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Browse Quality in Quaking Aspen (*Populus tremuloides*): Effects of Genotype, Nutrients, Defoliation, and Coppicing

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Abstract The consequences of interactions among genetic, ontogenetic, and environmental factors for the quality of winter-dormant tissues as food for browsing herbivores is poorly understood. We conducted two sequential common garden studies to assess the impacts of intraspecific genetic variation, nutrient availability, prior defoliation, and ontogenetic stage on the chemical quality of winter-dormant tissue in quaking aspen (*Populus tremuloides* Michx.). In the first study, saplings of 12 aspen genotypes were grown under low and high soil nutrient conditions, with or without two successive seasons of defoliation. Quantity and quality of current year's twig growth were assessed. Twig production varied among genotypes and declined under low nutrient availability, but showed little response to prior defoliation. Chemical quality of sapling twigs varied substantially among genotypes, and in response to nutrient availability and prior defoliation. Overall, browse quality improved (nitrogen levels increased while phenolic glycoside and condensed tannin levels decreased) after defoliation. Growth and chemical variables exhibited low to moderate clonal repeatability (broad sense heritability) values. Our second study employed the same 12 genotypes, grown under high-nutrient conditions and with or without two seasons of defoliation. The trees were coppiced to produce root sprouts, which were chemically assessed 1 yr later. Rejuvenation via coppicing

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led to increased levels of nitrogen, phenolic glycosides (salicortin), and tannins in root sprouts, and the magnitude of change varied among aspen genotypes. Signatures of defoliation nearly 2 yr earlier persisted in terms of elevated levels of phenolic glycosides in root sprouts of previously defoliated trees. Aspen forests likely present browsing herbivores with chemically heterogeneous environments because of the interactions of genetic, ontogenetic, and environmental factors that vary over space and time.

Keywords Condensed tannins · Defoliation · Genetic variation · Heritability · Ontogeny · Phenolic glycosides · Phytochemistry · Plant–mammal interactions · Winter

Introduction

The quality of plant tissues as food for herbivores is determined by the complex interplay of plant genetics, ontogenetics, biotic factors such as prior feeding damage, and abiotic factors such as resource availability. The independent and interactive effects of these factors on the quality of foliage for herbivorous insects have been assessed in numerous studies (reviewed by Berenbaum and Zangerl 1992; Fritz and Simms 1992; Karban and Baldwin 1997; Agrawal et al. 1999; Nykanen and Koricheva 2004). Much less is known, however, about how these factors influence the quality of woody tissues for browsing mammals.

During winter periods in north temperate and boreal forests, herbaceous vegetation is of low nutritional quality because of senescence and is often inaccessible because of snow. Mammalian herbivores rely heavily on dormant woody plants for food (Bryant and Kuropat 1980). Browsing mammals typically feed on the current year's growth (CYG), including woody stems and buds. Such damage alters the quantity and quality of foliage produced in the following growing season, with attendant impacts on the performance of folivorous insects (Danell and Huss-Danell 1985; Den Herder et al. 2004; Hochwender et al. 2005; Valkama et al. 2005). The reciprocal interaction, i.e., the effects of defoliation on the subsequent quantity and quality of woody browse, has rarely been investigated.

Quaking aspen (*Populus tremuloides*) and European aspen (*Populus tremula*) are circumpolar in distribution, and are a major food source for browsing mammals in boreal, north temperate, and montane regions. In North America, aspen provides winter food for browsing mammals such as hare, beaver, deer, elk, and moose (Bryant 1981; Perala 1990; Romme et al. 1995). Aspen is also subject to regular and expansive insect outbreaks by species such as forest tent caterpillars, gypsy moths, and large aspen tortrix (Mattson et al. 1991). In the Great Lakes Region, for example, forest tent caterpillars defoliated nearly seven million hectares of forest in 2001. Much of this was aspen forest, where early summer defoliation rates averaged nearly 100% (Donaldson 2005). Quaking aspen thus provides an ideal experimental organism for addressing how winter browse quality is influenced by defoliation in preceding summers.

A characteristic form of asexual reproduction in aspens is the production of new sprouts from adventitious buds of roots, after death of the primary stem. "Coppicing" of aspen trees by natural agents (e.g., browsing mammals or fire) or by timber harvest typically causes prolific suckering, with densities of ramets reaching 30,000–100,000/ha (Zasada et al. 2001). Loss of competing apices in the crown and access to nutrients and growth-promoting cytokinins in the remaining roots leads to "rejuvenation" and rapid growth of root sprouts (Dickmann et al. 2001). In essence, coppicing restarts the ontogenetic clock of sprouts. The juvenile sprouts typically express high levels of chemical defenses, ostensibly as protection

against browsing mammals (Bryant 1981; Bryant et al. 1991; Bryant and Julkunen-Tiitto 1995; Boege and Marquis 2005). Then, as trees grow beyond the reach of mammalian herbivores, levels of chemical defenses typically decline. The effects of prior defoliation on the quality of winter-dormant sprouts and how these may vary among plant genotypes are unknown.

