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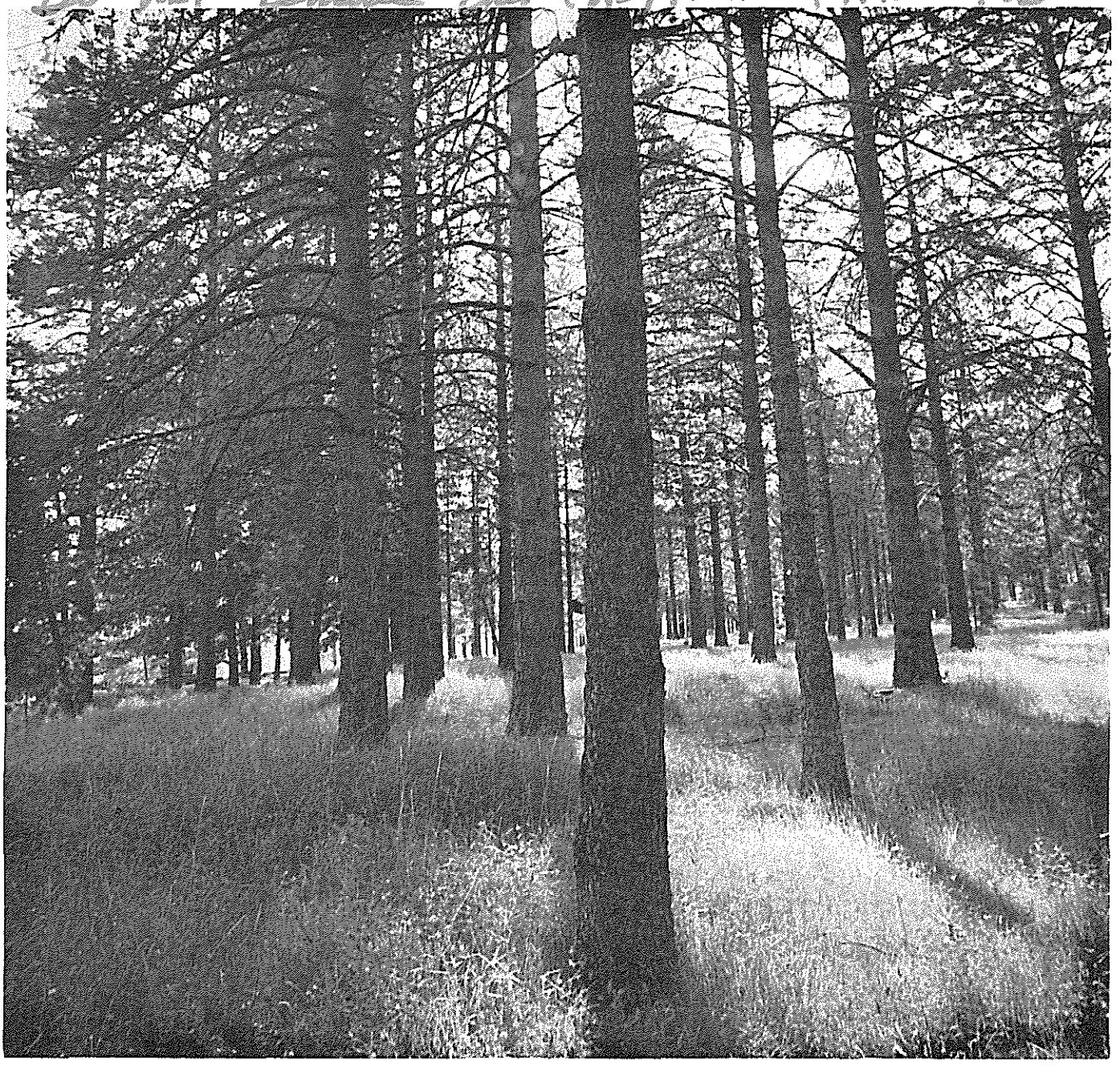
Xylem Monoterpenes of Some Hard Pines of Western North America: three studies

Richard H. Smith



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IN BRIEF...

