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An examination of the genetic control of Douglas-fir vascular tissue phytochemicals: Implications for black bear foraging

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Abstract

Silvicultural practices can influence black bear (*Ursus americanus*) foraging preferences for Douglas-fir (*Pseudotsuga menziesii*) cambial-zone vascular tissues, but little is known about the role of genetics. To study the impact of genetic selection, vascular tissue samples were collected from Douglas-fir trees in six half-sib families from five different sites in north central Oregon. Four replications of three-tree non-contiguous plots were sampled at each site to examine inter- and intra-site variation. Tree growth was measured as tree diameter at breast height and the absolute concentrations of 26 different terpenoids were determined by gas chromatography/flame ionization detection from ethyl acetate extracts. The simple carbohydrates fructose, glucose, and sucrose, and the phenolic glycoside coniferin were quantified using anion-exchange chromatography with pulsed amperometric detection. Cluster analysis was used to reduce the number of variables used in analyses of variance. Results for the families studied here indicate that tree growth and some terpenoids were under some level of genetic control. Furthermore, allocation of constitutive terpenoids in vascular tissues was not at the expense of tree growth. The sugars present in vascular tissue were affected by environment (site) and genetics (family) and their interaction.

Keywords: Forest management; *Pseudotsuga menziesii*; Sugars; Tree genetics; Terpenoids; *Ursus americanus*

1. Introduction

Relationships between Douglas-fir (*Pseudotsuga menziesii*) genotypes and mammalian herbivory have been well established. For example, Douglas-fir resistance to snowshoe hare feeding has been demonstrated to be under strong genetic control (Dimock et al., 1976). Deer preferences for certain clones of Douglas-fir have been attributed to differences in monoterpenes present in the foliage (Radwan and Ellis, 1975). In fact, mammalian preferences have frequently been linked to the repellent qualities of monoterpenes (Bryant et al., 1991, 1992; Bell and Harestad, 1987; Reichardt et al., 1990; Farentinos et al., 1981) in many species. The heritable nature of monoterpenes has led to the practice of using them as biomarkers for clonal

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identification (Kossuth et al., 1988; Schaefer and Hanover, 1986).

Phytochemicals present in the cambial zone vascular tissues of Douglas-fir may play a significant role in foraging preference of black bears (*Ursus americanus*; Kimball et al., 1998a). Black bears forage on cambial-zone vascular tissues in the spring before more diverse forage materials are available. Bioassay studies have shown that free-ranging bears prefer high sugar (non-structural carbohydrates) diets to low sugar diets of identical monoterpene concentration (Kimball et al., 1998a). Furthermore, bears preferred low terpenoid diets to high terpenoid diets of identical sugar concentration. In selecting trees, bears may rely on post-ingestive feedbacks to weigh the energetic value (sugars) of the vascular tissues against the cost of terpenoid detoxification (Kimball et al., 1998a). Previous studies have demonstrated that mammals regulate intake of foods varying in nutrients and toxins (Wang and Provenza, 1997).

Silvicultural practices are known to impact some of these same compounds that have been implicated in black bear foraging preference. Bear preferences for certain tree species have been reported (Radwan, 1969) as well as a preference for trees in thinned stands (Kanaskie et al., 1990; Mason and Adams, 1989; Schmidt and Gourley, 1992), fertilized stands (Nelson, 1989), and unpruned trees (Kimball et al., 1998b). Chemical analyses of vascular tissues have demonstrated that tissues from trees in thinned stands had significantly higher sugar concentrations in comparison to trees in adjacent unthinned stands (Kimball et al., 1998c). However, thinning did not significantly impact the terpenoid concentration of vascular tissues. Vascular tissue sugar concentrations were also increased immediately following nitrogen fertilization, however, this effect was short-lived and fertilization did not impact vascular tissue terpenoid concentrations. Pruning of the live crown had a negative impact on the vascular tissue concentration of sugars, while having no impact on the terpenoid concentration (Kimball et al., 1998b). These data indicate that bear preference for thinned trees and rejection of pruned trees may result from changes in the sugar chemistry of the vascular tissue brought about by silvicultural practices.

Genetic selection is another commonly employed silvicultural management practice. Tree breeding programs have been developed to select stock with preferred traits (e.g. King et al., 1998; Woods et al., 1995). It is possible that the practice of selecting fast growing genotypes for timber production congruently produces poorly defended trees that are highly preferred by bears. This effect is predicted by plant defense theory (see Berenbaum, 1995; Coley et al., 1985).