Two of the major determinants of tissue quality for browsing herbivores are nutrients (e.g., protein) and secondary metabolites (Lindroth 1989; Iason 2005). In aspen, the major secondary compounds are phenolics, including salicylate phenolic glycosides and condensed tannins (Lindroth and Hwang 1996). Concentrations of these compounds in aspen foliage are highly genetically variable, as well as differentially responsive to resource availability and prior feeding damage (Osier and Lindroth 2004, 2006; Stevens and Lindroth 2005). These compounds strongly influence interactions of *Populus* species with herbivores (Hemming and Lindroth 1995; Bailey et al. 2004, 2007; Donaldson and Lindroth 2007) and mediate ecosystem processes such as litter decomposition and nitrogen mineralization (Schweitzer et al. 2004; Madritch et al. 2006). Levels of these compounds also exhibit striking ontogenetic variation in aspen foliage, with high levels of phenolic glycosides and relatively low levels of tannins in young ramets, and the reverse in mature trees (Donaldson et al. 2006).

The research reported here had several objectives. First, we were interested in evaluating how aspen genetic variation, soil nutrient availability, prior defoliation, and interactions among those factors influence the quantity and quality of dormant winter browse in aspen. Plant defense theory suggests that levels of carbon-based secondary metabolites should increase in response to both relatively low soil nutrient availability (Herms and Mattson 1992) and prior defoliation (Bryant et al. 1991). Moreover, phenotypic plasticity in response to resource availability and defoliation is likely to vary among plant genotypes, especially in highly genetically variable species such as aspen (Perala 1990; Mitton and Grant 1996). We predicted that (1) current year's twig growth would be reduced under conditions of low nutrient availability or prior defoliation; (2) levels of nitrogen (an index of protein) would decline in twigs in response to low soil fertility or prior defoliation; (3) levels of phenolic glycosides, which are relatively nonplastic in response to environment (Osier and Lindroth 2004, 2006), would change little in response to experimental treatments; (4) levels of condensed tannins, which are highly plastic in response to environment (Osier and Lindroth 2004, 2006), would increase under low soil nutrients and prior defoliation; and (5) aspen genotypes would vary in response to nutrient availability and prior defoliation (significant gene × environment interactions).

Our second objective was to assess genetic variation in tissue quality of dormant juvenile sprouts after coppicing of saplings, and whether chemical profiles of the sprouts retain a signature of prior defoliation of the original (parent) ramet. No soil nutrient treatment was included in this study. We predicted that (1) sprouts would exhibit strong genetic variation in expression of chemical traits; (2) chemical profiles of sprouts would differ from those of parent ramets because of rejuvenation (increased concentrations of phenolic glycosides and decreased concentrations of tannins); and (3) prior defoliation to parent ramets would have no influence on chemical profiles of sprouts a year after removal of all aboveground biomass.

Methods and Materials

Experimental Design

We established a common garden of potted trees on the campus of the University of Wisconsin, Madison. The split-plot experimental design consisted of two soil nutrient

environments, two levels of defoliation (0% and 75%), and 12 aspen genotypes (details below). The defoliation rate of 75% corresponds to rates experienced by natural stands of aspen during outbreaks by forest tent caterpillars (Donaldson 2005). The soil nutrient and defoliation treatments were crossed at the whole plot level, with genotype incorporated at the subplot level. The 48 treatment combinations were replicated across 5 blocks for a total of 240 trees.

Genotypes

We micropropagated root material originally collected from 12 wild aspen genotypes growing in south-central Wisconsin. Micropropagation allows for the replication of many ramets from a single root source and decreases nongenetic effects (analogous to maternal effects) from source tissues (Wright 1976). Microsatellite DNA markers were used to verify that each genotype was unique (Cole and Lindroth, unpublished data).

Propagation

The micropropagates were planted outside into 5-l pots containing a 40:40:20 mix of sand/silt loam field soil/perlite in spring of 2001. All pots received Osmocote 3–4 mo slow release fertilizer (14:14:14 N/P/K + micronutrients) at a rate of 4.5 g/l of soil. In spring 2002, saplings (average height=1.1 m) were transplanted into the experimental garden comprised of 80-l pots. Each pot contained a 70:30 mixture of sand and silt loam field soil. High-nutrient pots received Osmocote 8–9 mo slow release fertilizer (18:6:12 N/P/K + micronutrients; 4.5 g/l) in the spring of both 2002 and 2003. Low-nutrient pots received no fertilizer.

Defoliation

Our defoliation treatment modeled an insect outbreak in both duration and intensity (Mattson et al. 1991; Parry et al. 2003). We severely defoliated experimental trees in two successive summers using both forest tent caterpillars and scissors. Insects were employed to provide natural cues (e.g., frass and saliva) that may be important to trigger tree responses to defoliation (Karban and Baldwin 1997; Havill and Raffa 1999), whereas scissors were used to ensure that all genotypes were damaged similarly regardless of their chemical quality (Stowe et al. 2000; Siemens et al. 2003). In early June of both 2002 and 2003, we exposed a subset of branches on each sapling in the defoliation treatment to forest tent caterpillar herbivory for 10 d. Scissors were then used to remove 75% of each leaf (for details see Stevens et al. 2007). Background levels of natural herbivory at our common garden were low.

