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PLANT ANIMAL INTERACTIONS

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Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*)

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Abstract Studies of induction in trees have examined rapid induced resistance (RIR) or delayed induced resistance (DIR), but have not examined induction that occurs in leaves produced by indeterminately growing trees subsequent to, but in the same season as, damage. We refer to induction that occurs during this time period as intermediate-delayed induced resistance (IDIR). We assessed the influences of genetic and environmental factors, and their interactions, on temporal and spatial variation in induction and on tradeoffs between induced and constitutive levels of resistance in indeterminately growing saplings of aspen (*Populus tremuloides*). We utilized a common garden of 12 aspen genotypes experiencing two levels of defoliation and two levels of soil nutrients. We assessed concentrations of phenolic glycosides and condensed tannins in damaged leaf remnants collected 1 week after defoliation to examine rapid and local induction, and in undamaged leaves produced 8 weeks after defoliation to assess intermediate-delayed and systemic induction. In general, tannins showed RIR, while phenolic glycosides expressed IDIR. For both classes of allelochemicals, we found high estimates of broad-sense heritability and genetic variation in both induced and constitutive levels. Genetic variation may be maintained by both direct costs of allelochemicals and by costs of inducibility (phenotypic plasticity). Such costs may drive the tradeoff exhibited between induced and constitutive levels of phenolic glycosides. IDIR may be important in reducing total-season tissue loss by providing augmented resistance against late summer herbivores in trees that have experienced damage earlier in the season. Herbivore-resistant compensatory growth is especially beneficial to young trees growing in competitive environments.

Keywords Defoliation · Genotype × environment interaction · Herbivory · Phenotypic plasticity · Plant defense

Introduction

The induction of plant resistance chemicals in response to herbivory varies temporally and spatially (Karban and Myers 1989). In terms of timing, studies of induction in trees have examined rapid induced resistance (RIR) or delayed induced resistance (DIR). RIR occurs within days in leaves present at the time of damage and affects the herbivore generation responsible for damage, while the effects of DIR are expressed in leaves produced in the following growing season and affect subsequent herbivore generations (Haukioja 1990; Clausen et al. 1991; Parry et al. 2003). A critical gap in our understanding of induction concerns the time period between RIR and DIR. Specifically, how do levels of resistance respond to defoliation in leaves produced by indeterminately growing trees subsequent to, but in the same year as, damage? We refer to induction that occurs during this time period as intermediate-delayed induced resistance (IDIR). In spatial terms, RIR can occur locally (in damaged leaves) or systemically (in undamaged leaves), while DIR and IDIR can occur only systemically.

Genetic and environmental factors and their interactions play important roles in affecting temporal and spatial patterns of induction. Genetic variation in inducibility was demonstrated experimentally (Zangerl and Berenbaum 1990; van Dam and Vrieling 1994; Robison and Raffa 1997; Ruuhola et al. 2001; Agrawal et al. 2002), and plant defense theories predict environ-

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mental effects on induction (Coley et al. 1985; Herms and Mattson 1992). According to these theories, plants adapted to high-nutrient environments are fast-growing, have low constitutive levels of resistance, and are more likely to show inducibility than slow-growing plants that have high constitutive levels of resistance and are common in resource-poor environments.

Tradeoffs between inducible and constitutive levels of resistance may have developed due to costs of resistance (Karban and Baldwin 1997), which are requisite to most plant defense theories (Feeny 1976; Coley et al. 1985; Herms and Mattson 1992). Induction may reduce costs of resistance by allowing plants to deploy high levels of resistance only in response to damage (Rhoades 1979; Clark and Harvell 1992), but inducibility (phenotypic plasticity) itself may come at a fitness cost (Relyea 2002). Cost saving may be particularly important to young trees that often grow in light-competitive environments where growth is paramount.

A characteristic common among herbs and fast-growing trees is indeterminate growth. It is characterized by primary extension and tissue formation from apical and lateral meristems that is sustained until late in the growing season. Indeterminately growing plants that experience herbivory early in the season produce leaves later in the same season that are both temporally and spatially separated from damage. We know of no other research that has addressed induction in leaves produced by indeterminately growing trees subsequent to, but in the same season as defoliation, despite the fact that indeterminate growth is exhibited by many tree species.

