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Secondary Plant Compounds in Seedling and Mature Aspen (Populus tremuloides) in Yellowstone National Park, Wyoming

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ABSTRACT.—Widespread establishment of seedling aspen (*Populus tremuloides*) occurred in Yellowstone National Park (YNP) following the extensive1988 fires. Aspen stands occupy ~2% of YNP and aspen stems are intensively browsed by native ungulates. Chemical composition, especially secondary compounds, may influence levels of herbivory and, hence, survival of aspen, but concentrations of such compounds in aspen in the northern Rocky Mountains are not known. Quantitative profiles of foliar nitrogen and secondary compounds (condensed tannins and the phenolic glycosides, salicortin and tremulacin) in aspen were assessed to address the following questions: (1) Do concentrations of secondary compounds differ between seedling and mature aspen stands? (2) Do concentrations of secondary compounds in seedling aspen differ between unbrowsed and artificially browsed seedlings? (3) Among mature aspen stands, do concentrations of secondary plant compounds differ among, (a) burned and browsed, (b) unburned and browsed and (c) unburned and unbrowsed stands? Concentrations of phenolic glycosides were significantly higher in seedlings than in mature stands, although condensed tannin concentrations and leaf nitrogen were higher in mature stands. Concentrations of leaf nitrogen and all secondary compounds were greater in unbrowsed seedlings than in seedlings subjected to simulated browsing. Concentrations of secondary compounds did not differ between mature aspen stands that were unburned regardless of whether they were browsed; however, burned stands (all of which were browsed) had significantly greater concentrations of secondary compounds and leaf nitrogen than the unburned stands. Results from this research suggest that foliar phenolic glycosides and tannins are not active defenses induced in response to browsing by large mammals. Rather, variation in levels between juvenile and mature ramets represents ontogenetic shifts in expression of defense, whereas variation between clipped and unclipped seedlings results from shifts in carbon/nutrient availability.

INTRODUCTION

Extensive crown fires in 1988 affected ~45% (400,000 ha) of Yellowstone National Park (YNP) and surrounding lands, producing long-lasting effects on the park's flora and fauna (Christensen *et al.*, 1989; Knight and Wallace, 1989; Turner and Romme, 1994; Turner *et*

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al., 1994a, b; Turner *et al.*, 1997). The fires resulted from an unusually prolonged drought and strong persistent winds (Christensen *et al.*, 1989; Renkin and Despain, 1992). Such fires are major, but infrequent, natural disturbances in the YNP area, occurring at 100 to 300-y intervals (Romme, 1982; Romme and Despain, 1989; Millspaugh and Whitlock, 1995). The fires created a striking mosaic of burned and unburned forest across the landscape, and postfire succession has proven to be complex (Turner *et al.*, 1997; Turner *et al.*, 1998).

A surprising result of the 1988 fires was the unexpected recruitment of aspen (*Populus tremuloides*) seedlings. Aspen seedlings established within burned areas that were previously dominated by lodgepole pine (*Pinus contorta* var. *latifolia*) and in places well beyond the prefire distribution of aspen (Kay, 1993; Tuskan *et al.*, 1996; Romme *et al.*, 1997). Aspen is the only deciduous forest type in YNP and it comprises only ~2% of the forested area of YNP (Despain, 1990). In the Rocky Mountains aspen is primarily a clonal species which reproduces almost exclusively via root sprouting and produces large stands composed of genetically identical stems (Barnes, 1966; McDonough, 1985). Most Rocky Mountain aspen genets are thought to be hundreds or thousands of years old (Baker, 1925; Barnes, 1966), although genetic analyses indicate infrequent episodes of new genet establishment (Jelinski and Cheliak, 1992; Jelinski, 1993; Tuskan *et al.*, 1996). A rare aspen seedling recruitment event followed the 1988 fires which created extensive areas suitable for germination and were followed by several relatively moist summers. The seedling aspen are genetically diverse (Tuskan *et al.*, 1996; Stevens *et al.*, 1999) and have persisted to date in many areas, despite heavy ungulate browsing. However, the long-term fate of aspen stands and seedlings in YNP is uncertain.