Dormant Twig Collection

In March 2004, 9 mo after the second defoliation, we collected winter-dormant twig samples from 4 of the 5 experimental blocks (192 trees). We haphazardly selected five branches from each tree, and ensured that our sample included branches from lower, middle, and upper portions of the crown. The terminal leader was avoided. We measured the length and diameter of the CYG on each selected branch. Diameter was measured at the proximal end of the CYG using calipers. For chemical analyses, we collected the most distal 10-cm portion of each of the five branches per tree. For branches with less than 10 cm

of growth, the entire CYG was collected. If branches had dead tips, those portions were removed and discarded. In the few cases where the entire CYG was dead, length and diameter were measured but chemical analyses were not done.

Coppicing and Dormant Root Sprout Collection

In May 2004, 3 yr after the trees were planted outside, all of the high-nutrient trees were coppiced (severed at the soil surface with loppers). The coppiced trees were allowed to regenerate via root sprouting. In March 2005, we collected the terminal 10 cm of winter-dormant CYG from 3–4 sprouts per pot (1–2 sprouts per pot for 6 trees with little regeneration). Trees from all five experimental blocks of the high-nutrient treatment were sampled.

Phytochemical Analyses

Aspen samples consisting of multiple twigs or spouts per pot were freeze-dried and ground through a Wiley mill (no. 20 mesh). To improve homogeneity of samples, highly fibrous woody particles were removed via sifting through a no. 25 mesh screen. Samples were stored frozen (−20°C) until chemically analyzed.

We analyzed the tissue samples for chemical constituents likely to influence the feeding preferences of browsing mammals. These included nitrogen (an index of protein), phenolic glycosides (salicortin and tremulacin, which typically comprise >95% of the phenolic glycoside pool; Lindroth and Hwang 1996), and condensed tannins. Total nitrogen concentrations were determined with an FP452 LECO (St. Joseph, MI, USA) elemental analyzer. Glycine *p*-toluenesulfonic acid was used as a standard. Phenolic glycosides were quantified by high-performance thin layer chromatography using salicortin and tremulacin purified from aspen as standards (Lindroth et al. 1993). Quantification was effected on the basis of peak heights, and peak identity was confirmed via UV spectral scanning. Condensed tannins were extracted from tissues in 70% acetone (with 10 mM ascorbic acid, 4°C) and quantified by the butanol–HCl assay (Porter et al. 1986). Purified aspen condensed tannins (Hagerman and Butler 1980) served as the standard.

Statistical Analyses

Differences in sapling growth and in concentrations of chemical constituents in CYG among aspen genotypes, and between nutrient and defoliation treatments, were assessed in a three-factor split-plot analysis of variance, using JMP version 5.0.1a. Defoliation and nutrient treatments were whole plots (crossed), and sapling genotype was analyzed as a subplot effect nested within whole plots. In separate analyses, the effects of "ontogenetic stage" (i.e., sapling vs. postcoppice root sprouts), genotype, and defoliation on chemical constituents were assessed using PROC MIXED in SAS. The statistical model was similar to that described above, except that there was no nutrient treatment, and ontogenetic stage was analyzed as a repeated measure. Pairwise relationships among phytochemical variables (among saplings), as well as correlations between sapling and root sprout chemical composition, were assessed using simple correlation analyses performed with JMP. For the sapling–sprout correlations, we used data from the four experimental blocks for which we had paired measurements from "parent" and "offspring" (root sprout) plants.

In studies with multiple ramets of different genotypes, variance component analysis can be used to partition the variance in traits of interest into within- and between-genet

components (Falconer 1989). Because clonally derived plants of a single genotype have identical genetic material, differences among them can be attributed to environmental variation or sampling error. Differences among genets estimate V_G (total genetic variance), which is the sum of V_A (additive genetic variance, the component of genetic variance that responds to selection), nonadditive sources of genetic variance such as dominance and epistasis, and maternal effects. The proportion of total phenotypic variance (V_P) that is genetically based is known as broad-sense heritability ($H^2 = V_G/V_P$). When clonally derived plants are the subject of research, the term "clonal repeatability" is preferred in lieu of "broad-sense heritability" (Falconer 1989). Clonal repeatability is an indirect index (and upper bound) of V_A (Mitchell 2004). We calculated clonal repeatabilities as described by Falconer (1989). Standard errors for repeatability values were estimated following Becker (1984).

Results

Growth indices differed among aspen genotypes and in relation to environmental factors. Similarly, chemical constituents differed among aspen genotypes, and in relation to both environmental and ontogenetic factors. In the following text, descriptions of changes among treatment factors are provided in relative, not absolute, terms, unless noted otherwise.

