

NOTE

Variation in the root bark phenolics/sugar ratio of Douglas-fir grown in two plantations in northern Idaho

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Abstract: Several studies have linked high phenolics/sugar ratios in the inner root bark tissue of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) to decreased susceptibility to *Armillaria* spp. While these studies have identified environmental factors that influence root chemistry, none have examined whether the phenolics/sugar ratio is genetically controlled. In this study, we investigated the effects of genetics and environment on the root bark chemistry of 20 families of 15-year-old Douglas-fir planted in two sites in northern Idaho. Only sugar concentrations varied significantly among families, but site was a significant source of variation for phenolics and the phenolics/sugar ratio. Family \times site interactions were significant for the concentrations of all measured root bark compounds as well as for the phenolics/sugar ratio. Phenotypic correlations between height and the phenolics/sugar ratio and between height and sugar concentrations were not significant. However, families with superior height growth and below-average sugar concentrations could be found at both sites. Should a high phenolics/sugar ratio prove effective in selecting genotypes for resistance to *Armillaria* infection, these results suggest that gains could be made more efficiently by selecting for low sugar concentrations.

Résumé : Plusieurs études ont établi une relation entre un rapport composés phénoliques/sucres élevé dans les tissus de l'écorce interne des racines du douglas de Menzies (*Pseudotsuga menziesii* (Mirb.) Franco) et une diminution de la susceptibilité à *Armillaria* spp. Bien que ces études aient identifié les facteurs environnementaux qui influencent la chimie des racines, aucune n'a examiné si le rapport composés phénoliques/sucres est sous contrôle génétique. Dans cette recherche, nous avons étudié les effets de la génétique et de l'environnement sur la chimie de l'écorce des racines de 20 familles de douglas de Menzies âgés de 15 ans et plantés à deux endroits dans le nord de l'Idaho. Seule la concentration en sucres variait significativement entre les familles mais le site était une source de variation significative pour les composés phénoliques et le rapport composés phénoliques/sucres. Les interactions entre les familles et les sites étaient significatives pour la concentration de tous les composés mesurés dans l'écorce des racines de même que pour le rapport composés phénoliques/sucres. Les corrélations phénotypiques entre la hauteur et le rapport composés phénoliques/sucres et entre la hauteur et la concentration de sucres n'étaient pas significatives. Cependant, des familles avec une croissance en hauteur supérieure et une concentration de sucres inférieure à la moyenne étaient présentes dans les deux sites. Même si un rapport composés phénoliques/sucres élevé s'avère efficace pour sélectionner des génotypes résistants à l'infection par *Armillaria*, les résultats montrent que des gains pourraient être obtenus plus efficacement en sélectionnant pour une faible concentration en sucre.

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Introduction

Several studies have suggested a link between *Armillaria* infection rates and variation in root bark chemistry. Species

with higher phenolics/sugar ratios in the root bark tend to be less susceptible to *Armillaria* infection than those with lower ratios (Entry et al. 1991a, 1992). Likewise, within a single species, stands with higher ratios of phenolics to sugars tend to be less susceptible to disease and insects than stands with low phenolics/sugar ratios (Entry et al. 1991b, 1994; Dudd and Shure 1994).

If variation in root bark chemistry is indeed linked to *Armillaria* susceptibility and it is at least partially controlled by genetics, it may be possible to decrease susceptibility in planted stands of species like Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) by selectively breeding for favorable chemical attributes. Should these favorable chemical at-

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tributes also be genetically correlated with favorable growth traits, it may also be possible to simultaneously increase growth rate while selectively breeding for favorable root bark chemistry. The objectives of this study were (i) to determine whether differences in root bark chemistry, in particular differences in the concentrations of sugars, phenolics, and the phenolics/sugar ratio, are genetically controlled in Douglas-fir and (ii) to examine the relationship between height growth and root bark chemistry.