Many aspen stands throughout the west, including YNP, appear to have been declining in vigor and abundance through the 20th Century; the causes and consequences of this apparent decline have received considerable discussion (Krebill, 1972; Loope and Gruell, 1973; Schier, 1975; Hinds and Wenger, 1977; Olmstead, 1979; Bartos and Mueggler, 1981; Hinds, 1985; Boyce, 1989; Kay, 1990; Bartos *et al.*, 1994; Romme *et al.*, 1995; Baker *et al.*, 1997). Browsing by herbivores, primarily elk, has been implicated in the decline of aspen in the YNP area (Kay, 1993; Wagner and Kay, 1993; Romme *et al.*, 1995; Ripple and Larsen, 2000). Some authors have suggested that willows (*Salix* spp.) and aspen in YNP have inadequate secondary compounds to defend against elk browsing (*e.g.*, Singer *et al.*, 1994; Singer *et al.*, 1998), but the chemical composition of aspen and its relationship with browsing have not been studied in detail in YNP. In this study we examined the concentrations of secondary plant compounds in leaves of mature and seedling aspen in YNP. Elk feed on shoots of mature aspen primarily in winter, as mature aspen are largely found on ungulate winter ranges (Romme *et al.*, 1995). However, elk also eat the leaves and shoots of the aspen seedlings that grow extensively throughout the summer range (Romme *et al.*, 1997).

The dominant secondary metabolites of aspen are phenolic products of the shikimic acid pathway. These include condensed tannins, phenolic glycosides (the salicylates salicin, salicortin, tremuloidin and tremulacin) and coniferyl benzoate (Palo, 1994; Lindroth and Hwang, 1996a). Tannins and phenolic glycosides occur in leaf, stem and root tissues, whereas coniferyl benzoate occurs only in flower buds. The roles of tannins and phenolic glycosides with respect to feeding by insect herbivores, particularly Lepidoptera, have been the subject of considerable investigation. In short, tannins are ineffective defenses, whereas phenolic glycosides are effective at moderate to high concentrations against many aspen-feeding insects (Bryant *et al.*, 1987; Hemming and Lindroth, 1995; Lindroth and Hwang, 1996a; Hwang and Lindroth, 1998). Coniferyl benzoate provides protection against herbivorous birds such as grouse (Jakubas *et al.*, 1989). Little is known, however, about the influence of aspen secondary metabolites vis à vis the foraging behavior of mammals, particularly

ungulates. Phenolic glycosides and their derivatives, but not tannins, deter feeding by hares (Tahvanainen *et al.*, 1985; Reichardt *et al.*, 1990). Aspen tannins may be similarly ineffective against browsing ungulates, a prediction consistent with the production of tannin-binding salivary proteins in such animals (Austin *et al.*, 1989; Hagerman and Robbins, 1993).

In the north-central U.S.A. aspen exhibit striking variation with respect to quantitative profiles of secondary compounds (Lindroth *et al.*, 1987; Hemming and Lindroth, 1995; Lindroth and Hwang, 1996b). This variation has both genetic and environmental bases (Hwang and Lindroth, 1997; Hemming and Lindroth, 1999). Levels of tannins differ markedly among genotypes, and, in accordance with carbon-nutrient balance theory (Bryant *et al.*, 1983), are highly responsive to resource (nutrient, light) availability. Levels of phenolic glycosides also differ greatly among genotypes, but are much less responsive to resource availability. Defoliation induces production of tannins (Roth *et al.*, 1998), whereas effects on levels of phenolic glycosides are equivocal (Clausen *et al.*, 1989; Lindroth and Kinney, 1998; Roth *et al.*, 1998). The effects of browsing on levels of secondary metabolites in aspen are unknown. According to the carbon-nutrient balance model of woody plant response to browsing (Bryant *et al.*, 1991), we would expect pruning of stems to increase the pool of nutrients available per leaf, thereby increasing foliar concentrations of nutrients (*e.g.*, nitrogen) while decreasing concentrations of tannins and phenolic glycosides. In this model changes in chemistry are not an active response to damage, but a passive response to shifts in resource availability.

