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PATTERNS OF MONOTERPENE VARIATION WITHIN INDIVIDUAL TREES IN PONDEROSA PINE

ROBERT G. LATTA,[1] YAN B. LINHART,* LAURA LUNDQUIST, and MARC A. SNYDER[2]

Department of Environmental, Population and Organismic Biology
University of Colorado
Boulder, Colorado 80309-0334

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Abstract—We surveyed variation in both the concentration and composition of monoterpenes in six tissues within individuals of ponderosa pine to determine (1) whether variation exists between different tissues; (2) whether variation occurs between samples from the north and south sides of the tree within a tissue, and (3) whether composition of one tissue is correlated with that of others. Wood, roots, and resin exuded from the trunk have similar monoterpene compositions within trees. Phloem and cones contain a higher proportion of α-pinene with less carene than resin, while needles are distinguished by high proportions of β-pinene. Samples of the same tissue taken from the north and south sides of the tree differed little and were strongly correlated. Correlations were moderate across tissues within the groups sharing similar monoterpene compositions (e.g., phloem vs. cones), but correlations between tissues in different groups were weak (e.g., phloem vs. needles).

Key Words—Oleoresin, secondary compounds, pinene, carene, intraindividual variation, plant defenses, *Pinus ponderosa*.

INTRODUCTION

Monoterpenes are a highly variable class of plant secondary compounds that occur in a wide diversity of angiosperm and gymnosperm species. Well-known examples include the constituents of essential oils in pines, mints, and citrus fruits. Monoterpenes are typically highly volatile, imparting a characteristic fla-

*To whom correspondence should be addressed.
[1]Current address: Department of Biology, Dalhousie University, 1355 Oxford St. Halifax, Nova Scotia, B3H 4J1 Canada.
[2]Current address: Department of Biology, Colorado College, Colorado Springs, Colorado 80903.

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vor and odor to the tissue in which they occur. Many can be toxic to herbivores. For these reasons, there exists a strong role for monoterpenes in mediating the interactions between plants and their herbivores and pathogens (Harborne, 1991; Gershenzon and Croteau, 1991; Lewinsohn et al., 1991; Raffa, 1991; Langenheim, 1994). In general, this role is defensive, where monoterpenes serve to discourage feeding by particular herbivores. In other examples, however, the interaction can be more complex, as for example, when bark beetles attacking pines use particular monoterpene compounds present in the tree as precursors to create an aggregation pheromone, which attracts additional beetles to the tree (Mitton and Sturgeon, 1982; Wood, 1982).

The monoterpene composition of a given species is usually characteristic of that species, although there is often considerable variability among individuals within a species in the relative amounts of the different monoterpenes present (Smith, 1977; Vernet et al., 1986; Harborne, 1991; Hanover, 1992). This variability both within and among plant species, coupled with the role of monoterpenes in defense, provides an opportunity for herbivores to use monoterpene composition as a criterion in selecting plants as food. For a number of plant–herbivore systems, there is a demonstrable relationship between amounts and kinds of monoterpenes present and the intensity with which individual plants are attacked by herbivores. Within one species, ponderosa pine, examples include *Dendroctonus* spp. (Sturgeon, 1979; Cates and Alexander, 1982; Mitton and Sturgeon, 1982), gouty pitch midge (Hoff, 1988), mule deer (Schwartz et al., 1980), and Abert's squirrel (Snyder, 1992). Similar examples have been documented for other conifers and for many angiosperms (reviewed in Langenheim, 1994).

The focus of this study is ponderosa pine (*Pinus ponderosa* Laws.), a dominant constituent of mid-elevation forests throughout western North America. Over 200 different insect species attack ponderosa pine in some part of its range (Furniss and Carolin, 1977), in addition to attacks by a variety of birds, mammals, fungi, and parasitic plants (e.g., Baumgartner and Lotan, 1988). Most of these herbivores attack only one part of the tree, with different herbivores attacking different tissues. For example, bark beetles of the genera *Dendroctonus* and *Ips* bore through the bark and into the wood, and their larvae feed on phloem. Aphids, spittlebugs, scale insects, and their allies (Homoptera) feed on sap obtained either from phloem or needles, while several insects including lepidopterans, coleopterans, hymenopterans and hemipterans will focus on cone tissues and seeds. In attempting to understand the feeding preferences of herbivores attacking ponderosa pine, we must recognize that different herbivore species will encounter the defenses of different tissues. Thus, it is relevant to ask how the monoterpene content and composition of the tissues they attack differ. Variation within trees has been documented in several species of conifers. This can often arise from the differentiation of tissues, such that particular tissues have characteristic monoterpene profiles (Roberts, 1970; Hrutfiord et al., 1974;

Bufler et al., 1990). In addition, some variation has been documented over the course of a season (Hrutfiord et al., 1974) and over the development of a tissue (Zavarin et al., 1971), as well as with position in the tree (Smith, 1968; Roberts, 1970; Moore and Hanover, 1987).

