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Root Chemistry of Mature Douglas-fir Differs by Habitat Type in the Inland Northwest

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ABSTRACT

This paper describes root biochemical characteristics of naturally regenerated Douglas-fir trees growing on a wide variety of sites in the inland Northwest. Roots of trees growing on dry, warm Douglas-fir habitat series had significantly greater concentrations of sugar and tannins, higher tannin/sugar ratios, and significantly lower phenolic concentrations and phenolic/sugar ratios than the roots of Douglas-fir trees growing on grand fir habitat series or western red cedar habitat series. Possible links between Douglas-fir root biochemical characteristics and susceptibility to root disease, particularly Armillaria, and tree resistance to drought are discussed.

INTRODUCTION

Concentrations of storage and secondary compounds in plant tissue such as sugars, starches, phenolics and tannins, depend to a considerable extent on the environment in which plants grow (Waring et al. 1985; Huber and Arny 1985). The concentrations of these compounds and the balance among them help to determine the resistance of plants to herbivores and pathogens (Wargo 1972; Garraway 1975; Ostrofsky and Shigo 1984; Larsson et al. 1986; Mwangi et al.1990; Dudt and Shure 1994). Therefore, there has been recent increased interest in developing a better understanding of the growing environment and biochemical characteristics for forest trees (Bryant et al. 1983; Entry et al. 1991a; Moore et al. 1994; Gebauer et al. 1998; Penuelas and Estiarte 1998; Shaw et al. 1998). Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Bessn.) Franco) was selected for our study because it is commonly managed in the forests of the inland Northwest and has wide ecological and geographic distributions.

Resource availability may influence production of secondary compounds like phenols and tannins (Mooney 1972; Bazzaz et al. 1987). In our study, we wanted to ascertain the influence of resource availability, specifically differences in temperature and moisture growing conditions, on the level of carbon-based compounds in the roots of forest-grown, mature Douglas-fir trees. Stands growing on a variety of habitat types (Daubenmire and Daubenmire 1968; Pfister et al. 1977) should represent a range of temperature and moisture conditions. Furthermore, habitat type classification of forest land in the inland Northwest is common and ecological interpretations are well developed (Cooper et al. 1991; McDaniel et al. 1994; Neiman 1988). Thus, the principal objective of this study was to determine differences in the chemical composition of Douglas-fir roots among habitat types.

METHODS AND MATERIALS

Site Selection

The Intermountain Forest Tree Nutrition Cooperative (IFTNC) maintains a large set of long-term Douglas-fir nutrition field experiments (see Moore et al. 1991 for more details). We selected eight IFTNC locations for root chemistry sampling. The eight study sites included Douglas-fir (dry and warm growing conditions), grand fir (moderate temperature and moisture regimes), and western red cedar (cool and moist growing conditions) habitat type series. Our intent was to use habitat type series as a selection criteria to ensure a broad range of temperature and moisture growing conditions for our study. Selected site and stand characteristics for the 8 study sites are provided in Table 1. The sites are approximately evenly divided among the 3 habitat types and consequently span a wide range of site quality as estimated by Douglas-fir site index (Table 1). All sites are populated by local Douglas-fir seed sources naturally regenerated after fire or natural harvest regeneration systems.

Tree Growth and Plot Measurements

Each untreated control plot sampled in the present study is 0.1 ac. in size, and each plot contains at least ten Douglas-fir sample trees. All live plot trees were tagged and measured for diameters at the beginning of the experiment. Every two years diameters were remeasured on all trees for a 10 year period and any incidence of damage or mortality along with probable cause was noted. Basal areas were summed over all trees (not just Douglas-fir) to obtain plot totals. Douglas-fir site index estimates for each plot were made based on height-age observations from stem analysis of destructively sampled trees located near each plot (see Vander Ploeg and Moore 1989 for more details.)