Methods

Two Inland Empire Tree Improvement Cooperative (IETIC) Douglas-fir progeny tests were sampled in this study. Both are located in northern Idaho in the midelevation (915–1219 m) breeding zone for Douglas-fir and both are on *Thuja plicata* Donn. ex D. Don – *Paxistima myrsinites* (Pursh) Raf. habitat types (Daubenmire and Daubenmire 1968). The Rimrock test (48°26'N, 116°47'W) is located near the Priest River Experimental Forest, southeast of Coolin, Idaho (Fins and Rust 1997). It has a mean elevation of 1143 m and a west to northwest aspect. The Bussel Creek test (47°05'N, 116°09'W) is located northeast of Clarkia, Idaho. It has a mean elevation of 1189 m and a southern aspect.

Rimrock and Bussel Creek were established as sister tests to evaluate long-term progeny field performance. A third sister test was also established, but with the exception of 12-year height measurements, it is not included in this study. Each site contains progeny of the same 260 families from eastern Washington, northern Idaho, and western Montana. In 1981, one-year-old container seedlings were planted in the tests at 1.5 × 1.5 m spacing. Families were randomly assigned to one of five sets. Family members were distributed in three blocks, each containing randomized, noncontiguous, nine-tree plots, (27 test trees per family per site) (Fins 1983). “Family”, in this case, refers to the open-pollinated progeny of a single, identified parent tree. Parent trees were naturally regenerated Douglas-fir in undomesticated stands and were phenotypically selected in 1979 for superior growth and form characteristics. The progeny in set 1, which included 59 families, were selected for use in this study because of high survival (90%) and fairly low damage (14%) across sites relative to the other sets. The 20 families examined in this study were selected, because they had been included in an earlier IETIC study relating foliar nutrient concentrations and height growth (Walker 1995).

Samples of inner root bark tissue were collected from 5 to 11 members of each family at each site. Mortality in the progeny tests prior to sampling prevented the use of a balanced design so samples were taken irrespective of block. Samples were collected during the first 3 days of October 1993 at Bussel Creek and from late September to early October 1994 at Rimrock. The root collar of each sampled tree was uncovered until two roots with a minimum diameter of 1.27 cm were exposed. Then, a 3.81 × 7.62 cm rectangular section of inner root bark tissue was removed from each root. Samples were taken approximately 20–30 cm below the root collar and, wherever possible, from opposite sides of the tree. When only smaller (<1.27 cm diameter) roots were uncovered, two roots were clipped and sections of bark

comparable in size with the chiseled samples were peeled from the roots. In some instances, this resulted in girdling of the root pieces. For two trees, only one root large enough to provide a sample was found so samples were taken from opposite sides of the same root. Samples were transported from the field in coolers and stored at –15°C at the College of Natural Resources, University of Idaho, Moscow, until April 1995. Samples were then divided into two batches (10 families each) to facilitate laboratory schedules and submitted for analysis to the Institute of Biological Chemistry, Washington State University, Pullman. The first set of samples was analyzed in December 1995; the second was done in August 1996. Sugar concentrations were determined according to the methods of Hansen and Moller (1975). Phenolics were extracted using the methods of Julkunen-Tiitto (1985).

In 1992, total tree height was measured at the three sister tests. Because of the negative correlations between height and previous damage, only values from trees expressing no signs of root rot were used to calculate the percentile height rankings for each family, and only values from trees with no history of damage were used to calculate heritabilities.

A randomized block design with a statistical model containing family, site, and the family × site interaction terms was applied to estimate genetic and environmental effects on root bark chemistry. To account for uneven sample sizes (5–11 trees per family per site), a general linear model analysis of variance (PROC GLM; SAS Institute Inc. 1990b) was used to identify significant sources of variation in the individual root compounds and in the phenolics/sugar ratio. The “test” option was used to treat all variables as random effects. Family and site means were calculated using the PROC MEANS procedure and phenotypic correlations were calculated using PROC CORR (SAS Institute Inc. 1990a).