In previous work, we analyzed the ecological and evolutionary consequences of interactions between ponderosa pine and diverse herbivores and parasites and also the roles played by intertree variation in monoterpene composition as a factor mediating these interactions (Sturgeon and Mitton, 1986; Linhart, 1991; Snyder, 1992, 1993; Linhart et al., 1994; Snyder et al., 1996; Latta and Linhart, 1997; Snyder and Linhart, 1998). Because natural selection acts on the individual, we are most interested in differences among trees in their overall monoterpene composition and wish to know if this is consistent across different tissues. In this study, we document the patterns of variation within trees among six different tissues that represent potential sites of herbivore attack—phloem, wood, roots, cones, needles, and the exuded xylem oleoresin. Although no herbivore feeds on resin, it represents an important defense mechanism for conifers against certain herbivores and has been widely studied in the context of herbivore attack on ponderosa pine (Sturgeon, 1979; Cates and Alexander, 1982; Sturgeon and Mitton, 1986; Snyder, 1992; Latta and Linhart, 1997). Exuded resin should not differ from that present in the wood itself. However, since many trees do not have sufficient xylem resin pressure to exude enough resin for analysis, extraction directly from wood shavings may present a viable alternative method of assaying resin in these trees, provided that wood and resin monoterpene profiles are indeed the same. We will thus refer to the resin exuded from the trunk of the tree as a "tissue" for brevity, although this is not strictly accurate. We examine two aspects of intratree variation. First, we document characteristic differences among tissues that are consistent from tree to tree. Second, we document the patterns of correlation between tissues across trees, to determine whether knowledge of monoterpene composition in one tissue (e.g., phloem) allows inferences to be made concerning the composition in other tissues.

METHODS AND MATERIALS

Study System. Ponderosa pine is a long-lived conifer widespread in the montane regions of western North America. Variation in the monoterpene composition of the oleoresin was described by Smith (1977) for the entire range within the United States. The major monoterpenes of ponderosa pine resin are α-pinene, β-pinene, and δ-3-carene, limonene, and myrcene. Camphene, terpinolene, γ-terpinene, and β-phellandrene are also commonly present in detectable amounts. Smith identified five major geographic zones within the range of ponderosa pine based upon variation in the relative proportions of the five major

monoterpenes. Our study site occurs within region III (Cascade–Northern) of Smith (1977). Resin in this region is dominated by carene, with moderate amounts of β-pinene and limonene and relatively small proportions of α-pinene and myrcene.

Our study site is located in Boulder Canyon approximately 2 km west of Boulder, Colorado, at an elevation of 1740 m. The site contains an all-aged pure stand of ponderosa pine occupying approximately 1.5 ha on a dry, south-facing slope. All of the trees within the study area were permanently tagged in 1977, and this population has been the site of extensive long-term research into the ecological genetics of ponderosa pine (e.g., Linhart et al., 1981; Mitton et al., 1981; Linhart and Mitton, 1985; Linhart, 1988; Latta and Linhart, 1997). Trees within the site show considerable variation in monoterpene composition of their resin (R. H. Smith and Y. B. Linhart, unpublished; Latta and Linhart, 1997), especially in the relative amounts of β-pinene and carene. However, the average monoterpene abundances for the population are consistent with those described by Smith (1977) as characteristic for this region.

Sampling Methods and Chemical Analysis. In the summer of 1996, only 18 trees at the study site produced cones. We selected one branch bearing maturing cones (i.e., cones that had been pollinated the previous summer and that were ripening to release mature seeds in the fall of 1996) from the mid-crown level on both the north and south sides of each cone-bearing tree. To minimize volatilization between field and laboratory, branch tips (approx. 40–50 cm in length) were cut from the tree with a pole pruner, placed on ice in a cooler and returned to the laboratory for immediate processing. Once in the laboratory, tissue samples were taken from the distal end of the cut branch, as far from the initial cut as possible to reduce the influence of the cut on the monoterpene composition of the samples. The time elapsed between a branch being cut from the tree, and the sample being sealed in pentane could potentially affect the monoterpene concentration. To prevent this from introducing any systematic bias either between samples or between tissues, samples were processed in random order.

