989; Donaldson, 2005). Microcuttings were taken from culture in January and February of 2002, and rooted in fine-textured potting mix in the glasshouse in 115 ml RLC-7 'Cone-tainers' (Stuewe & Sons, Inc., Corvalis, OR, USA). Seeds of marsh reed grass [*Calamagrostis canadensis* (Michx.) Beauv., obtained from J&J Transplant Aquatic Nursery, Wild Rose, WI, USA] were germinated and grown in planting trays in fine-textured potting soil. Seedlings were thinned to two per cell and grown for 4 wk in the glasshouse. Aspen propagules and grass seedlings were fertilized once a week with Miracid fertilizer solution (30-30-10 N-P-K, Scotts Co., Marysville, OH, USA).

Competition arenas were established in 12-l pots in a common garden at the University of Wisconsin–Madison. Pots were filled with a 30 : 20 : 50 mix (by volume) of potting mix, silt-loam field soil and sand. An empty 'Cone-tainer' was pushed into the center of each pot to facilitate planting of aspen propagules at a later time. On 28 June, six grass 'plugs' from the 96-cell planting trays were transplanted into half the pots. Before transplanting, grass seedlings were sorted so that each pot received a total of 12 *c.* equal-sized plants (two per plug). After 25 d, grass roots had infiltrated much of the pot and were visible when the empty 'Cone-tainers' were removed. Aspen propagules were then assigned randomly and planted in the space left by the 'Cone-tainers'. Aspen had well established roots at this time, and transplanting caused minimal disturbance to the roots of either species. After all trees had been transplanted, the nutrient treatment was applied by amending half the pots with 4.5 g l^{-1} 18-6-12 (N-P-K + micronutrients) Osmocote 8–9-month slow-release fertilizer (Scotts Co.; Hemming & Lindroth, 1999; Osier & Lindroth, 2001).

At the onset of nutrient and competition treatments (22 July 2002), we harvested six trees per genotype. At this time we also measured stem basal diameter (D) and height from soil surface to the apical meristem (H) of all trees. For each genotype we regressed tree mass against a stem volume index, D^2H. We then used the resulting models to estimate the initial mass of all remaining trees (R^2 = 0.62–0.99, not shown; overall average for estimated initial mass = 0.75 ± 0.37 g). When aspen trees were transplanted, grass basal area (soil surface area occupied by grass tillers; Andariese & Covington, 1986) and height did not differ among treatments (P = 0.98, data not shown). Thus all trees grown with grass were initially exposed to a similar level of competition (average for initial grass mass = 6.46 ± 1.01 g). To minimize water stress in the experiment, pots were checked daily and watered regularly (e.g. high-nutrient and competition-treatment trees required more frequent watering).

Measures of growth and its determinants

On 7 September 2002, from 08:00 to 11:00 h, light-saturated rates of leaf gas exchange were measured using a Li-Cor 6400 photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). Leaves were measured at a cuvette temperature and CO_2 partial pressure of 25°C and 37 Pa, respectively, and a photosynthetic photon fluence rate of 1800 μmol m^{-2} s^{-1} (using a red–blue LED array). An undamaged, recently mature leaf (leaf plastochron index of 6–8) was arbitrarily selected for measurement from each of four replicate trees within each treatment–genotype combination. At the time of these measurements, all trees (regardless of treatment) were actively growing, had not set terminal buds, and < 10% of the lower canopy was senescent. As has been observed in previous work with aspen (Volin *et al.*, 2002), preliminary measurements indicated that the leaves chosen for measurement were functionally similar to > 65% of the remaining foliage on the seedling in all treatments.

On 9 September 2002, stem diameters and heights of all trees were remeasured (12 replicates per treatment × genotype combination), and the shoot (stem and foliage) was harvested from five replicates per genotype in each treatment (total of 80 trees). After total leaf area was measured for each tree, leaves (without petioles) were flash-frozen in liquid N_2 and freeze-dried in preparation for subsequent phytochemical analyses. Total leaf-area and mass data were used to calculate specific leaf area. Aspen stems, including leaf petioles, were clipped just above the root collar (at ground level) and oven-dried to a constant mass at 60°C. Aboveground grass tissues were harvested on 11 September 2002, oven-dried and weighed. Following the aboveground harvest, aspen and grass roots were washed free of soil, manually separated based on morphological characteristics, oven-dried to constant mass, and weighed. Final grass mass did not differ among treatments or genotypes (P = 0.93).