Collecting Tree Root Samples

Two undamaged and apparently healthy dominant trees on the untreated control plots at each of the 8 study sites were selected for root chemistry sampling. One square inch root bark samples of living tissues (including phloem, cambium, phellum and phelloderm) were collected from four main lateral roots for each tree. The soil was carefully excavated from each root and the samples obtained near the root collar (~1 foot from the tree bole) just under the mineral soil surface. Field sampling procedures are similar to those of Entry et al. 1991b. After collection, the four root samples were composited by tree, placed in plastic vials, quickly frozen on dry ice and placed in insulated containers for transport back to the laboratory for processing. At the laboratory, the samples were placed in an ultra-cold freezer until they could be freeze-dried awaiting laboratory analysis.

Quantification of Carbohydrates, Phenolics and Tannins in the Root Tissues

For the chemical analysis, all bark samples were put in liquid nitrogen overnight, dead outer root bark was removed to the phloem, then the living tissues of the inner bark were analyzed. Samples were then ground to powder in a mortar. Total phenols were determined from samples after extracting with aqueous acetone (80%), adding Folin-Ciocalteu's Reagent, and then measuring absorbances at 700 and 735 nm (Julkunen-Tiito 1985). Samples were analyzed for total soluble starch through an ethanol and perchloric acid method (Hansen and Moller 1975) and glucose was determined by adding anthrone solution for absorbance determination at 630 nm (Hansen and Moller 1975). Concentrations of glucose were measured using a standard curve established with a glucose standard (Hansen and Moller 1975). The Hansen and Moller method overestimates starch levels because carbohydrates other than starch are extracted during the process (Marshall 1986, Rose et al. 1991). Perchlorate-extractable carbohydrates were, therefore, corrected to yield starch concentrations and are expressed in their corrected form throughout this paper. Tannin acid was determined after extracting with 80% aqueous acetone then the extract was loaded into an agarose plate containing bovine serum albumin, and diffusion rings were measured (Hagerman 1987). Analysis of starch, sugar (glucose), total phenols and protein-precipitable tannins analysis were performed by the Institute of Biological Chemistry, Washington State University in Pullman, WA.

Statistical Analysis

Analysis of variance (PROC GLM) was used to test for differences between means by habitat type series for root chemistry data using the least-squares means procedure of the Statistical Analysis System (SAS Institute Inc. 1985). Additionally, correlations between the various root chemical constituents and several measures of stand growth rate were analyzed using PROC CORR of SAS.

RESULTS

Mean root concentrations and concentration ratios for the three habitat type series are shown in Figure 1. Root sugar concentrations were significantly higher for Douglas-fir trees growing on Douglas-fir habitat types than for those growing on grand fir or red cedar habitat types; however, starch concentrations showed no significant differences among the three habitat types. Phenolic concentrations and phenolic/sugar ratios were significantly higher in the roots of Douglas-fir trees growing on western red cedar habitat series compared to grand fir and Douglas-fir habitats. Conversely, trees on Douglas-fir habitat series showed significantly higher root tannin concentrations than trees on either grand fir or western red cedar habitats and tannin/sugar ratios were significantly higher than trees on the grand fir habitat series.

Results of correlation analysis between the root chemistry concentrations (i.e., sugar, starch, phenolics, tannins) and concentration ratios (i.e. phenolic/sugar, tannin/sugar) with various expressions of plot growth rates and Douglas-fir site index are provided in Table 2. Net basal area growth and relative net basal area growth (NBAI and RNBAI) were positively correlated with sugar concentrations in Douglas-fir roots. Douglas-fir site index was positively correlated with root phenolic/sugar ratios. All other correlations between plot growth rate expressions or site index and root biochemical concentrations or ratios were non-significant.