Aspen (*Populus tremuloides*) serves as a host to a diverse array of leaf-feeding insects and is favored by expansive outbreak folivores such as large aspen tortrix (*Choristoneura conflictana*), forest tent caterpillar (*Malacosoma disstria*), and gypsy moth (*Lymantria dispar*) (Mattson et al. 1991). Aspen produces two general classes of carbon-based allelochemicals, phenolic glycosides and condensed tannins. Much research (Hemming and Lindroth 1995; Hwang and Lindroth 1997; Osier and Lindroth 2001) implicates phenolic glycosides in the reduction of insect performance, while few studies support the role of condensed tannins as anti-insect compounds (Ayres et al. 1997). Tannins may function as resistance chemicals against bacterial and fungal pathogens (Kellam et al. 1992), or in other roles such as inhibitors of photodamage (Close and McArthur 2002). Levels of both types of allelochemicals are induced in aspen by defoliation. Clausen et al. (1989) and Osier and Lindroth (2001) found RIR of phenolic glycosides and tannins respectively, and DIR was documented for phenolic glycosides (Clausen et al. 1991) and for tannins (Osier and Lindroth 2004). In addition to producing inducible allelochemicals, aspen grows indeterminately when young. Thus, aspen is particularly well-suited for the study of temporal and spatial variation in induction.

In this study, we describe induction over two temporal and spatial scales. Induction that occurs within a week in damaged leaves will be described as *rapid* and *local*, while induction that is evident months later in undamaged leaves produced subsequent to defoliation will be described as *intermediate-delayed* and *systemic*. Our rapid, local induction is equivalent to the RIR described in the literature, while our intermediate-delayed, systemic induction occurs in the time period between RIR and DIR.

The objectives of this study were to assess temporal and spatial variation in the induction of phenolic glycosides and condensed tannins in aspen. We predicted that genotypes would vary in phenotypic plasticity (inducibility) and that growth under high-nutrient conditions would damp induction responses. We also hypothesized that genotypes with high constitutive levels of phenolics would be less inducible than those with low constitutive levels.

Materials and methods

Experimental design

The experiment was a split-plot common garden that utilized defined defoliation levels, soil resource environments, and genotypes. The 2×2×12 split-plot design had two levels of defoliation (0 and 75%), two levels of nutrients, and 12 genotypes. Defoliation and soil nutrient treatments were crossed at the whole plot level, with genotype as a sub-plot. The 48 treatment combinations were replicated with four independent saplings for a total of 192 trees.

Genotypes

The aspen genotypes used in this study were derived from genetic material originally field-collected from south-central Wisconsin. These genotypes represent a wide range of variation in growth and levels of phenolic glycosides and tannins. Roots from source trees were collected and processed to produce new shoots, which were then clipped and introduced into a micropropagation system. Micropropagation reduces the potential problems of topophysis (Wright 1976), where clones retain non-genetic effects from the source individual (analogous to maternal effects).

Propagation

The micropropagates were established outdoors in spring 2001 in 5 l pots containing a 40:40:20 mix of sand: silt-loam field soil:perlite with Osmocote 3–4 months slow release fertilizer (14:14:14 N–P–K + micronutrients; 4.5 g/l of soil). They were watered as needed during the summer and overwintered outside under leaf mulch. In spring 2002, just prior to budbreak, trees were transplanted into 80 l pots containing approximately 60 l of a mixture of 70% sand and 30%

silt-loam field soil, and arranged into experimental blocks. To manipulate nutrient availability, Osmocote 8–9 months slow release fertilizer (18:6:12 N–P–K + micronutrients) was added to the high-nutrient pots at a rate of 4.5 g/l of soil; low-nutrient pots received no fertilizer. Trees at this time averaged 1.1 m in height.

Defoliation

We imposed heavy defoliation to mimic the intensity of an insect outbreak (Mattson et al. 1991). The use of artificial defoliation ensures that all genotypes receive the same level of defoliation regardless of their leaf chemistry. However, because actual herbivory has been shown to sometimes elicit a stronger response than artificial defoliation (Karban and Baldwin 1997; Havill and Raffa 1999), we used a combined approach with both insects and scissors.

Defoliation commenced on 7 June 2002 on half of the 192 trees. Twenty percent of the branches on each experimental tree were bagged collectively, and one third-instar forest tent caterpillar per branch was placed into each bag. The insects were allowed to feed for 10 days. This exposure of experimental trees to insects provided the saliva, frass, and related signals that may be requisite for induction. The majority of defoliation, however, was conducted manually. To ensure that all trees received the same level of defoliation, artificial defoliation using pinking shears began on 10 June 2002 and was completed within a week. We removed 75% of each leaf by cutting near each leaf base. Pinking shears were chosen because the blade provides a jagged edge that maximizes leaf damage and more closely mimics the damage imposed by a folivorous insect than does a standard blade.