Variance components and coefficients for the heritability estimates and the genetic correlations were calculated using PROC VARCOMP (SAS Institute Inc. 1990b). Family and single-tree narrow-sense heritabilities were estimated using equations adapted from those in Zobel and Talbert (1991) for estimating heritability in half-sib tests:

$$[1] \quad h_i^2 = \frac{3\sigma_{\text{fam}}^2}{\sigma_{\text{fam}}^2 + \sigma_{\text{fam} \times \text{site}}^2 + \sigma_{\text{error}}^2}$$

$$[2] \quad h_{\text{fam}}^2 = \frac{\sigma_{\text{fam}}^2}{\sigma_{\text{fam}}^2 + (\sigma_{\text{fam} \times \text{site}}^2 / s) + (\sigma_{\text{error}}^2 / ts)}$$

where h_i^2 is the single-tree narrow-sense heritability, h_{fam}^2 is the family mean heritability, σ_{fam}^2 is the family variance component, $\sigma_{\text{fam} \times \text{site}}^2$ is the family × site interaction variance component, σ_{error}^2 is the tree within families (error) variance component, s is the coefficient representing the number of sites, and t is the coefficient representing the number of trees per family. When calculating h_i^2 , a coefficient of relationship equal to 3 was used in place of 4, because the families included in this study were open pollinated and, therefore, probably not true half-sib families (i.e., some of the progeny within the families may be full-sibs or even selfs).

Results and discussion

The site mean phenolics/sugar ratios (5.36 at Bussel Creek and 4.85 at Rimrock; Table 1) in our study were lower

Table 1. Site means and ranges for compounds in the inner root bark tissue of 15-year-old Douglas-fir at two progeny test sites in north Idaho (based on individual tree data).

Compound	Site	N	Mean	SD	Minimum	Maximum
Sugar	Bussel Creek	179	4.40	0.78	2.45	8.38
	Rimrock	166	4.23	0.69	2.14	5.94
Phenolics	Bussel Creek	179	23.28	4.64	8.59	37.11
	Rimrock	166	20.51	6.26	4.69	44.90
Phenolics/sugar	Bussel Creek	179	5.36	1.00	1.33	8.20
	Rimrock	166	4.85	1.36	1.68	10.49

Note: Values for sugar and phenolics are in grams per 100 g root bark tissue.

Table 2. Mean squares, df, and *p* values from the analyses of variance for sugar, phenolics, and the phenolics/sugar ratio.

Source	df	Sugar		Phenolics		Phenolics/sugar	
		MS	<i>p</i>	MS	<i>p</i>	MS	<i>p</i>
Family	19	2.44	0.0337	102.20	0.2722	5.37	0.2464
Site	1	2.16	0.1639	575.02	0.0133	18.97	0.0001
Family × site	19	1.03	0.0003	77.07	0.0001	3.90	0.0001
Error	305	0.39		22.55		1.02	

Table 3. Phenotypic correlation coefficients for height growth and root bark chemistry in Douglas-fir.

	Sugar	<i>p</i>	Phenolics	<i>p</i>	Phenolics/ sugar	<i>p</i>
Phenolics	0.4220	0.0001				
Phenolics/sugar	-0.1718	0.0014	0.8002	0.0001		
Height	0.0912	0.0908	0.1128	0.0362	0.0506	0.3490

than those reported by Entry et al. (1991b) for stands of Douglas-fir. Foliar nutrient data (on file with the IETIC) suggest that the site mean ratios may be low as a result of nutrient deficiencies. Over 75% of the trees sampled at Bussel Creek had foliar N levels below those considered adequate for Douglas-fir in the Inland Northwest (Garrison and Moore 1998). Entry et al. (1991a) found that trees grown with limited N tend to have lower phenolics/sugar ratios than trees grown with adequate amounts of N.