Genetic and Environmental Effects on Winter-dormant, Sapling Twigs

Current year growth (i.e., twig production) varied among aspen genotypes and in response to nutrient availability, but only weakly in response to prior defoliation. The length of CYG twigs varied by 2.6-fold among genotypes (averaged across nutrient and defoliation treatments; Fig. 1). Twig length increased an average of 76% in high-nutrient trees relative to low-nutrient trees, and genotypes responded differently to changes in nutrient availability (significant genotype × nutrient effect). Twig length did not change significantly, however, in response to defoliation. The diameter of CYG twigs varied by 2.1-fold among genotypes and increased 58% in high-nutrient relative to low-nutrient trees (Fig. 1). Twig diameter declined by 10% in defoliated trees (a marginally significant response). When we incorporated both twig length and diameter into a metric of total twig production (calculated as diameter^2 × length), we found strong effects of genotype, nutrient availability, and genotype × nutrients, but no significant effect of defoliation (data not shown).

Chemical composition of CYG twigs also varied among genotypes and in response to nutrient and defoliation treatments. Nitrogen levels differed among genotypes, especially under high-nutrient conditions (Fig. 2; significant genotype × nutrient interaction). Nitrogen levels averaged 73% higher in aspen twigs under high-nutrient, relative to low-nutrient conditions. Prior defoliation influenced twig nitrogen concentrations, but differently so for the two nutrient treatments (significant nutrient × defoliation effect). Under low-nutrient conditions, defoliation led to a similar increase (29%) in nitrogen levels across genotypes. Under high-nutrient conditions, however, defoliation slightly increased, slightly decreased, or did not alter nitrogen concentrations in aspen genotypes.

Phenolic glycoside concentrations varied markedly among genotypes, but responded less strongly to environmental factors. Levels of salicortin in twig tissues increased slightly (8%) in high-nutrient relative to low-nutrient conditions, with some genotypes responding more strongly than others (Fig. 2; marginally significant genotype × nutrient interaction). Responses to defoliation also varied among genotypes, and included increases, decreases,

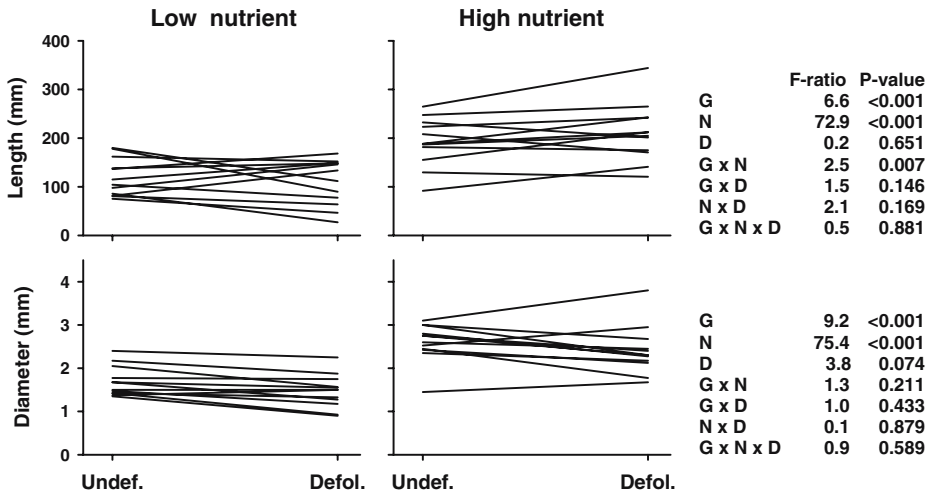

Fig. 1 Genetic and environmental effects on CYG of terminal branches (twigs) in quaking aspen saplings. Norm of reaction plots reveal responses for branch length and diameter after two successive seasons of 75% defoliation. Each line represents the mean response (N=4 replicate trees) of a single aspen genotype in the undefoliated vs. defoliated condition, under low and high soil nutrient availability. Summary statistics are provided to the right of the figure; G = genotype, N = nutrient treatment, D = defoliation treatment

and no change in concentrations (significant genotype × defoliation interaction). Levels of tremulacin showed a more pronounced increase (19%) under high-nutrient conditions, and again some genotypes responded more strongly than others (Fig. 2; significant genotype × nutrient interaction). With the exception of one genotype, tremulacin concentrations declined (an average of 15%) in response to prior defoliation.

As was the case for phenolic glycosides, condensed tannin concentrations varied most among genotypes and less in response to environment (Fig. 2). Levels varied an average of 1.6-fold among genotypes, and this variance was greater under high-nutrient than low-nutrient conditions (significant genotype × nutrient interaction). The marginally significant genotype × defoliation interaction reflects the fact that most genotypes exhibited slight declines in tannin levels in response to defoliation, whereas a few showed small increases or no change.

Genetic and environmental factors influenced sapling tissue quality in a similar manner for several chemical constituents (Table 1). Concentrations of both salicortin and tremulacin were positively correlated with concentrations of nitrogen. As expected, levels of the two phenolic glycosides were strongly and positively correlated with each other. Concentrations of condensed tannins were weakly and positively correlated with concentrations of tremulacin.

Clonal Repeatability of Sapling Twig Growth and Chemistry Variables

Both twig length and diameter exhibited moderately high clonal repeatabilities (Table 2), indicating a substantial genetic component in expression of those traits. The genetic component of variance in twig chemical characteristics varied from low to moderately high, even between structurally similar constituents (i.e., salicortin and tremulacin).