Smith, Richard H. **Xylem monoterpenes of some hard pines of Western North America: three studies.** Res. Paper PSW-160. Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture; 1982. 7 p.

Retrieval Terms: xylem monoterpenes, *Pinus ponderosa*, *P. coulteri*, *P. jeffreyi*, *P. torreyana*, *P. sabiniana*, *P. montezumae*, *P. hartwegii*, *P. rudis*, *P. pseudostrobus*, *P. durangensis*, *P. cooperi*, *P. engelmannii*

Copious production of xylem resin is characteristic of pines. The terpene fraction of this resin is suspected of being a defense mechanism in some pines; it also appears to be under strong genetic control. Thus, investigations of it can be useful for information on both genetics and host relationships. Studies were made to determine (1) the within-tree monoterpene composition of single stems, forks, and grafts; (2) the monoterpene composition of seven species of pines from Mexico growing in California; and (3) the number of trees required in a plot for estimates of local variation, and the comparative accuracy of short- and long-column gas chromatographic analysis.

All analyses were of normalized monoterpene composition of xylem resin using a thermal conductivity gas chromatograph and pentane solutions of whole resin.

Within-tree studies showed negligible and insignificant variation with varying vertical location in both single and forked stems of 40-year-old Jeffrey and Coulter pines and in forked stems of ponderosa pine. Within-tree constancy of single stems of ponderosa pine was reported earlier. Much of the intertree variation in Jeffrey pine is found only in heptane, which varies from 85 to 96 percent; the remainder of the composition is small amounts of nonane, α-pinene, camphene, β-pinene, 3-carene, sabinene, myrcene, limonene and β-phellandrene. All of these vary from less than 1 percent to 3 percent but with β-phellandrene as high as 6 percent. Coulter pine, however, has significant intertree variation: α-pinene (29 to 48 percent), sabinene (2 to 5 percent), myrcene (5 to 23 percent), limonene (2 to 10 percent), β-phellandrene (24 to 35 percent), and terpinolene (0 to 5 percent); five other components—heptane, nonane, camphene, β-pinene, α-phellandrene—are usually less variable and less than 5 percent. Ponderosa pine had a large intertree range in the percent of α-pinene, β-pinene, 3-carene, myrcene, and limonene. Hybrids of Jeffrey × ponderosa also showed little intratree variation in single stems and forks.

Analysis of 20-year-old grafts of Jeffrey on ponderosa and Digger on ponderosa (with graft unions at about 2 m) showed negligible variation in the monoterpene composition of the scion, but the root stock had monoterpene characteristics of both species. The effect of the scion on the root stock diminished with distance below the graft union. A graft of ponderosa on Jeffrey was quite different in that both scion and root stock showed only monoterpene characteristics of ponderosa. A graft of Torrey on ponderosa was made near the ground line. The scion showed only Torrey pine characteristics and variation was negligible; samples could not be obtained from the root stock.

Seven species of pines from Mexico were studied using 6-year-old trees growing in the nursery at the Institute of Forest Genetics near Placerville, California. Of these seven, only *P. durangensis* showed little intertree variation; but only two trees were available and both had about 96 percent α-pinene. Three of the remaining six species showed large intertree variation in α- and β-pinene as follows: *P. engelmannii*—α-pinene 49 to 94 percent, β-pinene 3 to 49 percent; *P. cooperi*—α-pinene 26 to 96 percent, β-pinene 2 to 69 percent; *P. montezumae*—α-pinene 76 to 98 percent, β-pinene 1 to 22 percent. The other three showed large variation in four or more components as follows: *P. hartwegii*—α-pinene 2 to 94 percent, β-pinene 1 to 60 percent, 3-carene 0 to 80 percent, limonene 0 to 64 percent; *P. rudis*—heptane 0 to 32 percent, α-pinene 6 to 79 percent, β-pinene 0 to 69 percent, 3-carene 0 to 33 percent, sabinene 0 to 30 percent, limonene 0 to 76 percent, terpinolene 0 to 19 percent; *P. pseudostrobus*—heptane 0 to 42 percent, nonane 2 to 10 percent, α-pinene 22 to 98 percent, β-pinene 1 to 37 percent, 3-carene 0 to 30 percent, limonene 0 to 34 percent. In addition to this variation in individual components, there is considerable range in types of composition.