Another possible factor in finding low site mean phenolics/sugar ratios is higher than expected sugar concentrations. Mean sugar concentrations were 4.40 and 4.23 g/100 g root bark tissue at Bussel Creek and Rimrock, respectively. These are over 33% higher than the sugar concentrations of 3.0 reported by Moore et al. (2000) for thinned stands of naturally regenerated Douglas-fir growing on *T. plicata* habitat types in the interior northwestern United States. In that study, the mean phenolics/sugar ratio was 6.7.

Seasonal variation in root bark chemistry may also contribute to the low phenolics/sugar ratios. Nerg et al. (1994) found that phenolics concentrations in *Pinus sylvestris* L. shoots are significantly lower in September than in spring. Similarly, Nozzolillo et al. (1990) found that phenolics in *Pinus banksiana* Lamb. needles were lower in the autumn months than in winter. Root sugar concentrations, by contrast, are higher during the autumn months. In *Pinus elliottii* Engelm., sugar concentrations in fine roots are lowest in

spring, then increase from late summer to winter (Gholz and Cropper 1991). Likewise, coarse root sugars in *Acer saccharum* Marsh. are highest in August through October (Liu and Tyree 1997). These studies suggest that the phenolics/sugar ratio would be at its lowest in fall, potentially making the trees most susceptible to *Armillaria* during that time.

Site differences in phenolics, and the phenolics/sugar ratios were highly significant (Table 2). Within sites, family differences in phenolics and the phenolics/sugar ratio were also highly significant. However, because of significant family × site interactions, family differences were not significant across sites. We suspect that genetic differences in phenolics concentrations and in the phenolics/sugar ratio really do exist across locations, but the prevalence of nutrient deficiencies within the test sites, the fact that sites were sampled in different years and the fact that roots were sampled when the phenolics/sugar ratios are likely to have been low may have hindered our ability to detect them.

The phenotypic correlation between phenolic concentrations and 12-year height growth was positive (Table 3), which appears to contradict traditional hypotheses regarding carbon allocation (Herms and Mattson 1992). However, several other studies have found positive relationships between growth and defense compounds as well (e.g., Briggs and Schultz 1990; Kimball et al. 1999). One explanation is that nitrogen deficiencies at the progeny test sites are limiting growth, making carbon available for phenol and tannin pro-

duction. Studies have shown that this carbon allocation pattern can occur without noticeable impact on growth when environmental resources limit growth (Bryant et al. 1985; Larsson et al. 1986; Mihaliak and Lincoln 1989). However, when adequate resources are available, carbon allocation shifts towards growth, and little carbon is available for defense compounds (Bazzaz et al. 1987).

Single-tree and family heritability estimates were moderately high for 12-year height growth (0.40 and 0.61, respectively). These values are higher than heritability estimates for 8-year height growth in the same 20 families (0.27 and 0.54, respectively) (Walker 1995), but the narrow-sense heritability estimate for 12-year height growth is consistent with values reported by Campbell (1972) for height in coastal Douglas-fir.

Single-tree and family heritability estimates were also moderately high for sugar concentration (0.48 and 0.60, respectively). Wargo (1980, 1981) found that when glucose concentrations are low, defense compounds such as phenols and tannins inhibit fungal growth and can be toxic to *Armillaria*. However, when glucose concentrations are high, *Armillaria* is capable of oxidizing defense compounds and using them as a carbon source (Wargo 1980, 1981). Thus, sugar concentration may be the key component of the phenolics/sugar ratio.

Because the phenotypic correlation between 12-year height and sugar concentrations was small (0.09) and not significant, it was possible to identify five families with superior height growth (>70th percentile) and below-average sugar concentrations at both test sites. With moderately high heritabilities for both traits and a negative genetic correlation (-0.18) between the two, selection for relatively low sugar concentration among families with above-average height growth could potentially decrease susceptibility to *Armillaria* sp. while at least maintaining or even increasing height growth in Douglas-fir in northern Idaho.

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