Chemical analyses

To assess levels of foliar phenolics and nitrogen, we collected leaves from all four experimental blocks twice during the 2002 growing season. The first collection occurred in June, 1 week after the 10-day defoliation event was completed. From the defoliated trees, we haphazardly collected 10–15 leaf remnants from throughout the crown. We followed the same protocol for the control trees except we collected 10–15 undamaged leaves (as the control trees were not exposed to defoliation and the background level of damage was quite low). The leaves collected from both defoliated and undefoliated trees derived from the initial leaf flush and were the same in terms of age and stage of development. The second collection was made in August, 8 weeks after defoliation. From the defoliated trees, we haphazardly collected 10–15 undamaged leaves produced subsequent to defoliation on indeterminately growing branches. We collected likewise for the control trees. Leaves produced on indeterminately growing branches could easily be distinguished (based on phenology) from leaves of the initial flush and were the same age in both defoliation treatments. Indeterminate growth was substantial during the summer growing season and in 1 month (mid-June to mid-July) represented an average 43% increase in leaf area among low-nutrient defoliated trees and a 120% increase among high-nutrient defoliated trees, relative to the initial June leaf flush area.

Samples were kept under ice in the field, then flash-frozen in liquid N_2 and freeze-dried in order to preserve the integrity of leaf chemicals (Lindroth and Koss 1996). Phenolic glycoside (salicortin and tremulacin) levels were determined by high performance thin layer chromatography (Lindroth et al. 1993). Condensed tannins were extracted from leaf tissue in 70% acetone at 4°C (with 10 mM ascorbic acid as an antioxidant) and quantified by the acid butanol method of Porter et al. (1986), using purified aspen tannins as standards. Nitrogen levels were assessed in a LECO elemental analyzer (St. Joseph, MI, USA) using glycine *p*-toluenesulfonic acid ($N = 5.665\%$) as a standard.

Statistical analyses

The effects of defoliation, genotype, and soil nutrient availability, and their interactions, on phenolic glycosides, condensed tannins, and nitrogen were analyzed with a mixed-model, three factor split-plot ANOVA using JMP IN Version 4.0.4 (SAS Institute Inc. 2001). We considered defoliation and nutrient level as fixed effects and genotype as a random effect. Defoliation treatment and nutrient level were analyzed as whole plot effects, fully crossed, with block incorporated as a random variable. Genotype was incorporated as a sub-plot factor within whole plot treatments. All interactions between genotype and whole plot treatments were included as sub-plot interactions. In the split-plot analysis, a whole plot error term (replicates within whole plots error) was used to test whole plot effects, while a split-plot error term (residual error) was used to test sub-plot effects. Phenolic glycoside data displayed normality and equal variances, but the condensed tannin and nitrogen data were arcsin-square-root transformed to improve homoscedasticity. The two data sets (June and August) were analyzed separately.

Broad-sense (clonal) heritability (Harvell 1998) estimates were obtained by calculating the proportion of total phenotypic variance explained by genotypic variance within each damage/nutrient environment combination (Falconer 1985; Bailey et al. 2004). Broad-sense heritability estimates include maternal and epistatic effects, whereas narrow-sense heritability estimates, typically obtained from half-sibling families, remove maternal effects and estimate only additive genetic variation. Pearson correlation coefficients were calculated to assess the relationship between induction (absolute change in levels of phenolic glycosides or condensed tannins) and constitutive levels of these chemicals under both low- and high-nutrient conditions in both June and

August. An analysis of covariance (SAS Institute Inc. 2001) was used to compare the slopes of these relationships.

Results

RIR: rapid and local effects of defoliation (June)

Phenolic glycoside concentrations responded weakly to defoliation and nutrient availability, as expressed only by a marginally significant interaction between the two treatments (Fig. 1). In response to defoliation, concentrations decreased slightly under low-nutrient conditions, but increased 15% under high-nutrient conditions. Phenolic glycosides were strongly influenced by genotype and ranged from 6% to 18% dry weight when pooled across other treatments.

In contrast, levels of condensed tannins were influenced primarily by defoliation and nutrients, rather than by genotype (Fig. 1). Damaged leaves showed substantial rapid and local induction of tannins, increasing 118% when pooled across nutrient and genotype treatments. The effect of defoliation varied among genotypes and showed higher values and a larger absolute increase under low, compared with high, nutrient conditions. Levels of condensed tannins varied by genotype, ranging from 6% to 27% of leaf dry weight when pooled across defoliation and nutrient treatments. Nutrients affected tannin levels such that under low-nutrient conditions, tannin levels averaged 55% higher than under high-nutrient conditions.