Thus, our study was designed to determine if the constitutive terpenoids and sugars that play a role in black bear foraging preference were subject to some level of genetic control (i.e. to determine if family differences existed). The relationship between tree productivity (growth) and the abundance of constitutive terpenoids (defense) was also of interest. To best achieve these objectives, six half-sib families ranging from slow to fast growth were chosen for evaluation. It should be noted that quantification of genetic variance was beyond the scope of this investigation. Rather, family differences (genetic control) were exclusively examined among the six families to answer the simple question, "Is there evidence that Douglas-fir vascular tissue phytochemicals are under some level of genetic control?."

2. Methods

2.1. Study sites

Five progeny test sites from an operational tree improvement program located in the Cascade range of north central Oregon were chosen for this study (Table 1). Each site was established in 1971 with 2-0 seedlings at a uniform 3.05 m × 3.05 m spacing to assess growth and wood quality. Evaluation of these traits was concluded in 1985. No silvicultural treatments were applied to the test sites except for weed control which was applied during the first three years. The five sites used for our study were chosen from nine potential sites on the basis of achieving the greatest environmental variation as indicated by the site elevation and tree productivity (Height at age 15; Table 2). In sampling only five of nine potential sites, the objective became detection of potential variation in phytochemical abundance due to the environment, not the quantification of the variation. Samples for the study reported here were collected over the period of June 1–6, 1997.

Table 1
Operational progeny test sites studied in north central Oregon

Name	County	Lat/Long	Site elevation	15 year tree height
Bissel Loop	Clackamas	45°20′ N, 122°10′ W	600 m	8.18 m
Bee Creek	Clackamas	45°10′ N, 122°20′ W	500 m	9.33 m
CZ 1098	Clackamas	45°5′ N, 122°20′ W	800 m	5.24 m
Strawberry fields	Marion	44°50′ N, 122°35′ W	750 m	7.60 m
Camp 26	Linn	44°40′ N, 122°25′ W	800 m	7.56 m

Table 2
Growth rankings of test families at age 15 and multiple comparison results of current tree diameter (means with different letters are significantly different)

Family	Age 15 Rank	Age 28 DBH (cm)	Multiple comparison		
A	1	24.39	a	b	
B	4	25.25	a		
C	10	23.16		b	c
D	13	23.11		b	c
E	18	22.10			c
F	22	22.12			c

2.2. *Study families*

At each site, 12 trees from each of 25 half-sib families were originally planted in four replications of three-tree non-contiguous plots (i.e. three trees per family/replication). Eight trees from each family (two per replication) were chosen randomly and sampled at each of the five study sites. In some cases, only one tree was surviving in a family/replication, but overall at least 37 (out of 40) trees were sampled for each family. Tree mortality was typically due to wind break. To best achieve our objectives of detecting whether some level of genetic control existed and examining the relationship between growth and defense, test families were selected so that tree growth variability was maximized. Six families were selected for the study that ranged from slow to fast growth as evidenced by the height growth rankings at age 15 (Table 2).

2.3. *Vascular tissue collection*

Samples were collected according to the procedures described by Kimball et al. (1998c). Briefly, 800 cm^2 of bark was removed from each tree below breast height. Cambial zone vascular tissues were scraped into a freezer bag and immediately frozen in liquid nitrogen. To minimize unknown sources of within-tree variation, we arbitrarily chose to sample all trees on the east side. Tree growth was evaluated by measuring the diameter at breast height (DBH).

2.4. *Chemical analyses*

Frozen vascular tissue samples were analyzed for terpenoids according to the procedures of Kimball et al. (1995) except that flame ionization detection was employed with gas chromatography for the detection of the terpenoid compounds (Table 3). A portion of each vascular tissue sample was lyophilized and analyzed for glucose, fructose, sucrose, and coniferin by anion-exchange chromatography with pulsed amperometric detection (Kimball et al., 1998a).