DISCUSSION

Root phenolic, tannin and sugar and their ratios vary significantly by habitat types for natural, forest-grown, mature Douglas-fir trees. There are several alternative explanations for these results. Herms and Mattson (1992) suggest that plants face a carbon allocation dilemma; that is whether to allocate more C to sugar, starch and cellulose production, i.e., rapid growth; or whether to allocate relatively more C to the production of secondary metabolites, such as phenolics and tannins, which are important in plant defense against diseases and insects. We attempted to develop empirical support for Herms and Mattson's growth-differentiation balance (GDB) hypothesis as an explanation for our results by conducting the correlation analysis presented in Table 2. We expected to find significant negative correlations between stand growth rate and phenolic and tannin concentrations; however, none of those correlations were significant. We are not suggesting that our study is a rigorous test of the GDB hypothesis, merely that the expected relationships between growth and secondary metabolites were not evident in our data. Perhaps this result can be explained by non-linear effects of resource availability on secondary metabolism and resultant levels of root phenols and tannins (Mattson and Haack 1987; Tuomi et al. 1988; Horner 1990). Given our limited number of observations, we are unable to fully detect possible non-linear relationships between root chemical constituents and plot growth rates. The only consistent relationship represented in Table 2 is between root sugar concentration and stand growth rate: faster growing Douglas-fir stands accumulated higher sugar in their roots.

Wargo (1980), working with sugar maple, and Entry et al. (1991a; 1991b), working with Douglas-fir, reported that increased levels of glucose enable the *Armillaria* fungus to grow more rapidly in tree roots, making the fungus better able to break down phenolic compounds. Possible higher root sugar concentrations in faster growing Douglas-fir stands would cause them to be more susceptible to root rot infection; however, the concurrent level in the roots of defense chemicals, such as phenols, is also important in determining conifer susceptibility to root disease (Entry et al. 1991b; Gebauer et al. 1998).

Levels of resource availability-- light, moisture, and nutrients-- affect the rates of net assimilation, growth and secondary metabolism. The functional relationships between these processes have not yet been established for Douglas-fir, but our study provides insights to the outcomes of these processes. The habitat type series included provide a range of resource availability conditions in the forests of the inland Northwest. The hot and dry Douglas-fir habitat types represent the most resources limited conditions, while the cool and moist western red cedar types represent the highest resources levels in our study (Cooper et al. 1991; Neiman 1988). Douglas-fir trees growing on Douglas-fir habitat types had significantly higher sugar, lower phenolics, and lower phenolic/sugar ratios in their roots than did Douglas-fir growing on western red cedar habitat types. Entry et al. 1991b showed that the sugar concentration and phenolic/sugar ratio in Douglas-fir roots were strongly related to the incidence of Armillaria infection in the same trees. Furthermore, McDonald et al. 1987 found that "incidence of pathogenic Armillaria showed a strong tendency to decrease as habitat type productivity increased. . . . The relatively less productive Douglas-fir series exhibited high incidence of root disease and the relatively more productive grand fir, western red cedar, and western hemlock series significantly less." The high sugar concentrations and low root phenolic/sugar ratios we found, coupled with Entry et al.'s (1991b) results provide a plausible explanation for these results.

Douglas-fir root tannin concentration and tannin/sugar ratios were significantly higher for Douglas-fir habitat series, just the opposite pattern than we observed for phenols and phenol/sugar ratio. Tannins are secondary metabolites, as are phenols, and may play a role in plant chemical defense against pests. For conifers however, the function of tannins in resistance to disease and insect infestation has not been as well established as it has been for phenols. Defense is not the only role identified for tannins in plants. Other functions include: structural support (Rhodes 1985; Haslam 1988); drought resistance (Rhoades 1977; Bariska and Pizzi 1986; Pizzi and Cameron 1986) and protection of roots from acidic soil environments (Kimura and Wada 1989). The average site indices by habitat types for our study sites were: Douglas-fir habitats = 57; grand fir habitats = 79; and red cedar habitats = 84. If we consider Douglas-fir site index as an expression of the degree of moisture limitation, then grand fir and red cedar habitats included in our study are similar and quite different from Douglas-fir habitats with respect to moisture limitations. We therefore speculate that the elevated root tannin levels of Douglas-fir growing on the dry Douglas-fir habitat series reflect the role of tannins in improved drought resistance. Furthermore, given the positive correlation that we found between site index and root phenolics/sugar ratios, our results suggest some affect of moisture limitation, i.e., low site index, on shifting carbon from phenolics to tannin production.