Aspen exhibits strong, negative genotypic correlations between growth and defense (total phenolic glycosides). Costs are most pronounced in resource-poor environments. Thus, we would predict that maintenance of high levels of phenolic glycosides would exact a significant cost (in terms of growth) for aspen inhabiting the generally infertile soils of YNP.

This research evaluated concentrations of phenolic glycosides and tannins in seedling aspen (which germinated in 1989, after the 1988 fires) and mature aspen (genets present before the fires) in YNP. Some mature aspen stands burned in 1988 now consist of dense stands of shrubby root sprouts that appear to be unable to grow into tree-sized stems because of heavy browsing every winter. Unburned aspen stands contain large stems with foliage and terminal buds out of reach of elk. Hypotheses were tested in the context of three questions: (1) Do concentrations of secondary compounds in leaves differ between seedling and mature aspen stands? We predicted that concentrations of secondary compounds would be higher in immature aspen than in mature aspen because defense traits "relax" due to escape from browsing afforded by vertical growth. This prediction is consistent with the hypothesis of ontogenetic development of chemical defense against herbivory (Bryant and Julkunen-Tiitto, 1995); some species of early-successional deciduous trees have evolutionarily adapted to substantial herbivory during juvenile stages by the expression of high levels of constitutive defense. Alternatively, concentrations of secondary compounds might be higher in mature aspen because poorly defended stands would have been eliminated by past herbivory. (2) Among seedling aspen, do concentrations of secondary compounds differ between unbrowsed and "browsed" (clipped) seedlings? We hypothesized that concentrations of secondary compounds would be lower in clipped than in unbrowsed seedlings, if production of secondary compounds is mediated by resource availability as postulated by Bryant *et al.* (1991). Alternatively, compounds may be higher in clipped seedlings if ungulate browsing induces their synthesis as an active defense mechanism. (3) Among mature aspen stands, do differences exist in levels of secondary plant compounds among (a) burned-browsed, (b) unburned-browsed and (c) unburned-unbrowsed stands? We predicted that concentrations of secondary compounds would be highest in the stands

that were both burned and browsed because all ramets are below the browse line and exhibit juvenile growth forms.

STUDY AREA

Yellowstone encompasses 9000 km^2 in the northwest corner of Wyoming and is primarily a forested high plateau. Approximately 80% of the park is covered with coniferous forests dominated by lodgepole pine (Despain, 1990). This study focused on the western portion of the subalpine forest plateau that covers most of YNP and supports similar vegetation throughout. The subalpine plateau is composed pimarily of dry infertile habitats on rhyolite substrates, although some mesic, more fertile habitats are found on andesite and lake-bottom substrates (Despain, 1990). The climate is generally cool with relatively moist springs and dry summers (Dirks and Martner, 1982). Mature aspen stands are found mostly around the margins of the subalpine plateau, but post-1988 seedlings are found in burned areas throughout much of the plateau (Romme *et al.*, 1997).

METHODS

Field sampling.—Aspen leaf tissue was collected during August 1998. Samples from seedling aspen were obtained from a large population of postfire seedlings in west central YNP along the Old Fountain Trail [UTM northing-4943050, UTM easting-499500]. Samples from mature aspen were obtained from 10 stands located in the northwest area of the park along the western portion of the Gneiss Creek Trail and within 16 km of the seedling population [UTM northing-4959000, UTM easting-493000]. Samples consisted of mature leaves cut from the stems at the petiole.

In mature stands 25 to 30 leaves were taken from each of five randomly selected ramets (individual stems that share a common root system) representing a cross section of the stand. Three stands were sampled within each of three categories: burned-browsed, unburned-browsed and unburned-unbrowsed. A stand was considered burned if charred wood was observed and tree-sized aspen had been killed by fire; such stands consisted of abundant root sprouts ca. 1 m in height. A stand was considered browsed if the stems showed evidence of current browsing or scars from herbivory within the past five years. Most ramets of unburned stands were >2 m tall; in contrast, the ramets in burned-browsed stands were <1 m tall. Because all burned stands were also heavily browsed, no burned-unbrowsed stands could be sampled.