2.5. *Statistical design*

Raw data was examined by using the UNIVARIATE procedure to identify severe outliers among the chemical data (SAS, 1997). Data points which fell outside the three interquartile range were considered extreme

Table 3
Clusters generated from quantitative terpenoid data

Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
α-Thujene	α-Pinene	Glucose	Fenchyl alcohol	Caryophyllene
Sabinene	Camphene	Fructose	α-Terpineol	Humulene
α-Phellandrene	β-Pinene	Sucrose	Borneol	
3-Carene	Myrcene	Coniferin		
α-Terpinene	Limonene			
p-Cymene	Bornyl acetate			
γ-Terpinene	Geranyl acetate			
Terpinolene	Longifolene			
Linalool				
Terpinen-4-ol				
Citronellal				
Citronellyl acetate				

outliers and removed from the data set (1 to 3 individual data points were removed for several of the chemical variables). The VARCLUS procedure (SAS, 1997) was used to group the chemical variables into five related clusters. The VARCLUS procedure computes the first principle component for each cluster and 'tries to maximize the sum across clusters of variation accounted for by the cluster components' (SAS, 1997). VARCLUS is a type of oblique component analysis related to multiple group factor analysis (Harman, 1976). Component scores were generated for each cluster from the normalized quantitative data and the VARCLUS scoring coefficients using the SCORE procedure (SAS, 1997).

Analysis of variance was used to investigate family and site effects on tree growth (DBH) and the component scores in a mixed design, with site and family classified as fixed variables and replication (nested in site) as a random variable. Hence, the objectives were to detect family and site variation, not to quantify variation. Only the significant predictors ($\alpha = 0.1$) were retained in final models. Type III sums of squares were used and the replication by family within site interaction employed as the error term when significant. Multiple comparisons of the DBH data and selected individual phytochemicals were made on the least-squares means (PDIFF option; SAS, 1997). Correlations between growth and the vascular tissue phytochemicals were examined by determining the correlations of DBH with the cluster component scores using the CORR procedure (SAS, 1997).

3. Results

The VARCLUS procedure identified five clusters which described 56% of the total variation (Table 3). Examination of the component scores generated from these clusters indicated that component scores for clusters 1, 2, and 3 were normally distributed. Growth data (DBH) was also normally distributed.

Analysis of variance results indicated that within site variation was significant for cluster 3 and DBH data as indicated by a significant rep(site) effects (Table 4). Significant site effects for clusters 1, 2, 3, and 5 indicate these variables were subject to considerable variation among sites. Significant family effects were identified for DBH and clusters 2 and 3, indicating that these variables are under some level of genetic control. Cluster 3 was subject to the interaction of family and site, while cluster 5 had a significant family × rep (site) interaction.

Multiple comparisons of the tree diameter data demonstrated that measured growth at the time of this study was consistent with the rankings assigned to the families at age 15 (Table 2). Furthermore, growth and the chemical variables were not well correlated. Only cluster 5 was significantly correlated with DBH $r = -0.15$, $p = 0.026$), while the positive correlations of cluster 2 ($r = 0.11$, $p = 0.13$) and cluster 3 ($r = 0.11$, $p = 0.11$) only approached significance. Cluster 1 ($r = -0.022$, $p = 0.75$) and cluster 4 ($r = 0.086$, $p = 0.21$) were not even mildly correlated with DBH.

Table 4
Significance levels of statistically significant sources of variation

Response	Source				
	Site	Rep (site)	Family	Site × family	Rep × family (site)
Cluster 1	<0.0001				
Cluster 2	0.051		0.076		
Cluster 3	<0.0001	0.018	<0.0001	<0.0001	
Cluster 4					
Cluster 5	0.04				0.0023
DBH		0.054	0.014		

4. Discussion

Examination of explained variance from the VARCLUS procedure suggested that using more than five clusters served only to increase the number of variables while accounting for not much additional variation. Thus, each compound quantified in Douglas-fir vascular tissue was assigned to one of five clusters by the VARCLUS procedure (Table 3). The ANOVA results were most relevant for clusters 1, 2, 3, and DBH since these data were demonstrated to be normally distributed. Clusters 2 and 3 were also of great interest because the predominate terpenoids were represented in cluster 2 while the sugars were represented in cluster 3.

Clustering of terpenoids produced groups similar to those reported by White (1983) for lodgepole pine foliage (*Pinus contorta*). Bicyclic α- and β-pinene, camphene, and bornyl acetate were found in one cluster (Cluster 2), as were the bicyclic sabinene and thujene clustered with most of the monocyclic monoterpenes (Cluster 1). Of the terpenoid clusters, only cluster 2 was subject to genetic (family) effects (Table 4).