Harvest biomass data were used to analyze plant growth in the manner described by Hunt (1982): relative growth rate (RGR) was calculated as $(\ln M_2 - \ln M_1)/(t_2 - t_1)$, where M is total plant dry mass and t is time. At the final as well as initial harvest, M was estimated for all trees based on relationships between total mass and D^2H. For final harvest estimates, separate models for each genotype-by-competition treatment resulted in the best fit (R^2 = 0.94–0.99, not shown). Assessments of treatment and genotype effects on growth rate were based on 12 trees per genotype × treatment combination (Fig. 1). Effects on leaf morphology and organ biomass distribution (specific leaf area; leaf mass ratio; root : shoot ratio) were assessed using the subset of five trees per genotype × treatment combination from the final harvest.

Phytochemical analyses

Tree-level averages for foliar N, condensed tannins (CT) and PG concentrations were determined from freeze-dried leaf samples collected at final harvest (n = 5 per genotype × treatment combination). An arbitrarily selected subsample of foliage from each tree (*c.* eight to 10 leaves) was ground through a 40-mesh screen in a Wiley Mill. Total N was determined in a LECO elemental analyzer (St Joseph, MI, USA) using glycine *p*-toluenesulfonate as a standard. Tannins were quantified using the acid butanol assay described by Porter *et al.* (1986) with purified aspen CT serving as a standard. The PGs salicortin and tremulacin were quantified using high-performance thin-layer chromatography methods described by Lindroth *et al.* (1993). Purified salicortin and tremulacin served as standards. Total PG were calculated as the sum of salicortin and tremulacin. In order to make possible the most relevant assessment of whole-plant allocation costs (Koricheva, 1999), CT and PG mass ratios were calculated for each tree as total foliage CT or PG content divided by total final tree mass.

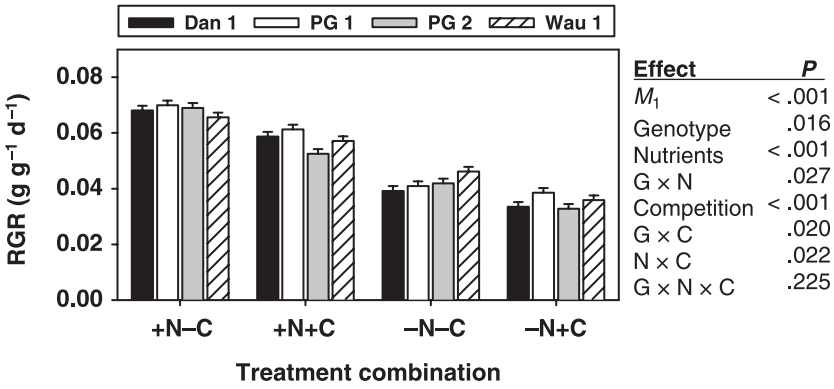

Fig. 1 Relative growth rate (RGR) of four aspen (*Populus tremuloides*) genotypes (Dan 1; PG 1; PG 2; Wau 1) (G) grown under different treatment combinations. Treatments are arranged in order of increasing stress: plus and minus nutrients (+N; –N, respectively), and minus and plus competition (–C; +C, respectively). RGR values were normalized for variation in initial mass (M_1) using ANCOVA (see Materials and Methods). Bar heights are genotype averages ± 1 pooled SE (n = 12 trees).

Statistical analyses and assessment of growth–defense tradeoffs

Independent and interactive effects of aspen genotype, nutrient availability and belowground competition on growth and phytochemistry were assessed by ANOVA. All analyses were conducted using JMP in version 4.04 (SAS Institute Inc., 2001). In our assessment of tradeoffs we were more interested in variation among genotypes (as genotype affects chemistry and growth) than in variation among individual trees. Therefore tradeoffs between growth and defense were examined using genotype × treatment means based on the same harvested subset of trees (as in Koricheva, 2002; Osier & Lindroth, 2006).