Seedling aspen were all sampled from within a set of experimental exclosures erected in 1996. Before the erection of the exclosures, the seedling aspen at this site had some natural protection from elk browsing since 1993 because many of the lodgepole pine trees killed by the 1988 fires had fallen and created a dense tangle of stems ca. one meter high that reduced elk access. We sampled 20 seedlings from reference plots that had no experimental treatments; we refer to these as "unbrowsed" seedlings. In addition, we sampled a total of 21 seedlings from within three experimental plots in which seedlings had been subjected to simulated browsing. The annual growth of each seedling had been clipped and removed during October of 1996 and 1997; we refer to this treatment as "clipped" seedlings. To avoid excessive damage to the seedlings from sampling, we restricted our tissue collection to 5–6 leaves from each individual. Leaf samples were quick frozen in the field on dry ice then stored at 0 C until analyzed.

Chemical analyses.—Chemical analyses were conducted to quantify levels of foliar nitrogen (an index of nutritional value), condensed tannins and phenolic glycosides. Nitrogen was measured using a micro-Kjeldahl assay. Leaf samples were digested in acid (Parkinson and Allen, 1975) and aliquots of the digests were subjected to a micro-Nesslerization tech-

TABLE 1.—Concentrations (mean and standard error) of foliar nitrogen and secondary compounds in seedling and mature aspen stands, Yellowstone National Park (August 1998)

Aspen	Nitrogen (% dry weight)	Condensed tannins (% dry weight)	Phenolic glycosides	
			Salicortin (% dry weight)	Tremulacin (% dry weight)
Mature (n = 9)	2.23 (0.09)	7.58 (0.45)	8.47 (1.32)	5.32 (0.72)
Seedling (n = 41)	1.56 (0.07)	4.03 (0.76)	14.58 (1.01)	9.20 (0.78)
	P = 0.0001	P = 0.0064	P = 0.0008	P = 0.0064

nique (Lang, 1958) for quantification of nitrogen. Glycine p-toluene sulfonate (5.67% N) served as a nitrogen standard. Condensed tannins were measured by the acid butanol method of Porter *et al.* (1986), which is based on the hydrolytic conversion of proanthocyanidins to anthocyanidins. Condensed tannins purified from aspen leaves (Hagerman and Butler, 1978) served as the standard. Concentrations of phenolic glycosides were quantified by high-performance thin-layer chromatography (Lindroth *et al.*, 1993), using purified glycosides as standards. We measured levels of only salicortin and tremulacin, as these compounds typically comprise >90% of the phenolic glycoside pool and are the most biologically active of aspen salicylates. Samples were checked for the presence of salicin and tremuloidin, levels of which increase during breakdown of salicortin and tremulacin. No significant amounts were detected, indicating that the freezing method employed did not cause appreciative degradation of the salicylates.

Data analysis.—Analysis of variance was used to compare leaf concentrations of nitrogen, condensed tannins, salicortin and tremulacin as a function of age (seedling or mature), differences in browse status among seedlings (unbrowsed or clipped) and differences in burn and browsed status among mature aspen stands. Each seedling was considered an individual (n = 41) because they are genetically distinct (Tuskan *et al.*, 1996; Stevens *et al.*, 1999). For mature stands, however, data from individual ramets were averaged by stand because they were likely to have come from the same genetic individual (Tuskan *et al.*, 1996). Thus, each stand (n = 9) was considered an individual, although 44 ramets were assayed. Significant differences among means were determined by using Tukey's studentized range test ($\alpha < 0.05$). All statistical analyses were conducted using SAS (SAS Institute, 1992).

RESULTS

Foliar nitrogen concentrations were 43% higher in mature aspen than in seedlings (Table 1). The total concentration of carbon-based secondary compounds was higher in seedlings than in mature trees, but the relative contribution of secondary compounds differed. Levels of tannins were lower than levels of (total) phenolic glycosides, and tannin values were nearly twice as high in mature trees as in seedlings (Table 1). In contrast, levels of phenolic glycosides were 72% higher in leaves of seedlings than in leaves of mature trees.