From each of the collected branches we took 1-year-old needles and ground them in liquid nitrogen. We stripped off the outer bark from each branch just proximal to the oldest needles and collected the underlying phloem, which we ground in liquid nitrogen. One cone was collected from each cone-bearing branch. Cones (along with other woody tissues described below) underwent an initial grinding in a coffee grinder before being ground in liquid nitrogen. Approximately 1.5 g of each powdered tissue was transferred to a glass vial, and soaked in 20 ml of pentane for at least five days to extract the monoterpenes.

Resin was collected from trees by drilling a small hole in the bark of the tree approximately 1 m above the ground on both the north and south sides. The wood shavings from this drilling were collected for analysis. The open end

of a vial was inserted into the holes, and left in place for 24 hr to collect the exuded resin. All trees were sampled for resin on the same day as they were sampled for solid tissues. Resin vials were capped when removed from the tree, immediately returned to the laboratory, and diluted 1 : 1 with pentane for storage. Prior to analysis by gas chromatography, samples were diluted a further 200-fold with pentane.

We examined an additional 18 trees that did not bear cones. These trees were selected from earlier information on resin composition to represent the extremes of the range of monoterpene compositions present in this stand. These trees were sampled as above for cone-bearing trees, but since there were no cones, we selected a branch at random from mid-crown, and we sampled these trees on the south side only. All trees were sampled on the same day and processed within 12 hr.

Roots could not be accessed easily from all trees. Instead, we selected nine trees from the above 36 that were sufficiently far from other trees so that we could identify their roots with certainty. We excavated a short length of root tissue from these trees and returned them on ice to the laboratory, where they were processed as for cones and wood, above.

After five days of extraction in pentane, we added 5 μl of fenchone to each sample as an internal standard. From each extract, 3 μl was analyzed with an HP5368 gas chromatograph containing a DB-Wax column (J&W Scientific). Since resin was more concentrated, only 2 μl of these samples were analyzed. Samples were injected into a split/splitless injector at a split ratio of 80 : 1 and injector temperature of 270°C. The temperature profile for the run was 3 min at 50°C, followed by a 4°C/min ramp to 68°C, then a 25°C/min ramp to 240°C. We calibrated the response factors of each monoterpene (Raffa and Steffeck, 1988) with a calibration standard containing 10 μl of each monoterpene (Sigma) and 10 μl of fenchone in 100 ml of pentane. The machine was recalibrated after every five samples. Signal peaks were integrated and concentrations were calculated with HP Chemstation software.

Statistical Analysis. In the analysis, we distinguish between variation in the monoterpene concentration (the total amount of monoterpenes in a tissue) and the monoterpene composition (i.e., the proportion of each of the nine monoterpenes, expressed as a percentage of the total). We tested first for differences among the tissues. Then we sought to quantify the correlations among the tissues. That is, if a tree has a higher concentration of, say, carene in its resin than other trees, will the monoterpene composition of its phloem also differ from other trees in a predictable way?

Rather than examine correlations across each tissue for each monoterpene fraction separately, we reduced the number of variables of the data set by taking factors of the monoterpene composition (Tabachnick and Fidell, 1989). Since monoterpene fractions expressed as percentages must sum to 100%, the mono-

terpene fractions are negatively intercorrelated with one another (i.e., an increase in the proportion of carene can only come at the expense of a reduced relative amount of some other monoterpene, such as α- or β-pinene) (Birks and Kanowski, 1988). Therefore, much of the variation can be summarized in a smaller number of factors. We used a principal-components initial factoring, with a promax rotation (Tabachnick and Fidell, 1989).

RESULTS

The factor analysis revealed three factors, which accounted for 81% of the total variation in monoterpene composition. Factor loadings are given in Table 1. Factor 1 summarizes variation between samples that were predominantly α-pinene and those that were predominantly carene. Factor 2 summarizes the variation in β-pinene and myrcene fractions, while factor 3 summarizes variation in the limonene fraction.

There was significant variation among the five solid tissues in the total monoterpene concentration on a fresh weight (FW) basis (Kruskal-Wallis $\chi^2 = 62.64$, 4 *df*, $P < 0.001$). This was primarily due to the high concentration of monoterpenes in phloem tissue (4.32 mg/g FW) versus the remaining tissues, which contained much less (Table 2).

The six tissues also differed in their composition (i.e., the relative proportions of the different monoterpenes they contained; Table 2). Multivariate ANOVA showed that these differences were highly significant ($\lambda = 0.185$, $P < 0.001$). Variation in the monoterpene composition divided the six tissues into three groups (Table 1, Figure 1). Resin, wood, and roots had similar compositions characterized primarily by lower proportions of α-pinene and higher proportions of carene than the other tissues. Thus, these three tissues scored lower

TABLE 1. FACTOR LOADINGS OF EACH MONOTERPENE FRACTION[a]

	Factor		
	1	2	3
α-Pinene	**0.881**	−0.166	0.243
β-Pinene	0.546	**−0.720**	0.089
δ-3-Carene	**−0.884**	0.599	−0.217
Myrcene	−0.164	**0.861**	−0.028
Limonene	0.269	−0.054	**0.984**
Terpinolene	−0.695	**0.778**	0.151

[a] The three factors accounted for 81% of the total variation in monoterpene composition. Monoterpenes loading most heavily on each factor are in bold type.