Growth rate and organ mass ratios (leaf mass ratio; root : shoot ratio) often vary with plant mass (Ledig *et al.*, 1970; McConnaughay & Coleman, 1999), and this can confound analyses of plant growth and allocation. Therefore, in our assessment of treatment and genotype effects, RGR and organ mass ratios were normalized to a common mass, using ANCOVA, when the following occurred: (1) RGR or organ mass ratios were found to be significantly related to tree mass; (2) sufficient overlap in the distribution of tree mass occurred across treatments and genotypes; and (3) no significant interaction occurred between tree mass and treatment.

Pearson's correlation coefficients were calculated to examine interrelationships among growth, its determinants, and phytochemistry. Potential defense costs were evaluated in two ways. First, direct phenotypic correlations between RGR and CT or PG mass ratios were calculated separately by fertility and competition treatments. Second, the potential costs of environmentally induced changes in phytochemistry were examined, based on the relationship between realized growth (RGR) and potential growth capacity. Photosynthesis per unit plant mass, calculated as the product of light-saturated leaf photosynthesis; interval average leaf mass ratio (mean of initial and final harvest values); and specific leaf area at final harvest, was used as an index of growth potential (Kaelke *et al.*, 2001; Volin *et al.*, 2002). The goodness of fit between RGR and photosynthesis per unit plant mass depends largely on variation in plant-level rates of dark respiration, which reflect costs of tissue construction and maintenance (Lambers & Poorter, 1992). To elucidate the implications of allocation to secondary metabolites for growth in the present study, residuals from the relationship between RGR and photosynthesis per unit plant mass were regressed against CT and PG mass ratios.

Results

Growth and its principal determinants

Manipulations of nutrient availability and competition led to marked differences in RGR (Fig. 1). On average, RGR in the most stressful treatment combination (low fertility with competition) was *c.* 45% less than that in the least stressful condition (high fertility without competition). Overall, differences in RGR among genotypes were subtle (< 5%). However, fertility and competition treatments had varying impacts on growth depending on genotype (significant genotype × environment interactions). Specifically, the RGRs of genotypes WAU 1 and PG 1 tended to be less responsive to nutrient and competition treatments than those of DAN 1 and PG 2.

As in the case of RGR, main effects of nutrient treatment and competition on light-saturated photosynthesis were considerable, and genotypes were affected differently by those treatments (genotype × environment interactions). Photosynthesis varied substantially among genotypes, and for all except WAU 1 it decreased by 40–50% from the lowest to highest stress treatment (Fig. 2). Photosynthesis of genotype WAU 1, which was lower than that of others in high fertility without competition, decreased by < 20% with increasing stress.

Nutrient limitation and belowground competition both had significant effects on biomass distribution (Fig. 3). Although aspen genotypes varied in root : shoot ratio (pooled across nutrient and competition treatments), the total range in this parameter was modest (from 0.50 to 0.57). Relative to genotypes DAN 1 and WAU 1, PG 1 and PG 2 had a greater increase in

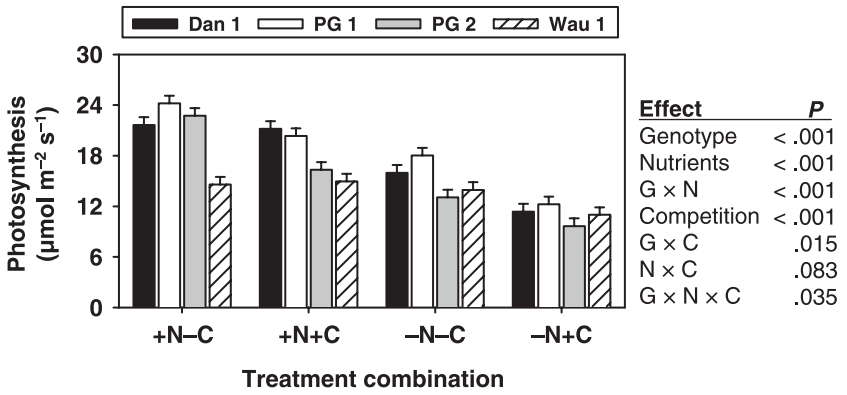

Fig. 2 Light-saturated rates of photosynthesis among four aspen (*Populus tremuloides*) genotypes (Dan 1; PG 1; PG 2; Wau 1) (G) grown under different treatment combinations. Format as for Fig. 1. Bar heights are genotype averages ± 1 pooled SE (n = 4 trees).