Growth, as measured by DBH at age 28 (the time of this study) was consistent with the family rankings assigned at age 15 (Table 2). The families evaluated in this study were chosen specifically to span the range of productivity. Over this range, the variation in growth was affected by intra-site variability (significant rep(site) effect) and the family from which it was sampled (Table 4). Thus, growth was also shown to be under some level of genetic control. It has been well established that heritabilities are moderate for most Douglas-fir traits, including growth (e.g. King et al., 1998; Woods et al., 1995).

A positive correlation between growth and cluster 2 ($r = 0.11$, $p = 0.13$) was indicated but not significant. Evaluation of mean concentrations of the terpenoids that made up cluster 2 indicated that some constitutive terpenoids were positively associated with growth for these families. The β-pinene, myrcene, and limonene concentrations of families A and B were significantly greater than the other families. Mean concentrations of α-pinene and camphene were also higher in families A and B, although not statistically significant. Growth data indicate that these families were the fastest growing at these sites (Table 2).

These results are not consistent with the resource availability hypothesis (RAH) which predicts that faster growing genotypes invest less in chemical defenses than their slower growing counterparts (Coley et al., 1985). In fact, the physiological tradeoff between growth and defense is the governing principle of most defense theories (Herms and Mattson, 1992). Our results, albeit for a limited system, indicate that fast growing genotypes allocate higher concentrations of certain terpenoids in the vascular tissue without sacrificing growth. The notion that growth is not the currency for defense has been demonstrated in many other species (Gershenzon, 1994).

In addition to the demonstrated role of tree genetics in the occurrence of cluster 2 terpenoids, every cluster, except 4, were subject to site effects. These results are not in agreement with recent results indicating that neither the abundance of hydrocarbon monoterpenes, oxygenated monoterpenoids, nor sesquiterpenes in Douglas-fir vascular tissues were subject to site effects (Kimball et al., 1998c). This apparent conflict may have resulted from the statistical approaches used to reduce the number of variables. The previous study examined three terpenoid variables generated from the sum of the individual compounds belonging in each class, while this study incorporated principle components analysis to reduce the number of variables.

Cluster 3 was significantly impacted by within site effects and the interaction of site and family (Table 4). Furthermore, a positive correlation between cluster 3 and DBH ($r = 0.11$, $p = 0.11$) was apparent but not significant. These results indicate that a relationship between the concentration of vascular tissue sugars and growth may exist for the families studied here, but environmental effects (both within and among sites) also had an effect on the presence of sugars. This interaction suggests that black bear preference for any of the families studied here could be based on differences in sugar concentration, but preferences would be site dependent.

Even small changes in the concentration of these compounds may lead to profound foraging preferences. Preferences for trees in thinned versus unthinned stands has been attributed in part to differences in sugar concentrations as small as 0.2% (Kimball et al., 1998c). Significant preferences due to small differences in energy availability have been demonstrated with other mammals. Cattle, sheep, and goats prefer tall fescue and alfalfa harvested in the afternoon to that harvested in the morning, evidently because of higher sugar concentrations (Fisher et al., 1997a, b). Likewise, sheep discriminate among foods based on energy content, even when the differences are as little as 2.5% of their daily requirement (Provenza et al., 1996; Villalba and Provenza, 1997).

No information is currently available regarding the effects of individual terpenoids on bear preference. However, increases in total monoterpenoid concentration have been shown by bioassay to deter food intake by bears (Kimball et al., 1998a). The terpenoid solution used in that bioassay consisted largely (82%) of α-pinene, β-pinene, limonene, and 3-carene in a $12:2:1:1$ ratio. Thus, significant increases in the concentration of cluster 2 monoterpenoids may similarly produce avoidance responses.

Melding these results with the knowledge that black bear preference is impacted by changes in sugar and terpenoid concentrations may allow predictions to be made regarding potential tree family preferences. When offered a choice, foraging bears are predicted to select tree families higher in sugars (cluster 3 compounds) and/or lower in cluster 2 terpenoids than other available genetic families.

This study indicated that the faster growing Douglas-fir families had higher vascular tissue sugar concentrations than their slower growing counterparts at many sites. However, the interaction of genetics and environment produced fast growing trees with relatively low vascular tissue sugar concentration at some sites. Some terpenoids were also found to be under genetic control (Table 4). However, no trade-off between growth and constitutive terpenoid concentrations was observed. Thus, it may be possible to select for characteristics which make trees less preferred by black bears (low sugars, high terpenoids) while not sacrificing other important traits such as growth.

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