This study does little to resolve the problem of the taxonomy of the pines of Mexico, but it does point out the large variation in monoterpene composition and the need for further study.

More than 1500 trees were used in a study of the local variation of the types of monoterpene composition of ponderosa pine in northeastern California. The results suggest that 80 trees are adequate for determining average composition, but are inadequate for determining the distribution and abundance in types of individual tree composition. About 350 trees are necessary for this last determination.

A short-column analysis (about 3 min) was found to be almost as accurate as a long-column analysis (about 12 min). The short-column analysis was based on converted peak height values. When a code system was used to express total monoterpene composition of a tree, the short column was 97 percent correct in determining individual code values and 86 percent correct for determining the types of composition of a five-component mixture. Thus, the short column is suitable for screening large numbers of trees in localized areas.

Copious production of xylem resin is a distinctive characteristic of the genus *Pinus*. The composition of the terpene fraction of the resin appears to be under fairly strong genetic control, and information about it can be useful, therefore, in genetic studies. Knowledge of monoterpene composition is also valuable in investigation of the role of the resin in general, and the terpenes in particular, as a defense mechanism in the ecology of pine (Smith 1972).

This paper reports results of several studies and relates them to earlier work. The first study examined within-tree variation in monoterpene composition of several California pine species. Evidence that xylem monoterpene resin is constant with varying positions within the tree would have application to both genetic theory and the design of biological studies. Both constancy and variation have been found in pines; for example, ponderosa (*Pinus ponderosa* Doug. ex Laws.) is constant in both time and place within a tree (Smith 1968); slash pine (*Pinus elliottii* Engelm.) is variable in certain trees (Squillace 1976).

The second study was an analysis of the monoterpene composition of 6-year-old trees of seven pine species growing at the Institute of Forest Genetics near Placerville, California, from seed collected in Guatemala and Mexico.

The third study examined two elements of procedure in the study of monoterpene composition: sample plot size and chromatographic analysis.

GENERAL PROCEDURES

Some of the procedures used to obtain, process, and analyze resin samples were similar in all three studies, and are described here. Procedures specific to a particular study are noted in the report of results.

Sample Collection

Resin was obtained in one of two ways depending on the size of the tree. Trees greater than 10 cm d.b.h., which included all the California species, hybrids, and grafts, were tapped with a 1.41 cm bit and brace. The hole was drilled, at a slight upward slant, through the outer bark and phloem and 0.6 to 1.3 cm into the xylem. The hole was cleared of debris, and a 5-cm^3 vial was placed in the hole so that the lip was past the phloem tissue. Except where noted in studies which sampled trees at varying heights, all taps were made 1.0 to 1.3 m above the ground. The vial was removed 6 to 24 hours after tapping. Up to 0.5 cm^3 of fresh resin was placed in a half-dram screw-cap vial with an approximately equal volume of chromatographic quality pentane. The vial was agitated to produce a homogeneous clear liquid ready for chromatographic analyses.

The resin sample from the Mexican pines, which were 6 to 7 years old, was obtained by cutting the tree off in the third internode back from the tip. A ring of cortex about 6 mm wide was removed from the wood just below the cut to prevent contamination of the xylem resin by cortex resin. The cross sectional surface was made clean and smooth by careful removal of a thin slice of about 0.5 mm of wood; the cut also increased resin flow. Within minutes resin began to exude onto the surface of the cross section. Within an hour or two after the cut was made, a drop of this resin was carefully picked up with a clean glass rod and placed in a half-dram vial. An approximately equal volume of pentane was added to the vial and agitated. The tightly sealed vials were held at about 3° C for periods as long as 4 to 6 months before analysis.

Chromatographic Analysis

All samples were analyzed by gas liquid chromatography using a thermal conductivity detector. Operating temperatures were 135° to 145° C on the injector, 60° to 70° C on the column, 145° to 155° C on the detector. There was a flow of helium of 30 to 40 ml per minute at the outlet port. All columns were stainless steel with a diameter of 3.1 mm; the short columns were 1.7 m in length and the long columns 4.0 m. All columns had a solid phase of 100 to 110 Chromosorb W/AW and a liquid phase of 5 percent b,b'-oxydipropionitrile. None of these variables was associated with any differences in the results of qualitative or quantitative analysis. The standard analysis required 12 to 15 minutes on the long column; the short analysis required about 3 minutes on the short column.