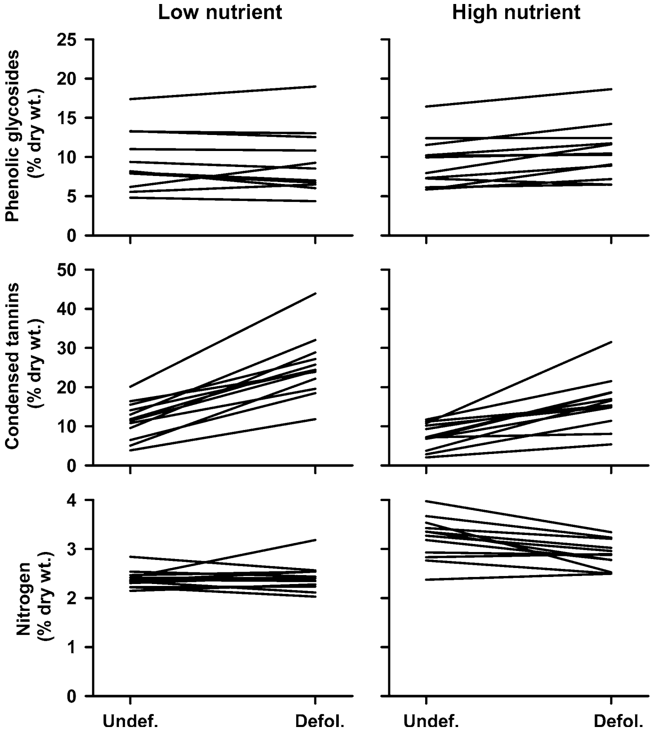
The response of leaf nitrogen levels to defoliation differed with regard to soil nutrient level (Fig. 1). Low-nutrient damaged leaves had nitrogen levels that were slightly higher than their controls. This response was largely due to a marked increase in one genotype. High-nutrient damaged leaves had nitrogen levels that were 11% lower than their controls. Nitrogen levels differed among genotypes across the two nutrient environments and as expected, increased (27%) in response to soil nutrient addition.

IDIR: intermediate-delayed and systemic effects of defoliation (August)

The effects of defoliation on phenolic glycosides were much more pronounced in August than in June (Fig. 2). Phenolic glycosides showed substantial IDIR (an average 36% increase) in undamaged leaves produced by previously defoliated trees. Defoliation interacted with nutrient level such that low-nutrient trees increased phenolic glycoside levels by 63%, while high-nutrient trees increased by only 15%. The response to defoliation differed among genotypes. Genotypic differences remained highly significant (ranging from 8% to 16% dry wt.), and differences among genotypes were influenced by nutrient environment.

In stark contrast to the response of phenolic glycosides, and in spite of a major RIR response in June, condensed tannins showed no IDIR in August (Fig. 2). In fact, previously defoliated trees produced leaves that had condensed tannin levels actually 18% lower than those of

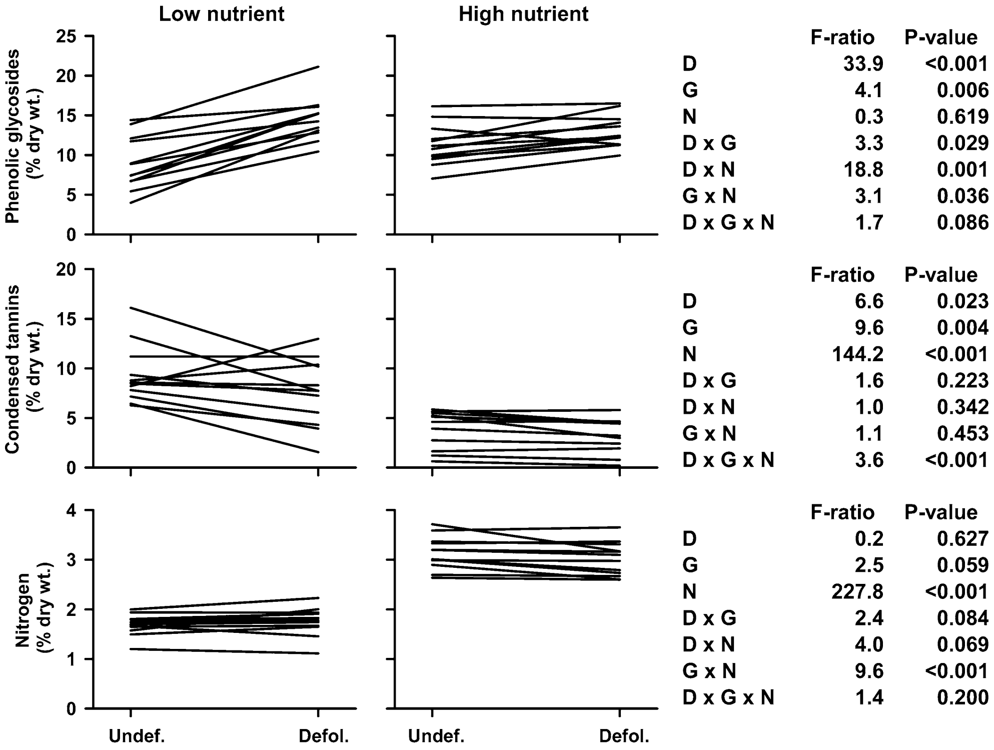
Fig. 1 Rapid induced resistance: norm of reaction plots for foliar concentrations of phenolic glycosides, condensed tannins, and nitrogen in June in relation to defoliation ("*D*," df = 1), genotype ("*G*," df = 11), and soil nutrient availability ("*N*," df = 1). *F*-ratios and *P*-values indicate the results of a 3-factor, split-plot ANOVA. *Each line* represents the mean response ($n = 4$ replicates) of a single aspen genotype in the undefoliated versus defoliated condition