TABLE 2. VARIATION IN CONCENTRATION (TOTAL MONOTERPENES IN TISSUE EXPRESSED AS mg/g OR mg/ml) AND COMPOSITION (PROPORTION OF EACH OF NINE MONOTERPENES EXPRESSED AS PERCENTAGE OF TOTAL) OF MONOTERPENES IN SIX TISSUES IN PONDEROSA PINE[a]

	Resin	$N = 38$	Wood	$N = 52$	Roots	$N = 11$	Phloem	$N = 51$	Cones	$N = 29$	Needles	$N = 49$
Total	254.41	(38.25)	2.65	(2.58)	1.93	(1.30)	4.32	(1.61)	2.20	(1.05)	2.23	(0.89)
% α-Pinene	11.17	(12.88)	8.91	(10.13)	6.04	(2.55)	27.26	(14.87)	33.83	(16.92)	27.28	(11.67)
% Camphene	0.07	(0.16)	0.02	(0.09)	0.00	(0.00)	0.31	(0.54)	0.07	(0.22)	0.40	(1.42)
% β-Pinene	22.07	(11.67)	20.47	(10.58)	25.21	(10.97)	25.86	(9.96)	19.41	(10.00)	53.94	(12.23)
% δ-3-Carene	50.51	(14.03)	51.18	(12.22)	60.07	(11.64)	27.68	(14.16)	34.88	(16.06)	7.91	(6.24)
% Myrcene	8.12	(4.48)	7.91	(4.45)	5.36	(5.24)	5.02	(2.86)	4.92	(3.87)	2.54	(1.40)
% Limonene	3.44	(3.41)	5.83	(4.50)	0.67	(1.23)	7.15	(4.82)	5.49	(4.69)	5.05	(2.89)
% β-Phelandrene	1.19	(0.21)	1.24	(1.30)	0.50	(0.71)	4.37	(4.41)	0.24	(0.87)	2.67	(2.33)
% γ-Terpinene	0.06	(0.16)	0.04	(0.14)	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)
% Terpinolene	3.33	(0.90)	4.35	(1.03)	2.11	(1.54)	2.30	(1.30)	1.11	(1.39)	0.17	(0.49)
Factor 1	−0.637	(0.760)	−0.824	(0.715)	−0.829	(0.420)	0.479	(0.562)	0.621	(0.628)	1.259	(0.261)
Factor 2	0.359	(0.864)	0.543	(0.631)	−0.141	(0.994)	0.082	(0.562)	−0.101	(1.006)	−1.212	(0.655)
Factor 3	−0.325	(1.017)	0.322	(1.011)	−1.47	(0.865)	0.484	(0.853)	0.031	(1.053)	0.094	(0.514)

[a] Total concentration is expressed in mg/ml for resin, and mg/g fresh weight for all other tissues. Composition is expressed as the percentage of the total monoterpene pool accounted for by each of nine compounds. (The absolute concentration of any individual monoterpene can thus be obtained by taking the given percentage of the total concentration.) Factor scores are dimensionless. Standard deviations in parentheses.

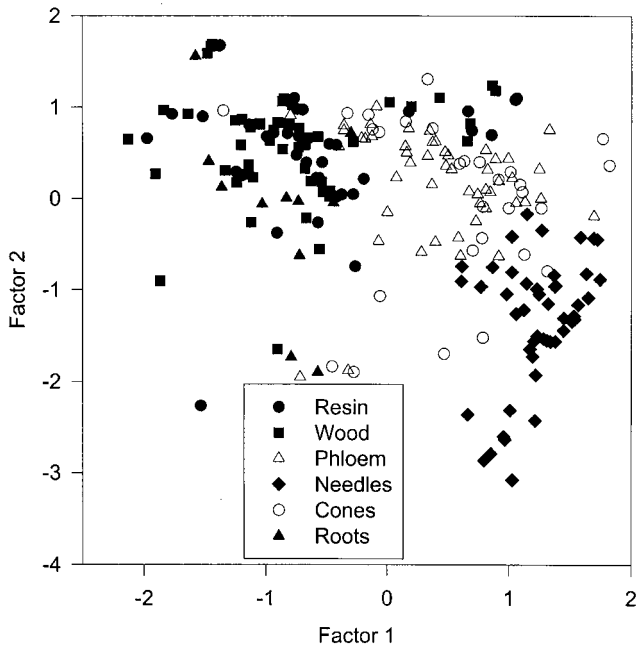

FIG. 1. Distribution of monoterpene composition (expressed as factor scores) across all tissues. Carene and α-pinene load heavily on factor 1, while β-pinene loads most heavily on factor 2 (cf. Table 1).