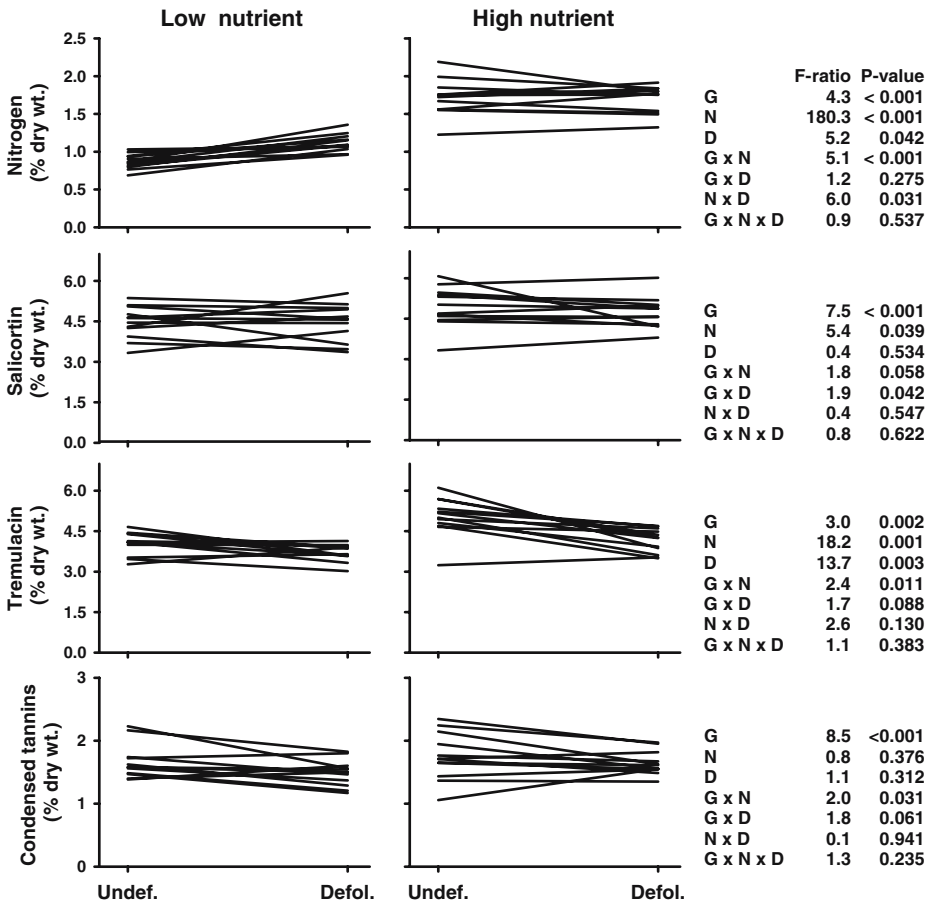

Fig. 2 Genetic and environmental effects on chemical composition of winter-dormant twigs in quaking aspen saplings. Norm of reaction plots reveal responses for nitrogen, salicortin, tremulacin, and condensed tannin concentrations after two successive seasons of 75% defoliation. Each line represents the mean response (N=4 replicate trees) of a single aspen genotype in the undefoliated vs. defoliated condition, under low and high soil nutrient availability. Summary statistics are provided to the right of the figure; G = genotype, N = nutrient treatment, D = defoliation treatment

Table 1 Correlation matrix (Pearson) for browse quality of dormant aspen saplings

	Nitrogen	Salicortin	Tremulacin
Salicortin	0.179*		
Tremulacin	0.352***	0.619***	
Condensed tannins	0.081	0.124	0.146*

*0.01<P<0.05

**0.001<P<0.01

***P<0.001.

Table 2 Clonal repeatability for dormant aspen twig productivity (CYG) and chemistry variables (SE = standard error)

Variable	Clonal Repeatability	SE
Twig length	0.318	0.045
Twig diameter	0.406	0.044
Nitrogen	0.214	0.044
Salicortin	0.353	0.045
Tremulacin	0.141	0.042
Condensed tannins	0.386	0.044

Genetic, Ontogenetic, and Defoliation Effects on Winter-dormant Root Sprouts

The chemical composition of dormant root sprouts shifted from that of parent saplings (i.e., as a function of ontogenetic stage), and differed both among genotypes and in response to defoliation history of the saplings. Nitrogen levels increased slightly (7%) in root sprouts, relative to sapling twigs, and genotypes varied in their magnitude of response (significant genotype × ontogenetic stage interaction; Fig. 3). Prior defoliation of aspen saplings did not significantly alter nitrogen levels in root sprouts. In general, nitrogen levels varied more among genotypes than in response to either coppicing or prior defoliation.