	F-ratio	P-value
D	2.3	0.151
G	29.5	<0.001
N	0.5	0.487
D x G	1.5	0.253
D x N	4.3	0.060
G x N	1.6	0.214
D x G x N	1.5	0.134

	F-ratio	P-value
D	75.9	<0.001
G	6.9	0.003
N	75.8	<0.001
D x G	4.6	0.009
D x N	4.5	0.051
G x N	1.0	0.532
D x G x N	2.2	0.020

	F-ratio	P-value
D	6.0	0.057
G	0.8	0.644
N	35.4	<0.001
D x G	1.7	0.184
D x N	23.4	0.035
G x N	5.1	0.006
D x G x N	1.1	0.375



	F-ratio	P-value
D	33.9	<0.001
G	4.1	0.006
N	0.3	0.619
D x G	3.3	0.029
D x N	18.8	0.001
G x N	3.1	0.036
D x G x N	1.7	0.086

	F-ratio	P-value
D	6.6	0.023
G	9.6	0.004
N	144.2	<0.001
D x G	1.6	0.223
D x N	1.0	0.342
G x N	1.1	0.453
D x G x N	3.6	<0.001

	F-ratio	P-value
D	0.2	0.627
G	2.5	0.059
N	227.8	<0.001
D x G	2.4	0.084
D x N	4.0	0.069
G x N	9.6	<0.001
D x G x N	1.4	0.200

Fig. 2 Intermediate-delayed induced resistance: norm of reaction plots for foliar concentrations of phenolic glycosides, condensed tannins, and nitrogen in August in relation to defoliation ("*D*," df = 1), genotype ("*G*," df = 11), and soil nutrient availability ("*N*," df = 1). *F*-ratios and *P*-values indicate the results of a 3-factor, split-plot ANOVA. *Each line* represents the mean response ($n = 4$ replicates) of a single aspen genotype in the undefoliated versus defoliated condition. Note that the scale for condensed tannins differs from Fig. 1

their controls. The response to defoliation varied among genotypes under low-nutrient conditions, but was similar under high-nutrient conditions. Main effects of genotype and nutrient level also were evident in August. Genotypes ranged from 3% to 9% dry wt. tannins, and low-nutrient trees had tannin levels 132% higher than high-nutrient trees, when pooled across other treatments.

The response of leaf nitrogen levels to defoliation was weak, and expressed only in terms of marginal interactions with genotype and nutrients (Fig. 2). Individual genotypic levels of nitrogen varied in the two soil nutrient environments. In June, leaves from high-nutrient trees had higher nitrogen levels, but in August, the differences were even more pronounced (78% higher).

Heritability of resistance factors

We determined broad-sense (clonal) heritability (Harvell 1998; Bailey et al. 2004) for both allelochemicals by calculating the proportion of total phenotypic variance explained by genotypic variance in each of the four damage/nutrient environment combinations for both June and August. We found that heritability was generally high for both phenolic glycosides and condensed tannins (0.48–0.89, and 0.71–0.90, respectively) (Table 1). Because our trees were exposed to ecologically relevant soil nutrient conditions and defoliation levels, our experimentally determined heritability values are likely representative of values for aspen in the field. Further, because our 12 genotypes were selected haphazardly from throughout south-central Wisconsin, they likely mirror natural levels of genetic variation in the region.