on factor 1 and higher on factor 2 than did the rest (i.e., these samples cluster in the upper left half of Figure 1, and are absent from the lower right). Among the remaining tissues, needles were further differentiated from phloem and cone monoterpene profiles by even lower proportions of carene and a higher proportion of β-pinene, thus scoring the lowest of the six tissues on factor 2 (Table 2, Figure 1).

Total monoterpene concentrations varied somewhat between north and south sides of the tree (Table 3). Although there is some tendency for the south side of the tree to contain slightly higher concentrations than the north, this difference was significant only for needles (paired $t = 3.04$, 13 df, $P < 0.01$). Rather, the weak correlation of total concentration between south and north sides of the tree suggests that variation with crown position is random (Table 3). By contrast, the monoterpene compositions were consistent between the north and south sides of the tree for most of the tissues. There was little difference in, and a high correlation of, factor scores between north and south sides (Table 3, Figure 2ab), although a few exceptions do exist, notably in needle tissue (Table 3).

TABLE 3. VARIATION IN MONOTERPENE CONCENTRATION (TOTAL MONOTERPENES IN TISSUE EXPRESSED AS mg/g OR mg/ml) AND COMPOSITION (PROPORTION OF EACH OF NINE MONOTERPENES EXPRESSED AS PERCENTAGE OF TOTAL) BETWEEN LOCATIONS IN CROWN OF TREE[a]

	Resin (N = 10)	Wood (N = 15)	Phloem (N = 16)	Cones (N = 13)	Needles (N = 15)
Concentration					
North	252	2.65	4.56	2.06	2.44
South	270	3.49	4.74	2.34	2.72
P	0.14	0.44	0.76	0.38	0.009
Corr	0.192	0.193	−0.051	0.594[b]	0.923[c]
Factor 1					
North	−0.631	−0.804	0.384	0.655	1.208
South	−7.069	−0.802	0.453	0.642	1.209
P	0.051	0.973	0.305	0.981	0.854
Corr	0.989[c]	0.936[c]	0.947[c]	0.946[c]	0.916[c]
Factor 2					
North	0.689	0.684	0.370	−0.067	−1.33
South	0.724	0.573	0.293	−0.21	−1.511
P	0.308	0.159	0.315	0.348	0.322
Corr	0.977[c]	0.960[c]	0.803[c]	0.853[c]	0.528[b]
Factor 3					
North	−0.275	0.284	0.654	−0.015	0.062
South	−0.414	0.205	0.668	−0.035	0.166
P	0.293	0.730	0.762	0.608	0.808
Corr	0.948[c]	0.540[b]	0.965[c]	0.966[c]	0.249

[a] Composition is expressed in terms of the three factors given in Table 1. Mean values for the north and south sides of the tree are given, along with the significance level of the difference (paired t-test P). Correlations between north and south sides are also given. Note, only those trees for which data were available from both sides of the tree are included, thus sample sizes (N) are smaller than those in Table 2.
[b] $P < 0.05$.
[c] $P < 0.001$.

The total monoterpene concentration was poorly correlated among tissues. Although the correlations were generally positive, few were statistically significant (Table 4, factor 1, below the diagonal). By contrast, the monoterpene compositions showed stronger correlations among tissues, especially for factor 1 (Table 4, Figure 2c–h). Correlations were strongest between tissues within the same group identified in Figure 1. For example, wood and resin are strongly correlated for factor 1 (Figure 2c) as are phloem and cones (Figure 2g). Correlations between these groups are weaker, but still significant. For example, phloem exhibits much higher factor 1 scores than does resin (Figure 1), because phloem has more α-pinene and less carene (Table 1). Thus in Figure 2e, the open circles plot above the 1 : 1 line. Despite this different composition, how-