Phenolic glycoside concentrations exhibited strong genetic and ontogenetic effects, as well as interactions between those factors and defoliation (Fig. 3). Rejuvenation increased salicortin concentrations by 49 to 153% among aspen genotypes (significant genotype × stage interaction). Prior defoliation affected salicortin levels differently among genotypes (significant genotype × defoliation interaction). Defoliation had little effect on salicortin levels in saplings, but slightly increased (8%) levels in root sprouts (significant stage × defoliation effect). Similar to salicortin, tremulacin levels varied among genotypes, and differently so for the two ontogenetic stages (significant genotype × stage effect; Fig. 3). Contrary to the pattern exhibited by salicortin, however, average tremulacin levels were similar between rejuvenated root sprouts and saplings. Moreover, in response to prior defoliation, levels of tremulacin decreased (an average of 18%) in saplings, but increased slightly (an average of 5%) in root sprouts (significant stage × defoliation effect).

Condensed tannin concentrations varied among genotypes and ontogenetic stages, but not in response to prior defoliation, and showed relatively few interactive effects (Fig. 3). Rejuvenation stimulated an increase in tannin concentrations, ranging from 5 to 72% among genotypes (significant genotype × stage interaction).

Correlation analyses revealed surprisingly few significant relationships between parent sapling and root sprout chemical constituents (Table 3). We found positive correlations between parents and sprouts for nitrogen (marginally significant) and salicortin, but only in trees that had previously been defoliated.

Discussion

Although much research has addressed the interactive effects of genetics, ontogenetics, resource availability, and herbivore damage on the quality of foliar tissues, remarkably little work has evaluated the consequences of such interactions for the quality of winter-dormant woody tissues. To our knowledge, this is the first attempt to address all of those factors in a

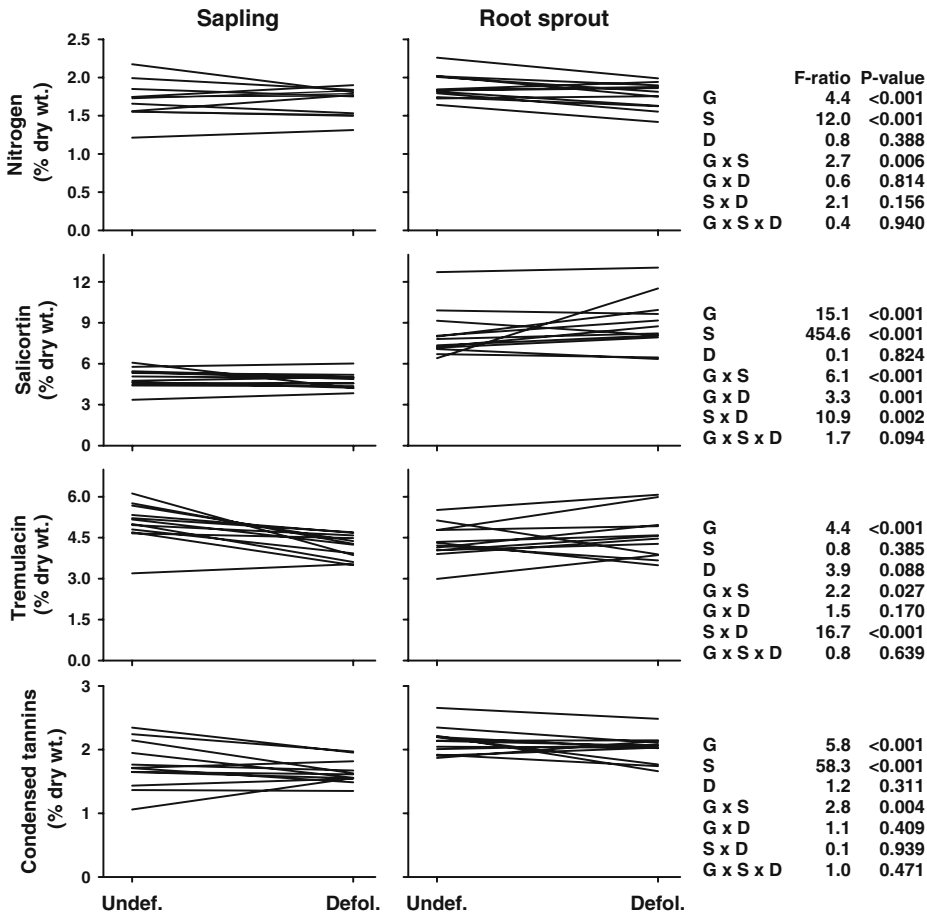

Fig. 3 Genetic, ontogenetic, and environmental effects on chemical composition of winter-dormant twigs in quaking aspen saplings and root sprouts. Norm of reaction plots reveal responses for nitrogen, salicortin, tremulacin, and condensed tannin concentrations after two successive seasons of 75% defoliation. Note the difference in scale for salicortin in Fig. 3 vs. Fig. 2. Each line represents the mean response of a single aspen genotype, as either saplings (N=4) or root sprouts (N=5), in the undefoliated vs. defoliated condition. Summary statistics are provided to the right of the figure; G = genotype, S = ontogenetic stage, D = defoliation treatment

Table 3 Correlations (Pearson) between sapling and root sprout chemistry

Variable	Undefoliated Trees		Defoliated Trees	
	r	*P*	*r*	*P*
Nitrogen	0.212	0.201	0.317	0.052
Salicortin	−0.095	0.570	0.399	0.013
Tremulacin	0.218	0.189	−0.070	0.678
Condensed tannins	0.036	0.828	0.089	0.605

single study. Here we document that for aspen, the production and chemical composition of dormant woody tissue is strongly influenced by a complex of independent and interacting genetic, ontogenetic, and environmental factors. The resulting temporal and spatial variation in food quality likely holds implications for the ecology and evolution of plant–mammal interactions.