Peak areas of the standard chromatogram were used for quantitative analysis; these were derived from disk integrator values and then normalized for each sample to

express each monoterpene component as a percentage of the total monoterpene content.

For the short column analyses, peak heights with appropriate conversion factors were used to obtain the percent composition (Smith and Greene 1971). The short column chromatogram was also used to guide and check the standard analysis.

WITHIN-TREE VARIATION IN COMPOSITION

Five groups of trees growing at the Institute of Forest Genetics, Placerville, California, were studied by sampling at different vertical positions on the main stem. The internode length varied from about 30 to 45 cm. Trees sampled were 8 Coulter pines (*P. coulteri* D. Don.) 35 years old; 10 Jeffrey pines (*P. jeffreyi* Grev. & Balf.) 35 years old; 3 Jeffrey × ponderosa pine hybrids 30 years old; 10 forked trees (7 ponderosa, 2 Jeffrey, 1 Jeffrey × ponderosa hybrid); and four 20-year-old grafts of Jeffrey, ponderosa, Digger (*P. sabiniana* Dougl.), and Torrey (*P. torreyana* Parry) pines.

The monoterpene composition of all species and hybrids examined is quite constant with differing locations within a tree (*table 1, fig. 1*). This result closely resembles findings for ponderosa pine (Smith 1968) and several other pines (Squillace 1976). The evidence now points to constancy rather than variation in this characteristic in many pines, although some pines do have variation (Squillace 1976) past the juvenile stage.

Considerable change in composition was seen in the grafts, depending on the point of sampling and the species (*table 2*). In all grafted trees, the scion held true to the general species composition, but the root stock was affected by the scion. This was also found for slash pine (*P. elliottii*) (Squillace and Fisher 1966). In Digger or Jeffrey pine grafts on ponderosa, the samples from the ponderosa root stock within 1 m of the union had all the characteristics of Jeffrey or Digger but none of ponderosa. More than 1 m below the union, however, the composition was a mixture of both scion and stock. This condition suggests possible movement of precursors from the scion to the stock, or the slow movement of scion resin across the union; in 20 years the scion resin had moved about 1 to 2 m. The fact that there was no evidence of root stock resin less than 1 m below the union suggests possible slow loss and replacement of resin over long periods.

Table 1—*Normalized percent monoterpenes in xylem resin by species from within-tree vertical samples selected to represent the trees studied*[1]

Tree and internode[2]	Heptane	Nonane	α-pinene	Camphene	β-pinene	3-carene	Sabinene	α-phellandrene	Myrcene	Limonene	β-phellandrene	γ-terpinene	Terpinolene
	Percent[3]												
	Coulter pine												
1-12	0.4	0.5	34.3	0.5	3.9		2.7	0.9	15.6	6.7	32.5		2.0
24	0.6	0.7	30.6	0.7	3.9		2.7	0.9	17.4	5.8	34.7		2.0
36	1.1	1.1	29.2	0.7	4.9		2.0	0.6	18.5	5.8	34.5	t	1.7
6-12	0.7	0.5	44.7	0.5	3.5		4.8	0.7	5.3	6.7	28.0		4.6
24	0.6	0.5	48.5	0.5	3.6		3.4	0.4	5.6	10.2	24.3		2.3
36	0.2	0.2	46.3	0.8	3.5		4.0	0.6	5.0	10.3	26.6		2.6
8-12	0.2	0.2	36.7	0.5	3.2		0.5	0.4	23.2	2.2	32.7		
24	0.4	0.4	37.7	0.7	3.4		0.5	0.4	21.1	2.7	32.6		
36	0.4	0.4	36.5	0.7	4.1		0.3	0.4	21.5	2.5	33.1		
	Jeffrey pine												
2-12	93.2	1.1	1.1	0.1	1.1	0.1	t		0.4	t	3.0		
24	93.0	1.4	0.6	0.2	1.4	0.3	t		0.3	t	2.9		
36	93.3	1.4	0.5	0.1	1.2	0.4	t		0.3	t	2.9		
6-24	83.7	1.1	1.0	0.3	3.4	1.2	0.9	t	1.6	0.6	6.1		
36	88.2	1.3	0.6	0.3	3.0	0.9	0.4	t	0.9	0.4	3.9		
7-12	96.5	0.2	0.2	0.1	0.9	0.1	0.1		t	0.5	1.6		
24	96.8	0.3	0.1	0.1	0.8	0.1	0.1		t	0.3	1.6		
36	96.4	0.5	0.2	0.1	0.8	0.1	0.1		t	0.4	1.6		
	Jeffrey × ponderosa pine[4]												
1-10	30.0	1.0	8.0		21.0	24.0			7.0	5.0	1.0	t	2.0
30	35.0	1.0	6.0		18.0	21.0			8.0	6.0	1.0	t	1.0