Among seedlings, concentrations of nitrogen were slightly (10%) higher in clipped than in unbrowsed individuals (Table 2). Levels of tannins were 2.4-fold higher in unbrowsed than in clipped seedlings (Table 2). Concentrations of salicortin did not differ significantly between unbrowsed and clipped seedlings, whereas concentrations of tremulacin were 53% higher in the former than in the latter (Table 2).

Burning and browsing treatments did not significantly alter levels of foliar nitrogen or condensed tannins in mature aspen stands (Table 3). Concentrations of phenolic glycosides,

TABLE 2.—Concentrations (mean and standard error) of foliar nitrogen and secondary compounds in postfire seedling aspen in Yellowstone National Park (August 1998). Clipped aspen were subjected to simulated browsing during 1996 and 1997 (see text). Unbrowsed aspen showed no evidence of browsing since 1993

			Phenolic glycosides	
Seedling aspen	Nitrogen (% dry weight)	Condensed tannins (% dry weight)	Salicortin (% dry weight)	Tremulacin (% dry weight)
Clipped (n = 21)	1.63 (0.08)	2.43 (0.77)	13.50 (1.36)	7.30 (1.04)
Unbrowsed (n = 20)	1.48 (0.06)	5.78 (0.75)	15.7 (0.84)	11.2 (0.52)
	P = 0.0030	0.0039	0.1653	0.0015

however, averaged 1.8- to 2.4-fold higher in burned-browsed stands than in unburned-browsed or unburned-unbrowsed stands (Table 3).

DISCUSSION

Overall, the magnitude of variation in levels of nitrogen among the different age and browse/burn classes was small in relation to that of the secondary compounds. This pattern mirrors that exhibited by aspen grown in common gardens and in the field in the Great Lakes region of the U.S.A. (Hwang and Lindroth, 1997, 1998; Lindroth and Hwang, 1996a, b). Levels of nitrogen were quite low in the aspen foliage we assayed, consistent with the nutrient-deficient status of volcanic soils in YNP. When compared with aspen leaf samples collected in the Midwest, the nitrogen concentrations observed in this study were considerably lower than those of aspen in fertile soils (~2.5–4%; Hemming and Lindroth, 1995) and even in infertile glacial outwash soils (~1.7–2.8%; Lindroth and Hwang, 1996b). Levels of tannins and phenolic glycosides were comparable to those measured in aspen of the Great Lakes region (Hemming and Lindroth, 1995; Lindroth and Hwang, 1996b).

Mature and seedling aspen differed in their concentrations of nitrogen and carbon-based secondary compounds. We hypothesized that concentrations of secondary compounds would be greater in the seedling aspen, and this was supported for phenolic glycosides but not for tannins. Although little is known about the protective role of aspen tannins and phenolic glycosides against mammalian herbivores, several studies with mammals and many with insects suggest that phenolic glycosides are more efficacious than tannins as defenses

TABLE 3.—Concentrations (mean and standard error) of foliar nitrogen and secondary compounds in leaf tissue of mature aspen in Yellowstone National Park (August 1998). Burned-browsed stands were burned during the 1998 fires and have responded with profuse vegetative sprouting. Unburned stands were not affected by the 1998 fires and were distinguished by whether they did or did not show evidence of ungulate browsing. Within columns, means followed by the same letter do not significantly differ

			Phenolic glycosides	
Aspen stand	Nitrogen (% dry weight)	Condensed tannins (% dry weight)	Salicortin (% dry weight)	Tremulacin (% dry weight)
Burned-browsed (n = 3)	2.22[a] (0.09)	6.96[a] (0.67)	12.84[a] (1.53)	8.46[a] (0.44)
Unburned-browsed (n = 3)	2.31[a] (0.08)	7.05[a] (1.45)	7.10[b] (1.67)	3.98[b] (1.41)
Unburned-unbrowsed (n = 3)	2.11[a] (0.11)	7.88[a] (0.54)	5.79[b] (0.77)	3.49[b] (0.32)
	P = 0.6711	P = 0.8867	P = 0.0313	P = 0.0068