Total current year twig production declined substantially under low-nutrient conditions, but weakly if at all in response to two successive seasons of heavy defoliation. This finding suggests that the availability of winter-dormant browse to vertebrate herbivores may not be significantly reduced in years after extensive defoliation by outbreak species such as forest tent caterpillars and large aspen tortrix. Moreover, growth responses to defoliation did not differ among aspen genotypes. This result is similar to those of a larger study (of which these trees were a part), which documented little genotypic variation in allocation of biomass to stem (aboveground woody) tissues, in response to defoliation of high-nutrient trees (Stevens and Lindroth, unpublished data). Finally, moderately high clonal repeatability values indicate that CYG twig production may show a strong response to selection, assuming no constraints beause of genetic correlations with other factors influencing fitness.

Nitrogen concentrations of dormant twigs increased in response to high-nutrient conditions and in response to prior defoliation in low-nutrient trees. The latter increase was contrary to our expectations; heavy defoliation typically causes fine root mortality and reductions in plant nitrogen concentrations (Bryant et al. 1991). These results suggest that defoliation may elicit changes in the sites of overwinter storage of nitrogen-containing compounds. The primary form of reduced nitrogen storage in perennating tissues of plants is vegetative storage proteins. In *Populus*, the major form of storage proteins is bark storage proteins, which accumulate during autumn senescence in tissues such as bark, wood, and roots (Cooke and Weih 2005). Extensive defoliation may shift allocation of bark storage proteins from roots and primary stems to CYG tissues, thereby elevating concentrations of nitrogen.

Levels of phenolic glycosides varied substantially among aspen genotypes, increased slightly to moderately under high-nutrient conditions, and exhibited mixed responses (salicortin) or declines (tremulacin) after defoliation. This pattern of substantial variation among genotypes, coupled with relatively small responses to resource availability and prior damage, is similar to that observed in previous studies of both rapid and delayed induced resistance in aspen foliage (Osier and Lindroth 2001, 2004). Overall, concentrations of phenolic glycosides in winter-dormant twigs were similar to those in leaf tissues of trees growing in the same common garden (Stevens et al. 2007) and in comparably aged trees (Donaldson and Lindroth 2007).

Condensed tannin concentrations also showed substantial genotypic variation, coupled with minimal and genotype-dependent responses to nutrient availability and defoliation. These latter, environmentally mediated changes were different from what we had predicted. Previous studies of aspen foliage have consistently demonstrated both substantial decreases in tannin concentrations when plants are grown in nutrient-rich environments (Bryant et al. 1987; Osier and Lindroth 2001, 2004, 2006; Stevens and Lindroth 2005; Donaldson and Lindroth 2007) and strong short- and long-term induction in response to defoliation (Osier and Lindroth 2001, 2004; Stevens and Lindroth 2005). This disparity in expression profiles between winter-dormant wood and summer leaf tissues may indicate differences in both the cost of metabolite production and the risk of tissue damage between the two seasons. Finally, overall levels of tannins in winter-dormant twigs were low in comparison with levels in foliage of comparably aged trees (Donaldson and Lindroth 2007; Stevens et al. 2007).

Although sapling aspen showed substantial genetic variation in terms of current year twig production and tissue chemistry, the genotypes tended to respond similarly to the

defoliation treatment imposed (i.e., relatively few genotype × defoliation interactions). Thus, phenotypic plasticity of woody tissue response to foliar damage may be constrained in aspen, and expressed more strongly in response to other environmental factors, such as resource availability. Such patterns have been observed for both short- and long-term induction responses in foliage (Osier and Lindroth 2001, 2004).

In summary, the general effect of prior, extensive defoliation was to not alter the quantity, but increase the quality, of winter-dormant twigs in sapling aspen. In this unique case of induced susceptibility, damage to one form of tissues (leaves), by one set of herbivores (outbreak insects), during the growing season, is likely to influence susceptibility of another set of tissues (CYG twigs), to another set of herbivores (browsing mammals), during winter dormancy. Whether preferences of and damage imposed by browsing herbivores in the field actually do change after major summer defoliation events remains unknown. We are aware of only one study that evaluated the impact of defoliation on subsequent mammal browsing. Hjältén et al. (1994) found that voles preferred previously defoliated birch seedlings over controls, despite the fact that defoliation did not significantly affect phenolic concentrations in birch shoots.