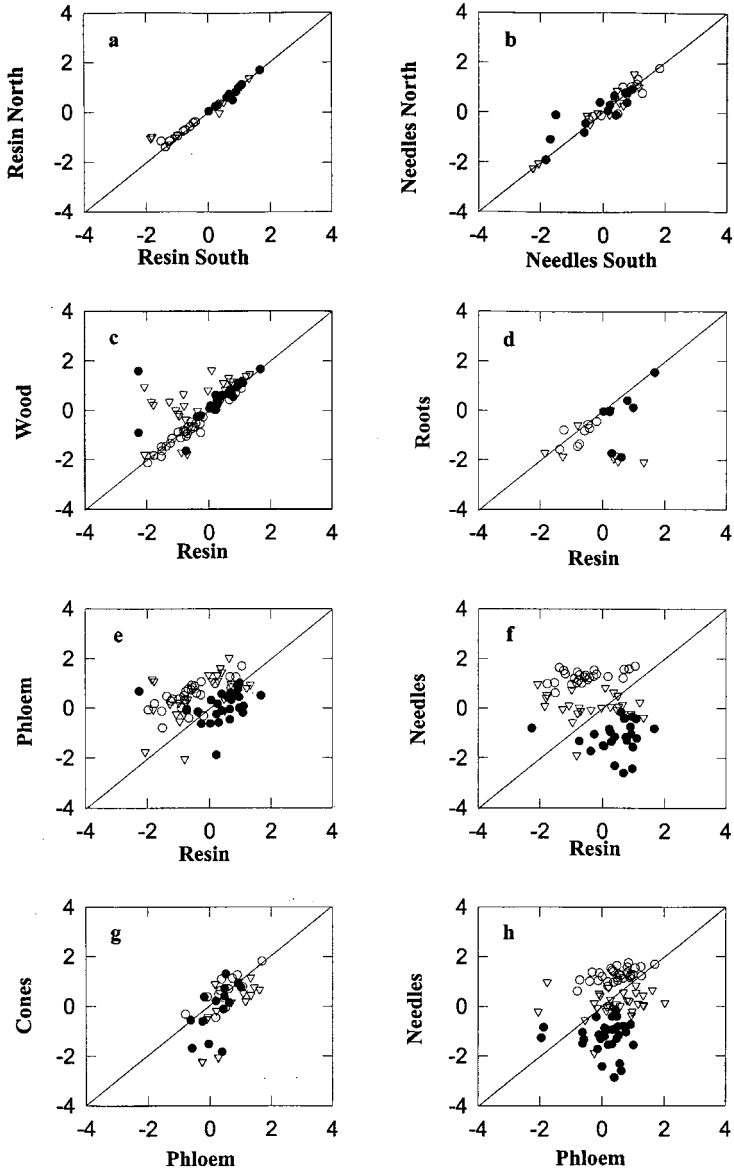

FIG. 2. Correlations among monoterpene composition (factor scores) in different samples taken from the same tree. a,b: Correlations between the north and south sides of the tree. c–h: Selected correlations among tissues. Open circles: factor 1 (α-pinene vs. carene); closed circles: factor 2 (β-pinene); triangles: factor 3 (limonene).

TABLE 4. CORRELATION OF MONOTERPENE CONCENTRATION (TOTAL MONOTERPENES IN TISSUE EXPRESSED AS mg/g OR mg/ml) AND COMPOSITION (PROPORTION OF EACH OF NINE MONOTERPENES EXPRESSED AS PERCENTAGE OF TOTAL) ACROSS TISSUES[a]

	Resin	Wood	Phloem	Cones	Needles
Factor 1 (below diagonal) and total concentration (above diagnonal)					
Resin		0.211	−0.042	−0.041	0.168
Wood	0.980[b]		0.431	0.945[b]	0.210
Phloem	0.712[b]	0.659[b]		0.529	0.111
Cones	0.778[c]	0.761[b]	0.784[b]		0.216
Needles	0.527[c]	0.411[c]	0.575[b]	0.568[d]	
Factor 2 (below diagonal) and 3 (above diagonal)					
Resin		0.638[b]	0.484[c]	0.432	−0.012
Wood	0.537[c]		0.455[c]	0.377	−0.218
Phloem	0.181	0.344[d]		0.684[c]	0.144
Cones	0.858[b]	0.661[c]	0.803[b]		−0.138
Needles	0.055	0.116	−0.033	0.465	

[a] Correlations are computed for samples from the south side of the tree only.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.

ever, factor 1 scores are moderately correlated between the two tissues (Figure 2e, Table 4). Needle monoterpene composition was not well predicted by the composition of other tissues for any factor.

To reduce the number of variables, we have conducted our analyses in terms of factors that we extracted from the raw data. To make the results more intuitively accessible, we illustrate our finding for two monoterpenes (β-pinene and carene) in Figure 3, where each line connects observations made on different tissues of the same tree. Differences between the tissues are seen in the fact that, for example, carene consistently makes up a greater proportion of the monoterpene pool in the resin of each tree than it does in the needles, while the reverse is true for β-pinene (Table 1). Such differences are not apparent between resin and wood. Thus the tissues can be divided into groups based upon their different monoterpene compositions (Figure 1). Group one consists of roots, resin, and wood. Group two includes cones and phloem, and needles form a third group on their own.