[1]Three of 8 Coulter pines, 3 of 10 Jeffrey pines, and 1 of 3 Jeffrey × ponderosa hybrids.
[2]Internodes are counted from the terminal.
[3]t = trace.
[4]Two percent undecane was found for both internodes.

The mixing pattern did not hold true in grafts of ponderosa pine on Jeffrey. Here, there was no evidence of the Jeffrey root stock resin even nearly 1.7 m below the union; both scion and stock had only ponderosa characteristics. Because the union of the Torrey pine scion with ponderosa stock was just above the ground line, it was not possible to get a sample of the stock. Above the union it was fully Torrey pine. Unfortunately, these trees were cut before sampling and analysis could be made to check on any changes in these conditions.

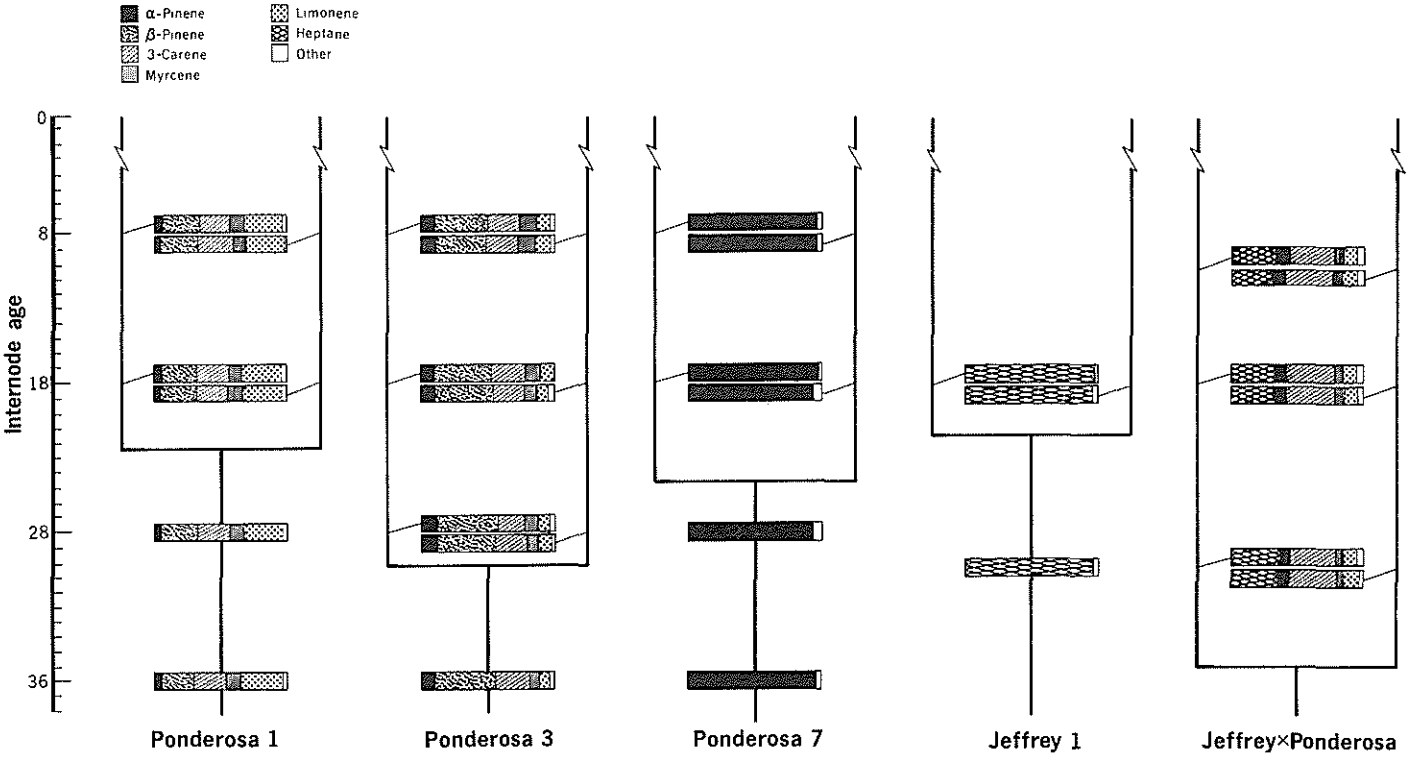

Figure 1—Normalized monoterpene composition of the xylem resin at different heights of forked trunks of selected pines. Each multishaded bar equals 100 percent of the monoterpenes.

Table 2—*Normalized xylem monoterpene composition of within-tree samples taken at specified distances above the ground on four 20-year-old grafts (arrow shows location of graft union)*

	Jeffrey on ponderosa				Ponderosa on Jeffrey					Digger on ponderosa				Torrey on ponderosa	
Monoterpene	5.0 m	3.3 m	2.0 m	0.7 m	7.0 m	5.0 m	3.3 m	2.0 m	0.7 m	5.0 m	2.8 m	2.0 m	0.7 m	2.0 m	0.7 m
	Percent[1]														
Heptane	98	98	↓94	70				↓		99	99	↓97	81		↓
α-pinene	t	t	t	2	8	8	8	8	8	t	t	t	1	3	3
Camphene	t	t	t	t	29	29	29	29	29	1	1	1	1	10	9
β-pinene	t	t	2	10	50	49	49	50	49			1	4	2	
3-carene	t	t	2	12	7	7	7	7	7			1	8	1	
Myrcene	2	2	2	4	3	3	3	3	4			8	2	4	4
Limonene	t	t	t	2	1	t	1	1	t			t	3	81	85
β-phellandrene		t	t	t	t	t	t	t	t				8		