Tradeoffs between induction and constitutive levels of resistance

In June, we found no evidence of tradeoffs between absolute induction and constitutive levels of either phenolic glycosides or condensed tannins. In August,

Table 1 Broad-sense heritability estimates with 95% confidence intervals for phenolic glycosides and condensed tannins in June and August in each nutrient/defoliation environment

	June		August	
	Phenolic glycosides	Condensed tannins	Phenolic glycosides	Condensed tannins
Low nutrient				
Undefoliated	0.83 ± 0.01	0.81 ± 0.02	0.81 ± 0.02	0.71 ± 0.03
Defoliated	0.89 ± 0.01	0.90 ± 0.01	0.70 ± 0.04	0.80 ± 0.02
High nutrient				
Undefoliated	0.81 ± 0.02	0.71 ± 0.03	0.75 ± 0.03	0.77 ± 0.02
Defoliated	0.79 ± 0.02	0.77 ± 0.03	0.48 ± 0.07	0.77 ± 0.02

however, induction of phenolic glycosides was negatively correlated with constitutive levels of the compounds, in both low- and high-nutrient conditions (Fig. 3). An analysis of covariance revealed that although phenolic glycoside induction was influenced by both soil nutrient environment ($P = 0.001$) and constitutive levels of phenolic glycosides ($P = 0.004$), the relationship between induced and constitutive levels was consistent across the two nutrient environments ($P = 0.984$). Tannins showed no tradeoffs between induced and constitutive levels of expression.

Discussion

Environmental, physiological, and genetic effects on RIR and IDIR

Although much research has addressed the effects of soil nutrient availability on constitutive levels of allelochemicals, less work has focused on how soil nutrient environment affects RIR in trees, and no prior work has addressed IDIR. Mutikainen et al. (2000) showed that soil fertilization enhanced RIR in birch, while Hunter and Schultz (1995) reported the opposite pattern in oaks. Glynn et al. (2003) found that the effect of soil nutrients on RIR can vary among insects. In their study of *Populus nigra*, gypsy moth growth was negatively correlated with foliar phenolics (tannic acid equivalents) while whitemark tussock moth growth was not affected by the compounds. Glynn et al. (2003) reported that soil nutrients increased the expression of RIR against gypsy moths, but decreased the response against whitemarked tussock moths. We found that RIR of phenolic glycosides was limited to high-nutrient environments, although RIR was pronounced for condensed tannins in both nutrient environments. With regard to IDIR, we found substantial induction of phenolic glycosides under low-nutrient conditions but only slight induction under high nutrients. Condensed tannins, on the other hand, showed no IDIR and decreased in response to defoliation under both nutrient conditions.

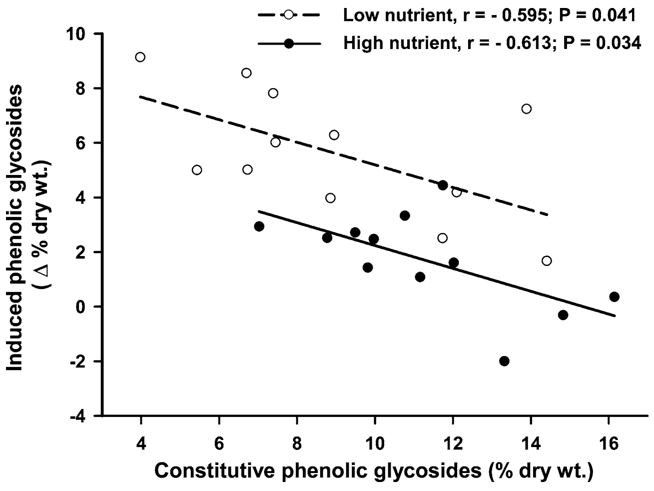


Fig. 3 Correlation between induced phenolic glycosides (absolute change in concentrations) in August and constitutive phenolic glycosides in August under both low- and high-nutrient conditions. *Each point* represents the mean response ($n = 4$ replicates) of a single aspen genotype

Although research addressing the physiological mechanisms of induction of phenolic glycosides is lacking, recent physiological work on the induction of condensed tannins in *Populus* may help explain why condensed tannins showed RIR, but not IDIR in our study. Arnold and Schultz (2002) and Arnold et al. (2004) reported that induction of tannins in hybrid poplar was linked to the establishment of a carbohydrate sink. In June, the damaged leaves that exhibited RIR were likely carbohydrate sinks, while the undamaged leaves produced in August subsequent to defoliation were likely sources of carbohydrates and, therefore, showed no tannin induction.