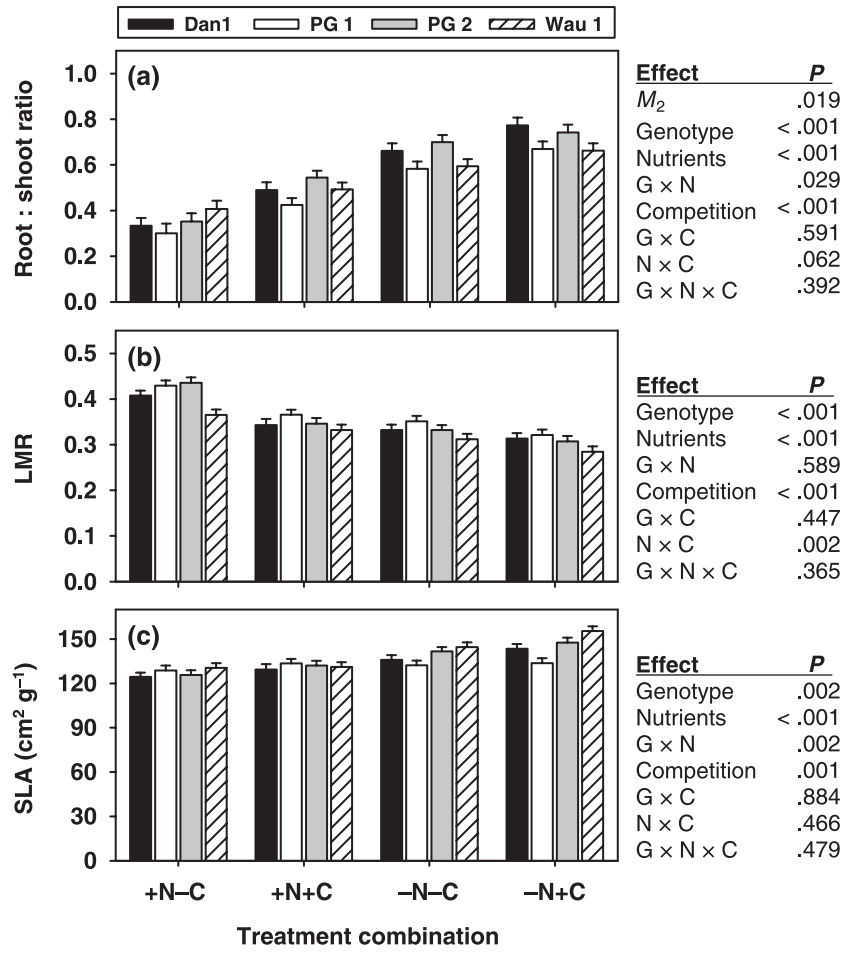

Fig. 3 Patterns of biomass distribution among four aspen (*Populus tremuloides*) genotypes (Dan 1; PG 1; PG 2; Wau 1) (G) grown under different treatment combinations. Format as for Fig. 1. (a) Root : shoot ratios were normalized by final mass (M_2) using ANCOVA. (b) Leaf mass ratio (LMR) means could not be normalized because of multicollinearity among independent variables. (c) Specific leaf area (SLA). Bar heights are genotype averages ± 1 pooled SE (n = 5 trees).

root : shoot ratio in response to low fertility (Fig. 3a), resulting in a significant genotype × environment interaction. Across genotypes, the combined effects of low fertility and competition led to a 40% increase in root : shoot ratio. This increase corresponded to a *c.* 25% decrease in leaf mass ratio (Fig. 3b). Aspen trees growing in low fertility with competition had a higher specific leaf area than those in high fertility without competition (Fig. 3c). Genotype PG 1 was unique, however, in that it showed almost no treatment variation in specific leaf area relative to the other three genotypes (significant genotype × environment interaction).