Our study is about the ecology of root biochemistry of native Douglas-fir forests growing on a wide variety of sites in the inland Northwest but we can currently only speculate about the physiological basis for our results. We certainly realize that the observed differences in root chemistry are the outcomes of myriad biological and physiological processes and their complex interactions (well reviewed by Herms and Mattson 1992). However, we feel our study results are directly useful in understanding the ecology of Douglas-fir and its interactions with native pests at a landscape level in the inland Northwest. Further, our results could help in the design of future physiological studies aimed at a better understanding of processes of net assimilation, growth rates and secondary metabolism that determine forest productivity across the wide variety of site conditions in the inland Northwest.

CONCLUSIONS

Native Douglas-fir growing on Douglas-fir, grand fir and western red cedar habitat type series showed significantly different concentrations and ratios of root chemical compounds. Trees growing on dry, warm Douglas-fir habitat types had significantly greater sugar concentrations and significantly lower phenol/sugar ratios. These root chemical characteristics suggest that Douglas-fir growing on Douglas-fir habitat types would be less resistant to root rot infection, particularly by Armillaria, than Douglas-fir growing on grand fir and western red cedar habitat types.

The roots of Douglas-fir growing on Douglas-fir habitat types were higher in tannin concentration and had higher tannin/sugar ratios. The higher tannin levels may improve Douglas-fir drought resistance on the dry, warm Douglas-fir habitat types.

This paper demonstrates that Douglas-fir root chemistry varies significantly according to presumed growing site moisture and temperature differences represented by forest habitat type series. This knowledge provides a better understanding of Douglas-fir species ecology across a wide range of growing environments in the inland Northwest.

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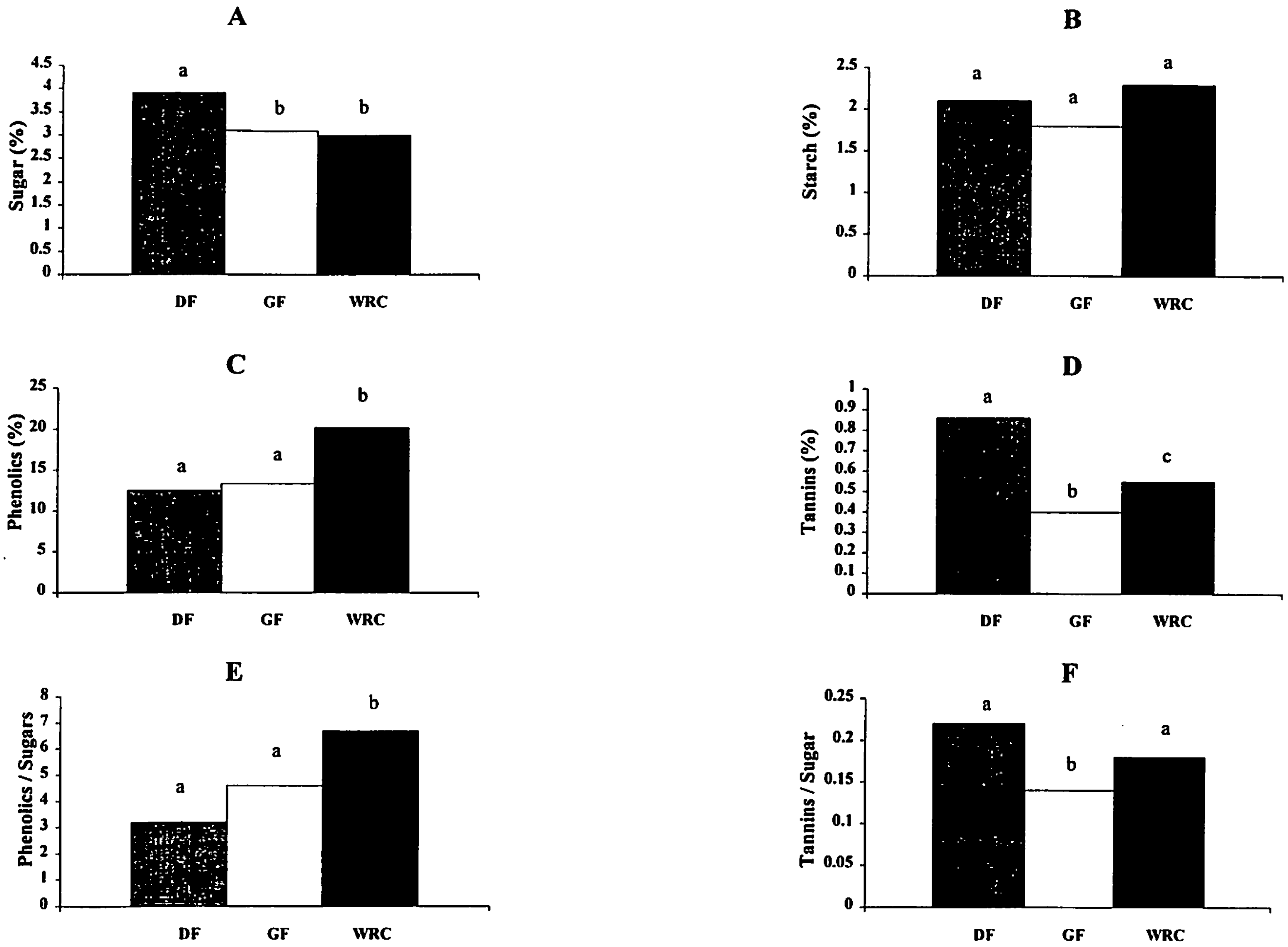
List of Figures