Estimates of heritability for quantitative traits indicate the proportion of phenotypic variance that is genetically determined. Heritability values calculated for concentrations of secondary metabolites in other experimental systems are highly variable, spanning the range from 0 to 1 (Berenbaum and Zangerl 1992; Laitinen et al. 2005). In this study, broad-sense heritability (clonal repeatability) values for secondary metabolites were low to moderate. Particularly low values (e.g., as for tremulacin) may reflect previous strong selection for the trait, and subsequent loss of genetic variation (Falconer 1989). Our value for tannins was similar to that reported by Laitinen et al. (2005) for birch condensed tannins, whereas our value for salicortin was similar to that reported by Orians et al. (1993) for the same compound in *Salix*. In contrast, Stevens and Lindroth (2005) and Donaldson and Lindroth (2007) reported high values (>0.70) of clonal repeatability for foliar concentrations of tannins and phenolic glycosides in aspen. This disparity may reflect a difference in the heritability of woody vs. foliar chemical constituents. Or, it may simply highlight the fact that estimates of heritability can vary greatly among habitats and studies. Because no general rules exist for extrapolating among environments (Pigliucci 2005), comparisons among studies must be interpreted cautiously.

During plant development, both the risks of herbivore damage and the costs of antiherbivore defenses are likely to change, leading to ontogenetic variation in the expression of defense traits (Boege and Marquis 2005). In general, levels of secondary metabolites tend to be higher in juvenile than in adult tissues (Bryant, 1981; Bryant and Julkunen-Tiitto 1995; Laitinen et al. 2005). Few studies, however, have evaluated genetic variation in defense traits across multiple developmental stages for plants growing in standardized environments (e.g., common gardens). Rejuvenation of biochemical and physiological characteristics in plant tissues is of particular ecological and evolutionary relevance in aspen, where damage to individual trees (ramets) because of browsing, logging, or fire typically elicits a dense flush of root sprout production.

We observed strong ontogenetic shifts in the chemistry of dormant root sprouts. Juvenile sprouts contained elevated levels of nitrogen, salicortin, and tannins, relative to the saplings from which they derived. Genotype × stage interactions were significant for all chemical variables, revealing that expression of genetic variation in defense differs among ontogenetic stages. That genotypes exhibit different ontogenetic trajectories (*sensu* Boege and Marquis 2005) explains why we observed few significant correlations in chemical traits between precoppice trees and their root sprouts. Somewhat surprisingly, signatures of

defoliation carried through to affect several chemical traits of sprouts, despite the facts that defoliation had occurred nearly 2 yr previously, and that all aboveground biomass had been removed 10 mo earlier. For nitrogen, the effects of prior defoliation on root sprouts mirrored those on saplings. For phenolic glycosides, however, concentrations generally increased in response to prior defoliation for root sprouts, but not for saplings.

Striking ontogenetic variation in biochemical and physiological function is a hallmark characteristic of *Populus* (Dickmann et al. 2001; Donaldson et al. 2006). Numerous studies relating the ontogeny of individual leaf development (within a season) to the biosynthesis, transport, and accumulation of secondary metabolites have employed *Populus* as a model system (e.g., Arnold and Schultz 2002; Kleiner et al. 2003; Orians 2005; Donaldson et al. 2006; Rehill et al. 2006). Additional studies have linked ontogenetic shifts in resistance (whole plant development) to variation in the distribution and abundance of herbivorous insects on poplars (Kearsley and Whitham 1989; Martinsen et al. 1998). Little research, however, has addressed ontogenetic variation in the chemical quality of woody tissues in *Populus*. Bryant (1981) showed that adventitious shoots of aspen produce increased amounts of extractable "resin" (chemical composition unknown), which deters feeding by snowshoe hares. Our results are consistent with that finding, and suggest that elevated levels of tannins and phenolic glycosides (salicortin) in dormant root sprouts may serve as deterrents to mammalian browsers. Indeed, consumption of aspen summer foliage and twigs by elk and porcupine is negatively related to concentrations of phenolic glycosides (Bailey et al. 2007; Diner et al., unpublished data; Wooley and Lindroth, unpublished data), and selection of *Populus* branches by beaver is inversely correlated with bark tannin levels (Bailey et al. 2004).

Given that levels of phenolic glycosides and tannins are strongly genetically determined in aspen, and confer defense against a variety of biotic and abiotic agents (Lindroth 2001), the question arises as to why genetic variation for these traits persists in natural populations. The conventional explanation is that the costs and benefits of chemical defense vary over time and space. Such appears to be the case with aspen. In young aspen, production of phenolic glycosides exacts a cost in terms of growth, particularly in resource-limited habitats (Osier and Lindroth 2006), whereas in older saplings, costs of chemical defense are greatest under favorable growing conditions (Stevens et al. 2007). How cost–benefit relationships are shaped by extensive root systems and clonal integration, particularly among aspen root sprouts after coppicing, is unknown.

In conclusion, aspen exhibits substantial genetic variation in expression of woody tissue chemistry, as has been established previously for aspen foliage. Browse quality is further influenced by ontogenetic stage, as well as by environmental factors such as soil nutrient availability and prior defoliation. Winter browsers, like summer defoliators, are likely to encounter a chemically heterogeneous environment because of the complex interplay of genetic, ontogenetic, and environmental factors that vary spatially and temporally in aspen forests.

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