Phytochemistry

Leaf nitrogen concentration ([N]) and its response to treatments varied among genotypes ($P \leq 0.06$; Fig. 4a). Leaf [N] decreased

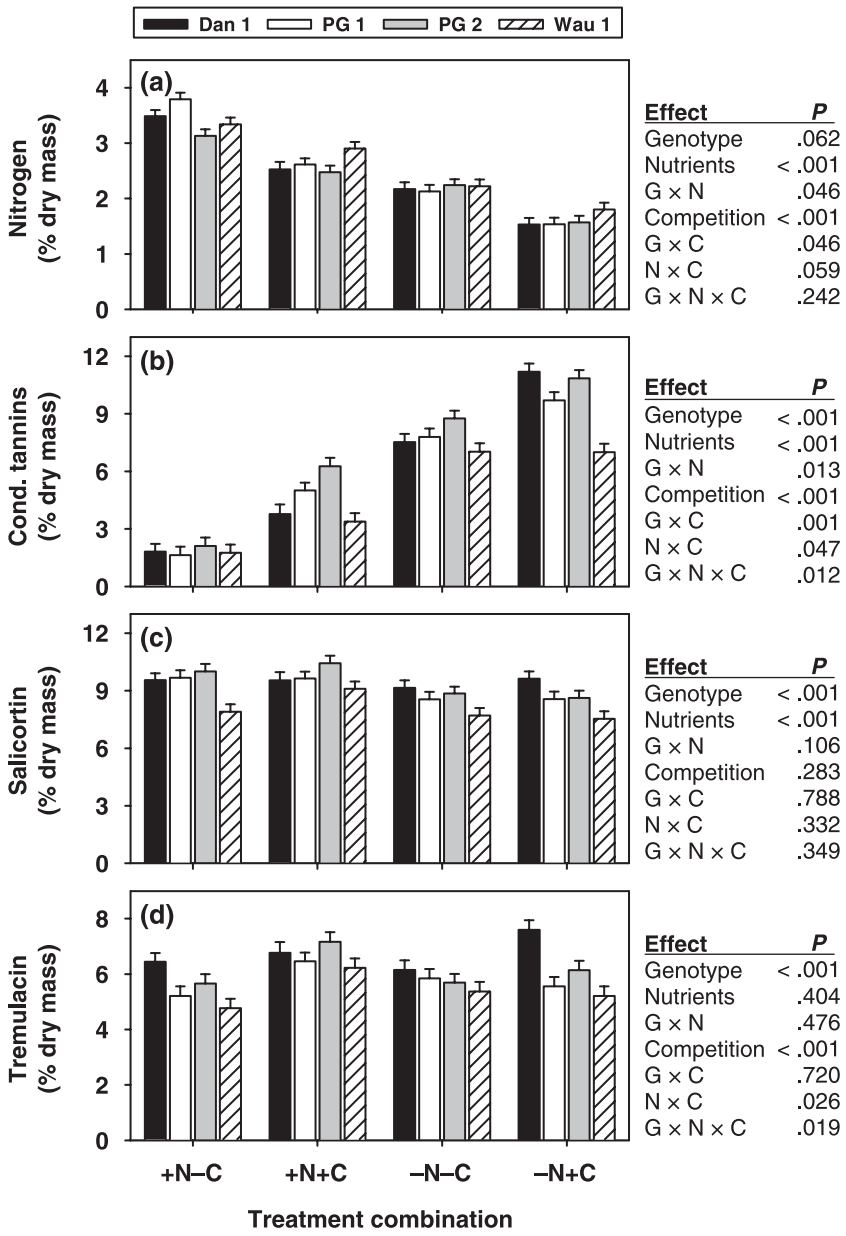

Fig. 4 Leaf phytochemical concentrations among four aspen (*Populus tremuloides*) genotypes (Dan 1; PG 1; PG 2; Wau 1) (G) grown under different treatment conditions. Format as for Fig. 1. Bar heights are genotype averages ± 1 pooled SE (n = 5 trees).