γ-terpinene			t	t											
Terpinolene			t	t	2	3	3	3	3						

[1]t = trace.

MONOTERPENES IN HARD PINES OF MEXICO

Earlier studies of some of the Mexican pines did not establish the value of the terpenes in resolving their taxonomy. In a preliminary study of the monoterpenes of Mexican pines (Mirov 1961), samples were obtained by open-faced collection, often bulked for several trees in order to obtain sufficient resin for analysis, which did not include gas chromatographic procedures. Later, gas chromatographic analysis provided reports on *P. hartwegii* Lindl. (Williams and Bannister 1962); on *P. durangensis* Martinez, *P. montezumae* Lamb, and *P. pseudostrobus* Lindl. (Manjarrez and Guzman 1964); and on *P. engelmannii* Carr. and hybrids of ponderosa with *P. engelmannii* and with *P. montezumae* (Smith 1967).

The present study made use of the five species mentioned above, with the addition of *P. rudis* Endl. and *P. cooperi* E. E. Blanco. All were grown in the 1966 and 1967 nursery of the Institute for general study. The identification of the seed-bearing parents is based on determinations by qualified botanists. Seed was collected in 1963 and 1964 from trees growing in Mexico; some additional seed of *P. pseudostrobus* was collected in Guatemala. Six-year-old plants were analyzed for monoterpenes in 1972-73 as a preliminary study of the species.

Xylem resin samples were obtained (see general procedures) from the 3-year-old internode of 182 6- to 7-year-old trees. Samples were analyzed on a short column for guidance and on the standard long column for calculations. For concise summary for each species, data were averaged in certain obvious appropriate groupings of composition.