In addition to the environmental and physiological effects, genetic factors influenced the induction of phenolic glycoside and condensed tannin levels. The genetics underlying certain biochemical mechanisms of induction in *P. tremuloides* have recently been elucidated. Peters and Constabel (2002) showed that tannin induction in aspen occurs in response to mechanical damage and forest tent caterpillar herbivory through increased expression of dihydroflavonol reductase and other phenylpropanoid genes. The multi-gene induction system is likely responsible for the genetic variation in inducibility we observed for RIR of tannins in June and for IDIR of phenolic glycosides in August.

Genetic variation in induction is especially likely when costs of resistance, costs of inducibility, and/or tradeoffs between constitutive and induced levels of resistance are present. For example, genetic variation in induction may be maintained by different types of herbivore pressure (Tiffin and Rausher 1999). Conditions where herbivore populations are stable, likely favor plants with high constitutive levels of resistance. Such plants are not likely to benefit greatly from induction. In contrast, variable herbivore populations may favor plants with low but inducible resistance factors. Genetic variation in induction is further maintained by induction responses that are modulated by the environment. Thus, genetic variation in induction (phenotypic plasticity) is to be expected, and has been reported in other studies of the Salicaceae, including hybrid poplars (Robison and Raffa 1997) and *Salix myrsinifolia* (Ruuhola et al. 2001).

Costs of resistance, costs of inducibility, and tradeoffs between constitutive and induced levels of resistance

Physiological costs of resistance (including both tradeoffs with growth and opportunity costs) may explain why genetic variation is maintained for constitutive and induced levels of allelochemicals. Physiological costs can be particularly high for saplings because growth is critically important for juvenile trees. Hwang and Lindroth (1997) showed that total allelochemicals (including both tannins and phenolic glycosides) are negatively correlated with growth in aspen and Osier (2001) reported that phenolic glycosides are negatively correlated with aspen growth. These correlations between resistance and growth can be very strong. For example, Osier (2001) found that a 1% increase in phenolic glycoside concentrations can decrease aspen growth by as much as 5%. These findings indicate that aspen genotypes with low constitutive levels of allelochemicals may out-compete aspen genotypes with high levels, especially if herbivores are effectively deterred through induction.

Inducibility, or phenotypic plasticity, also may exact fitness costs (Relyea 2002). Genetic variation in the induction response may be maintained by costs of plasticity, as the ability to respond chemically to defoliation may prevent the commitment of resources to growth and/or reproduction (Agrawal et al. 2002).

We calculated high estimates of broad-sense (clonal) heritability for both classes of allelochemicals. Heritability estimates for phenolic glycosides generally were higher among undamaged versus damaged trees, while the reverse was true for condensed tannins (Table 1). That we found genetic variation and high heritability estimates for both constitutive and induced levels of allelochemicals provides evidence that both direct costs of allelochemicals and costs of plasticity may exist.

Differences between costs of constitutive and induced allelochemicals can drive the tradeoffs between constitutive and induced levels of resistance predicted by plant defense theory (Herms and Mattson 1992) and recently confirmed by the meta-analysis of Koricheva et al. (2004). We found such tradeoffs for phenolic glycosides in August when induction was substantial (Fig. 3). Genotypes with high constitutive levels of phenolic glycosides may not benefit from induction, while genotypes that maintain low constitutive levels are likely to benefit from even modest levels of induction. Mattson and Palmer (1988) and Nef (1988) also found such tradeoffs for levels of total phenolics and inducibility in *Populus*.

Tradeoffs between constitutive and induced resistance can be affected by both environmental and genetic factors (Gatehouse 2002). Constitutive levels of phenolic glycosides under low-nutrient conditions were lower and more inducible than levels under high-nutrient conditions. Plants growing under low-nutrient conditions may be subject to more variable levels of herbivore pressure that select for low, yet responsive, levels of resistance. However, we found a very similar relationship between constitutive and induced levels of phenolic glycosides in both soil nutrient environments, as evidenced by the similar slopes of the correlations in Fig. 3. This result suggests that although constitutive concentrations and inducibility of phenolic glycosides are phenotypically plastic with respect to environmental conditions, the tradeoff observed is largely driven by genetics. A genetically driven relationship that is preserved across a range of soil nutrient conditions is evidence for a tradeoff canalized by selection.