Figure 3 also illustrates the pattern of correlation between tissues among trees. For any given tissue there is a wide range of variation among trees. For example, the resin of one individual contains almost no β-pinene, while that of another contains over 40% β-pinene. This variation in resin composition is

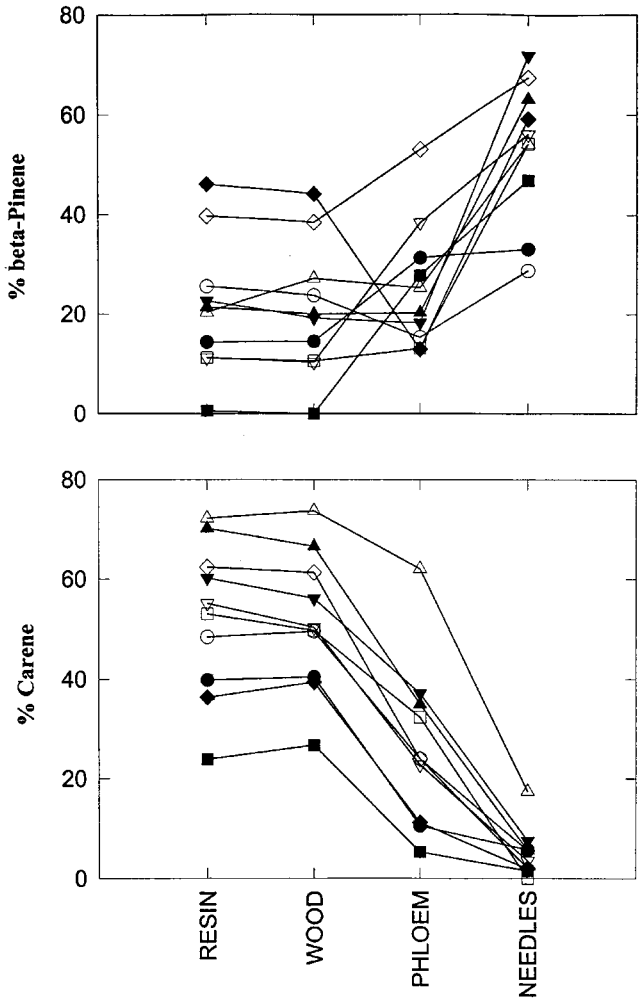

FIG. 3. Example of the changes in monoterpene composition across several tissues in twelve trees for which all data were available. Different symbols represent different individual trees.

strongly correlated with variation in the monoterpene composition of the wood (Table 4), such that the rank-ordering of the trees is preserved between the two tissues (Figure 3). There is a weaker, but still significant correlation between resin and phloem (Table 4). Thus, despite the difference between the tissues, some of the rank ordering is preserved (Figure 3). By contrast, since the rank

ordering of the trees is very different between resin and needles, little correlation between these two tissues in seen (Table 4).

DISCUSSION

Variation in the monoterpene composition of coniferous resin has been extensively documented between trees (von Rudloff, 1975; Smith, 1977; Hanover, 1992). In addition, numerous studies have documented variation among tissues within individual trees (Roberts, 1970; Hrutfiord et al., 1974; Zavarin et al., 1971; Moore and Hanover, 1987; Bufler et al., 1990; Wallin and Raffa, 1998, 1999). Our results suggest little variation with crown position (north vs. south) for most tissues but indicate systematic differences between tissues of ponderosa pine in both the concentration as well as the composition of monoterpenes. To our knowledge, however, this study represents the first that attempts to relate variation within trees to that between trees by examining the correlation among tissues. We have demonstrated that monoterpenes do not vary independently in different tissues. Rather, monoterpene composition of one tissue is generally correlated at least partly with the composition of other tissues. In general monoterpene composition is more strongly correlated among tissues in the same tissue group than in different tissue groups.

The monoterpene composition is much more consistent within trees than is the absolute concentration of monoterpenes. Total concentration is poorly correlated between north and south sides of the tree, and among tissues. This suggests that the size of the monoterpene pool is more strongly determined by environmental factors such as available resources than is the allocation to particular fractions within that pool. Resource availability is known to influence the production of monoterpenes in several species (Gershenzon and Croteau, 1990; Koricheva et al., 1998). Based upon the different amount of sunlight expected on the north vs. south sides of the tree, one might predict more resources to be available, and overall monoterpene concentrations to be higher on the south side of the tree. Although there is a slight tendency for monoterpene concentration to be higher on the south side of the tree than the north, this difference is only significant for needles.