Figure 1. Means for soluble sugar (A), starch (B), phenolics (C), protein-precipitable tannin (D), phenolic to sugar concentration ratios (E), and tannin to sugar concentration ratios (F) collected from the roots of mature Douglas-fir growing on western red cedar (WRC), grand fir (GF) and Douglas-fir (DF) habitat type series. Different lower case letters indicate that the respective means were statistically different at the α=.05 level.



Habitat Type Series

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Table 2. Correlation coefficients between site index, measures of plot growth rate, and chemical composition of Douglas-fir roots.

Table 1. Selected site and stand characteristics for eight Douglas-fir study sites in the inland Northwest.

Installation #	Habitat Type Series	Douglas-fir Site Index (ft. @ 50 yrs)	Stand Age (yrs @ BH)	Basal Area (ft^2/ac)	Basal Area Growth (ft^2/ac/yr)
218	Douglas-fir	48	110	120	3.35
248	Douglas-fir	66	74	152	4.82
255	red cedar	84	44	212	7.61
258	grand fir	77	82	197	2.21
264	red cedar	84	60	269	4.26
283	Douglas-fir	57	85	134	3.29
287	grand fir	71	67	205	4.28
288	grand fir	88	64	289	3.74

Table 2. Correlation coefficients between site index, measures of plot growth rate, and chemical composition of Douglas-fir roots.

	BAI	RBAI	SI[a]
Sugar	.75**	.86***	-.53
Starch	.05	-.11	.44
Tannin	.34	.51	-.46
Phenol	.20	-.10	.56
Tannin/Sugar	-.03	.09	-.19
Phenol/Sugar	-.32	-.58	.68*

(* significant $p \leq .10$; ** significant $p \leq .05$; *** significant $p \leq .01$)

a = Douglas-fir site index (Monserud 1979)

Note: BAI is 10 year plot basal area growth rates for living trees for the period just prior to root sampling. RBAI = BAI ÷ plot basal area at beginning of the period.