Ecological implications

Induction of allelochemicals after defoliation may be protective against various damaging agents. The substantial RIR observed in both nutrient environments for tannins may be a response to protect physically damaged leaves from opportunistic pathogens and/or fungi (Kellam et al. 1992). A damaged crown also allows for increased light penetration, so high levels of tannins may protect leaf remnants from ultraviolet light (Close and McArthur 2002). Phenolic glycosides showed only limited RIR, but in contrast to tannins showed substantial IDIR. Induction of phenolic glycosides in undamaged leaves produced after early season defoliation may be an antiherbivore response to protect against late-season herbivory and damage to compensatory growth.

Although the role of condensed tannins as anti-insect resistance chemicals may be limited, RIR of both condensed tannins and phenolic glycosides is likely most effective against late spring folivores such as forest tent caterpillars and gypsy moths (generalists), and large aspen tortrix (an aspen specialist). Hemming and Lindroth (1995, 1999) showed that phenolic glycosides reduced the growth of forest tent caterpillars and gypsy moths, while Bryant et al. (1987) showed that condensed tannins and a simple phenolic extract (including phenolic glycosides) reduced the growth of large aspen tortrix.

Rapid and local induction may be particularly effective against the large aspen tortrix, since tortrix larvae form webs around adjacent leaves and feed for multiple days on the same branch (Clausen et al. 1991). In fact, both RIR and IDIR may be important against large aspen tortrix. These insects are univoltine but have two feeding periods each summer. Second instars emerge from hibernaculae and feed early in the growing season (May and June). Their feeding may trigger an increase in phenolic glycosides that can offer resistance against first instars from the next generation that emerge and feed in July and August. Although first instars do less damage in August than the instars feeding during the early spring (Cerezke and Volney 1995), the first instars are very active and can disperse on spun threads (Prentice 1955). Thus, IDIR of phenolic glycosides may play a crucial role in protecting aspen saplings from damage, especially if tortrix larvae disperse from highly resistant clones.

Intermediate-delayed, systemic induction of phenolic glycosides is likely beneficial for young, indeterminately growing trees because phenolic glycosides negatively affect late summer folivores such as the generalist Canadian tiger swallowtail (*Papilio canadensis*) and the aspen specialist, big poplar sphinx moth (*Pachysphinx* *modesta*) (Hwang and Lindroth 1998). Using regression equations reported by Hwang and Lindroth (1998), we determined that the mean induction of phenolic glycosides observed under low-nutrient conditions in August (63%), would result in 34% and 23% decreases in larval weights of Canadian tiger swallowtails and big poplar sphinx moths, respectively. Multivoltine folivores such as the whitemarked tussock moth (*Orgyia leucostigma*) may also be adversely affected by IDIR because they feed both early and late in the same growing season and are negatively impacted by phenolic glycosides (Agrell et al. 2000).

In addition to the induced chemical resistance factors, plants can offset damage from herbivores through compensatory growth, expressing tolerance to herbivory (Strauss and Agrawal 1999). Plants that grow indeterminately can better compensate for tissue loss (Haile et al. 1998). In fact, indeterminately growing trees may be less susceptible to early season defoliation because they can continue to grow throughout the summer and compensate for lost tissue. This may explain why RIR of phenolic glycosides was so limited in June. Indeterminately growing trees, however, may not be able to tolerate late season herbivory because of the limited time available for regrowth before leaf senescence. Late season damage is likely to be particularly detrimental in trees that also have experienced damage early in the season. Hence, aspen responds to early damage with high induced levels of phenolic glycosides late in the season, and increases are greatest among genotypes with low constitutive levels. Chemically resistant compensatory growth may enhance the ability of aspen to tolerate herbivory.

In summary, we observed both RIR and IDIR in aspen, with tannins exhibiting primarily the former and phenolic glycosides exhibiting primarily the latter. Within-tree temporal and spatial variation in induction likely enhances the efficacy of the allelochemicals. Specifically, RIR of phenolic glycosides may benefit trees by spreading damage more uniformly throughout the crown (Karban and Baldwin 1997) while the pronounced RIR seen for tannins may protect damaged leaves from pathogens or ultraviolet light. The IDIR observed for phenolic glycosides likely lessens total-season tissue loss by providing augmented resistance against late summer herbivores in trees that have experienced damage earlier in the season. Although the propensity toward indeterminate growth (and thus IDIR) decreases in many trees as they age, IDIR likely benefits trees during a critical stage when competition with other plants is intense and growth is of paramount importance.

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