markedly as nutrient availability decreased and competition increased. Condensed tannin (CT) concentrations varied substantially among genotypes and increased sharply with decreased fertility and with competition (Fig. 4b). Tannin concentrations of WAU 1 showed a distinctly more conservative response to environment than did those in the other three genotypes (significant genotype × environment interaction). At both high and low fertility, differences among genotypes were much greater when grown with competition than without. Concentrations of salicortin and tremulacin were less variable among the four aspen genotypes than were tannins (Fig. 4c,d). Concentrations of salicortin decreased slightly in low fertility, and tremulacin levels increased when trees were grown with competition. Interactive effects of genotype and environment were largely insignificant for PGs except that tremulacin concentrations were significantly higher for genotype DAN 1 at low fertility when grown with competition.

Tradeoffs between growth and chemical defense

While genotypes varied significantly in allocation to foliar condensed tannins (CT mass ratio), RGR was not directly correlated with this variation (either within or across treatments). In both low-fertility treatments, however, RGR was negatively correlated with PG mass ratio (Fig. 5). The relationship was highly significant under the most stressful treatment combination (low nutrients plus competition), and marginally so under low-fertility conditions without competition. For example,

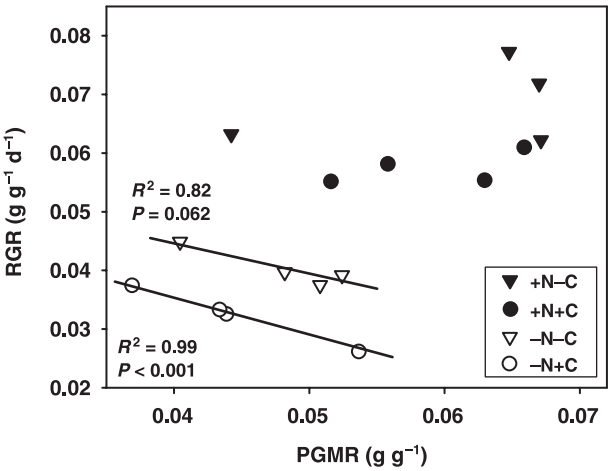

Fig. 5 Negative phenotypic correlations between relative growth rate (RGR) and phenolic glycoside mass ratio (PGMR). Each point represents the mean value for a single aspen (*Populus tremuloides*) genotype (n = 5 trees) within each of the four treatment combinations plus and minus nutrients (+N; –N, respectively), and minus and plus competition (–C; +C, respectively).