(Tahvanainen *et al.*, 1985; Reichardt *et al.*, 1990; Lindroth and Hwang, 1996a). Thus, the much higher concentrations of phenolic glycosides observed in seedling aspen compared to mature aspen may reflect "relaxation" of defense in mature trees as they grow above the browse line. Ontogenetic shifts (changes in gene expression with age) in chemical composition have been described for a number of early successional deciduous species and have typically been considered as an evolutionary response to varying levels of browsing between young and mature plants (Bryant and Julkunen-Tiitto, 1995). Indeed, Basey *et al.* (1988) reported that juvenile quaking aspen in the Sierra Nevada range are more heavily defended against feeding by beaver than are mature aspen, although the phenolic metabolite responsible was not identified. Our data are consistent with the notion that high levels of defensive compounds in young plants may be viewed as an evolutionary adaptation to age-specific variation in susceptibility to mammalian herbivory. How ontogenetic shifts in defensive capability may alter resistance to attack by herbivorous insects, which can extensively defoliate adult trees (Fitzgerald, 1995) is not known.

In addition to ontogenetic shifts in chemical defense capacity, resource availability influences defense traits. As we had hypothesized, concentrations of secondary metabolites were generally lower in clipped than in unbrowsed seedlings, whereas nitrogen values showed the opposite trend. These results are consistent with the carbon-nutrient balance model of woody plant response to browsing. According to Bryant *et al.* (1991), browsing decreases the number of subsequent leaves, leading to an increase in available nutrients per leaf. Clipped seedlings indeed did have slightly but significantly higher levels of nitrogen than did unbrowsed seedlings. Higher nutrient availability promotes leaf growth, which in turn reduces carbohydrate pools and accumulation of C-based secondary compounds. Accordingly, levels of tannins and tremulacin were lower in clipped than in unbrowsed seedlings.

We hypothesized that concentrations of secondary plant compounds in mature aspen stands would be highest in stands that were both burned and browsed, and this was observed for phenolic glycosides but not tannins. Similarity in nitrogen and tannin levels among mature aspen stands suggests similar nutrient status as well as reduced genetic variation with respect to tannin production among the nine stands sampled. Levels of phenolic glycosides, however, were higher in the burned-browsed stands than in unburned stands that were or were not browsed. This difference is likely due to ontogenetic shifts in expression of defense traits, as described previously for seedling and mature aspen. Root sprouts in the burned stands were nearly all browsed, resulting in maintenance of low-stature (~30 cm tall), shrubby individuals. These individuals may express juvenile defense traits; indeed, concentrations of phenolic glycosides in the burned-browsed stands were quite similar to those in the seedling population. These seedlings were also growing on burned sites and were of similar height. Unfortunately, the design of our studies does not allow us to distinguish between the influence of burning and the influence of browsing or maintenance of heights that are vulnerable to browsing.

The different patterns observed in concentrations of phenolic glycosides and tannins may reflect their differential effectiveness as a defense against ungulate browsing. Tannins, which may be deactivated by ungulate saliva (Austin *et al.*, 1989; Hagerman and Robbins, 1993) and which are strongly influenced by nutrient availability, had lower concentrations in seedling than in mature aspen and did not differ among treatments of mature aspen. Although little is known about phenolic glycosides as defense against mammals, several studies suggest they may be effective (*e.g.*, Lindroth and Hwang, 1996a). Levels of these compounds differed significantly between seedling and mature aspen, between unbrowsed and clipped aspen seedlings and among mature aspen treatments. Aspen twigs and bark also contain tannins and phenolic glycosides, but how levels of defenses in these tissues

vary with respect to clone, plant age or resource availability is unknown. Given that these tissues are consumed by browsing mammals, evaluation of the factors influencing their chemical composition is important.

This research demonstrates that the chemical defense profiles of aspen in YNP vary with respect to genotype (clone), age and browsing/burning. Our data suggest that foliar phenolic glycosides and tannins are not active defenses induced in response to browsing by ungulates. Rather, variation in levels between juvenile and mature ramets represents ontogenetic shifts in expression of defense, whereas variation between clipped and unclipped seedlings is a function of shifts in carbon/nutrient availability.

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