However, the composition of samples drawn from north vs. south sides of the tree were generally tightly correlated (needles are a notable exception), such that one sample could predict the composition at other locations around the crown of the tree with high accuracy. By contrast, other workers have found differences in composition correlated with height in the crown (Smith, 1968; Moore and Hanover, 1987), although the degree of correlation among positions is unknown.

Because of the variation among tissues, herbivores attacking different tissues within the tree will encounter different monoterpene profiles. This may affect not only the choice of which individual to attack, but also the choice

of specific tissue or attack site. Some have argued that an important factor contributing to the ability of trees to resist insect attack is that individual trees may consist of genetic mosaics produced by rapid mutation in different branch systems (e.g., Whitham and Slobodchikoff, 1981). Our results suggest instead that phenotypic heterogeneity within individual trees involves developmental, rather than genetic, variation among adjacent tissues of a single branch. Such tissue differences may involve evolved responses to the different interactions of each tissue. For example, in *Clarkia brewerii*, linalool is produced solely in the petals of the flower, where it functions in pollinator attraction, illustrating the possibility of tissue-specific regulation of monoterpene biosynthesis to perform tissue specific functions (Pichersky et al., 1994). Alternatively, intratree variation may represent either phenological differences as tissues develop (Zavarin et al., 1971) or chemical interactions that take place along a gradient within a tree. For example, several studies note a decrease in the relative abundance of α-pinene with height in the tree (Smith, 1968; Roberts, 1970; Moore and Hanover, 1987), a pattern that is consistent with differential volatilization of α-pinene due to its lower boiling point relative to other monoterpenes (Smith, 1968).

The correlations between monoterpene fractions in different tissues have important implications for studies of plant–herbivore interactions. Knowledge of the resin composition in one tissue contains at least some information about the monoterpene profile of other tissues in the tree. Thus, over a large sample of trees, those with, say, a high proportion of β-pinene in their resin are likely (on average) to also have high concentrations of β-pinene in their phloem. We have examined differences of resin composition between trees that are attacked and those that are not for several herbivores (Snyder, 1992; Snyder et al., 1996; Latta and Linhart, 1997; Snyder and Linhart, 1998). Measurable differences between attacked and nonattacked trees in resin composition are likely to reflect meaningful differences between the two groups of trees in the monoterpene profile in the tissue actually being attacked.

We must emphasize, however, that such correlations are not tight enough to be predictive. That is, attempts to predict the exact composition of a particular tissue in a given tree from knowledge of the resin composition will have a very high error associated with it. Even in the most strongly correlated tissues, resin and wood, there is considerable residual variation, especially with respect to the proportion of limonene. It is likely that this represents methodological difficulties—trees with low resin exudation pressures also have low monoterpene concentrations in the wood and other tissues. In this case many minor monoterpene fractions drop below the detection limit, biasing the measured composition of wood towards the more abundant monoterpene fractions. Such error variance precludes the substitution of one tissue for another on a single tree basis.

Ideally, therefore, studies of defense mechanisms should examine the tissue or perhaps the microsite being directly attacked. However, in broader studies of nat-

ural selection, large sample sizes are needed (often several hundred), which may prohibit the analysis of each possible site of herbivore attack. More importantly, if several herbivores attack the same host species, they may produce a diversifying selection pressure on the host population (Linhart, 1991; Linhart and Thompson, 1995). If each tissue had a separate monoterpene profile, uncorrelated with any other tissue, each could respond separately to the selective pressure of the herbivore that attacks it. However our results demonstrate that this is not the case—a response to selection in one tissue, say phloem, will produce a correlated change in the monoterpene composition of another, say resin. This correlated change may or may not be beneficial to the host in defending against attack in the second tissue. In these cases, it will be most useful to assess a single tissue that best characterizes the monoterpene concentration and composition in the tree as a whole. We suggest that the exuded xylem oleoresin presents such information, since resin shows the least variability within trees (Table 3) (Smith, 1968) between years (Smith, 1964), or in response to simulated herbivory (Snyder, 1992).

Needles, by contrast, are the most variable tissue within the tree. Monoterpene composition of the needles was weakly correlated with other tissues, and also weakly correlated between north and south sides of the tree. Moreover, needles are the only tissue to show a consistent difference in total monoterpene concentration between north and south sides. This suggests that monoterpene concentration and composition in needles is more strongly influenced by physiological processes within that tissue. Although we can only speculate here on the causes of the high intratree variability exhibited by needles, this variation indicates a much more labile monoterpene composition in needles than in other tissues. Thus the criteria for selective herbivory on this tissue are unlikely to be inferred from study of the resin, but must instead be studied directly.

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