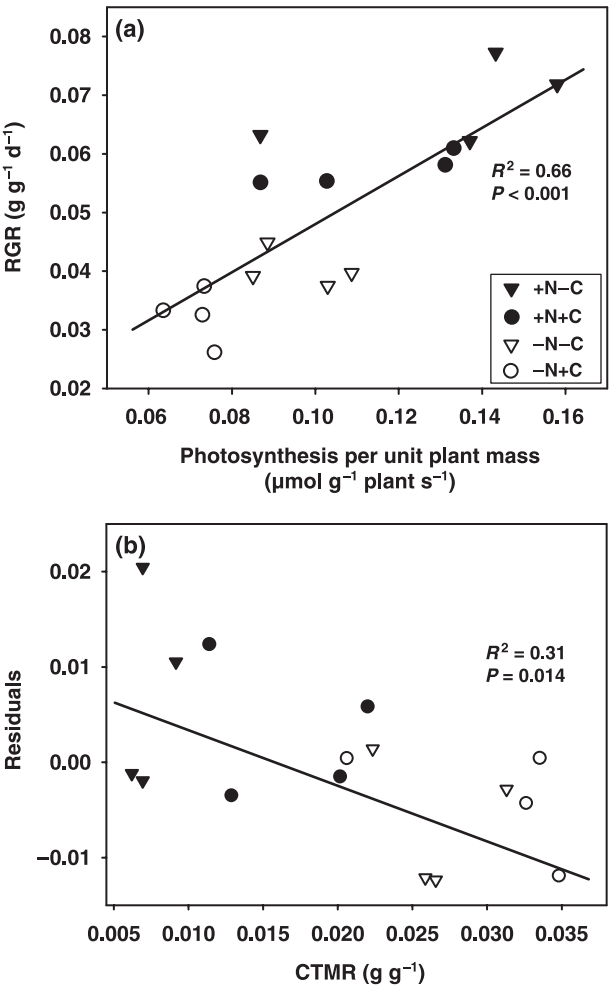

Fig. 6 Regression analyses of (a) relative growth rate (RGR) as a function of growth potential (photosynthesis per unit plant mass) at high and low fertility (+N; –N, respectively), and (b) residuals from (a) vs condensed tannin mass ratio (CTMR). Each point represents the mean value for a single aspen (*Populus tremuloides*) genotype (n = 5 trees) within each of the four treatment combinations (as Fig. 5).

under low fertility plus competition, a 29% range in RGR among genotypes was associated with 26% range in PG mass ratio. Under high fertility, with or without competition, no such tradeoff occurred.

Stress-mediated increases in allocation to foliar tannins also appeared to exact costs with regard to growth. Photosynthesis per unit plant mass, an index of growth potential, explained 66% of the variation in RGR across genotypes and treatments (Fig. 6a). A significant portion of the residual error from this regression was explained by condensed tannin mass ratio, indicating a potential growth–defense tradeoff (Fig. 6b). By adding the effect of fertility in a multiple regression model, we were able to explain 85% of the variation in RGR.

Discussion

Aspen typically exhibits considerable genetic variation with regard to plant chemistry and growth (Lindroth & Hwang, 1996; Hwang & Lindroth, 1997; Osier & Lindroth, 2006). This study sought to identify potential relationships between growth and allocation to secondary metabolites as they vary among genotypes and in response to nutrient availability and belowground competition.

Although growth varied modestly among genotypes, a significant negative phenotypic correlation between RGR and levels of PGs was nevertheless observed. Similarly to the results of Osier & Lindroth (2006), this tradeoff was evident only when resources were limiting. An examination of the relationship between PG concentration and other growth determinants (photosynthesis, leaf mass ratio, specific leaf area) did not reveal a mechanism for this putative defense cost. For example, a relative increase in allocation to nonstructural leaf mass (e.g. secondary chemicals, nonstructural carbohydrates) can lead to decreased specific leaf area and therefore a dilution of N and decreased assimilation efficiency (Roumet *et al.*, 1999). Although specific leaf area trended downward as levels of PGs increased (not shown) in this study, the relationship was not statistically significant. There is evidence that PG maintenance costs can be high in aspen (Kleiner *et al.*, 1999), although research with willow has shown that these compounds are surprisingly stable and turn over relatively slowly (Ruuhola & Julkunen-Tiitto, 2000). The fact that the tradeoff occurred only when resources were limiting suggests that allocation or maintenance costs, as predicted by the growth–differentiation balance hypothesis (Herms & Mattson, 1992) or protein-competition model (Jones & Hartley, 1999), may explain a portion of the patterns observed.

The effects of nutrient availability and belowground competition appear to be additive for the majority of traits measured in this study. Most variables either increased or decreased in a linear fashion with increasing stress (decreased fertility and increased competition). Although nutrient uptake was not measured, the lower leaf [N] in competition treatments, observed with both high and low fertility, indicated that growth losses resulting from the competition treatment were probably related to decreases in either nutrient availability (e.g. caused by nutrient usurpation by grass) and/or aspen nutrient uptake. There was some evidence implicating the latter. Soil testing at harvest indicated that, in the low-fertility treatment, soil NO_3^- levels were actually slightly higher with competition than without (data not shown). Grass could have interfered with aspen's nutrient acquisition in any of several ways (Aerts & Chapin, 2000). For example, competition-mediated decreases in tree transpiration might have led to declines in transport of mobile ions, such as NO_3^- to the root via mass flow (McDonald *et al.*, 2002).

Plant competition may affect plant growth and allocation indirectly via its influence on secondary metabolite production. Although Agrawal (2004) found that competition has no effect on levels of cardenolides or latex in milkweed, results of other studies indicate that competition decreases allocation to defensive compounds in *Brassica napus* (Cipollini & Bergelson, 2001) and tomato (Stamp *et al.*, 2004). The effect of competition may be dependent on the class of compounds produced in the plant. For example, Marak *et al.* (2003) found that competition may affect allocation to C-based compounds differently from allocation to non-C-based compounds. In their study of *Plantago lanceolata*, competition significantly increased allocation to iridoid glycosides. They suggest that plant carbon–nutrient balance may explain such increases. The carbon–nutrient balance hypothesis (CNB; Bryant *et al.* 1983) predicts that nutrient stress results in 'excess' fixed C and therefore increased allocation to C-based chemical defenses. The theory has been debated intensely (Hamilton *et al.*, 2001), but many plants, including aspen, appear to follow its predictions (Hemming & Lindroth, 1999; Lerdau & Coley, 2002). For example, in the present experiment CT concentrations increased with decreasing fertility and with competition.

We note, however, that a key exception to the predictions of CNB occurred in this study. Increased production of CTs under limited resource availability appeared to exact a cost for growth in aspen (increased CT was not solely a product of 'excess' C). Relative to that in high fertility, RGR in low fertility was generally less than would be expected for a given rate of photosynthesis per unit plant mass. This discrepancy was so large that we examined an additional index of growth potential, N mass ratio (calculated in the same manner as CT and PG mass ratios), to ascertain whether photosynthesis per unit plant mass underestimated the direct effect of N limitation on growth.

Nitrogen mass ratio was very similar to photosynthesis per unit plant mass in terms of its role as a predictor of RGR (regression not shown). Thus neither predictor explained all the growth loss exhibited by stressed trees. Rather, some correlate of N limitation constrained growth. We acknowledge that, because N, P and K were manipulated together in our soil fertility treatment, we cannot rule out the influence of P and K in explaining some of the growth differences among treatments. However, allocation and maintenance costs of CTs are second only to lipids and lignin in their relative cost (mg C g^{-1}) to plants (Chapin, 1989), and the more than twofold increase in levels of tannins in low-fertility trees was correlated with the difference between realized growth rates in low vs high nutrient trees. Still, the difference in RGR was greater than is likely based on CT construction costs alone (Chapin, 1989; Poorter, 1994), and other factors may have played a role (e.g. P, K, water availability). Although we are unable to explain fully the magnitude of such a reduction in realized growth, resource limitation and (to a lesser extent) plant competition appear to change allocation patterns in aspen, and may result in compounded costs of chemical defense.

Herbivory and plant competition are among the primary selective agents operating in plant communities. The growth–differentiation balance hypothesis (Herms & Mattson, 1992) provides a conceptual model that describes how these two selective agents interact to maintain genetic variation in plant chemical defense in plant populations. Physiological constraints within the plant lead to a tradeoff between competitive ability (high growth rates) and resistance to herbivory (high levels of defense). If the relative importance of plant competition and herbivory vary in time and space (as they may), then natural selection will maintain variation in a population (Herms & Mattson, 1992). Aspen experiences significant selection pressure via competitive interactions (Barnes, 1966; Landhäusser & Lieffers, 1998; Powell & Bork, 2004) and from herbivores (Lindroth & Hwang, 1996). Genetically variable allocation to chemical defense in aspen may, in part, be maintained by environmental heterogeneity in the relative importance of these two opposing selective pressures.

Results from this study support the hypothesis that there is a tradeoff between growth and defense in establishing (seedling) aspen, and that the tradeoff is mediated by resource availability. When resources are limiting, not only is the cost of chemical defense realized, but changes in allocation (increased condensed tannin concentrations) compound costs by further limiting growth. The effects of this tradeoff are likely to be particularly important during aspen establishment, in both their immediate effects and their long-term influence on aspen populations (Powell & Bork, 2004).

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