The types of composition that could be identified by inspection for each species (*table 3*) showed a fairly narrow range of values for a given assigned value for a component. The data in this preliminary study do not seem sufficient to justify listing of standard deviations. Some examples of the range of values for an assigned value are as follows:

Species (n):	Component	Assigned value	Range
P. engelmannii (5)	α-pinene	94	90-97
	β-pinene	3	1-7
P. cooperi (7)	α-pinene	47	38-54
	β-pinene	49	42-60
P. hartwegii (9)	α-pinene	30	20-40
	myrcene	3	1-4
	limonene	64	45-75

Two general observations can be made from the data in *table 3*. First, there is a wide range in composition and compositional types, despite the relatively small number of trees sampled, particularly in *P. hartwegii*, *P. rudis*, and *P. pseudostrobus*, which show large variations within a single State and from State to State. Second, the previously reported composition types were found and were fairly common in every species. However, many distinctly new types of composition were found for six of the species: *P. engelmannii*, 1; *P. cooperi*, 2; *P. montezumae*, 1; *P. hartwegii*, 6; *P. rudis*, 14; *P. pseudostrobus*, 11.

Five types of composition are of particular note:

1. A large percentage of limonene was found in several trees of *P. hartwegii*, *P. rudis*, and *P. pseudostrobus*. Some of these types also have moderate amounts of α-pinene, and are much like those found in a few ponderosa pines in Arizona.

2. Two high sabinene types were found in *P. rudis*, in a total of six trees. Again, similar types were found in several ponderosa pines in southeastern Arizona. The approximate one-to-one relation between sabinene and terpinolene, which has been noted for ponderosa pine (Smith 1977), prevailed in one set of these trees (13 to 15); in the other set, the relation was about one and one-half to one (30 to 19).

3. A number of trees of *P. rudis* and *P. pseudostrobus* had heptane and nonane along with several terpenes. This type might be expected of a hybrid of Jeffrey and ponderosa pine from southeastern Arizona, or a cross between ponderosa pine and *P. montezumae* reported by Smith (1967).

4. All species except *P. rudis* have trees which can be termed high in α-pinene (greater than 94 percent α-pinene).

5. Five of the species, *P. engelmannii*, *P. cooperi*, *P. hartwegii*, *P. rudis*, and *P. pseudostrobus*, have trees with nearly equal amounts of α-pinene and β-pinene, with small amounts of other terpenes.

The pines of Mexico are rich in variety of composition types, but until further and more detailed studies are made, the value of monoterpenes in clarifying the taxonomy of these pines remains uncertain.

Table 3—*Normalized monoterpene composition of the xylem resin of assigned types of composition of seven hard pines native to Mexico*

Pinus species	Trees[1]	Heptane	Nonane	α-pinene	Camphene	β-pinene	3-carene	Sabinene	Myrcene	Limonene	β-phellandrene	Terpinolene	Seed source[2]
		Percent[3]											
P. engelmannii	5(m,s)			94	l	3		t	l	t	t		Ca
	l			49	t	49			l	l	l		Ca
P. durangensis	2(m,mg)			96	l	l		t	t	l	l		Ca
P. cooperi	7(m)			47	l	49	l	t	l	l	l		D
	2			26	t	69		t	l	l	2		D
	l			96	l	2			l	l	t		D
P. montezumae	23(m,mg)			98	l	l	t	t	t	t			MS
	3			76	l	22			l	l	t	l	MS
P. hartwegii	9(m)			30	l	2			3	64		t	MS,NL
	8(w)			12	t	l	80		2	l	t	5	MS
	7			32	t	l	43		3	19	t	2	NL
	6			66	l	3		2	2	26	t	t	MS,NL
	4			39	l	2	53		l	t	t	3	MS,NL
	4			34	t	60	3		l	l	l	t	NL
	2			94	l	2		t	3				MS,P
	2			18		l	57		4	17	t	3	MS
P. rudis	15(m)			28	l	69			l	l	l		NL,T,P
	4			34	l	6	t	30	5	4	t	19	NL
	3	9	3	79	l	3	2		3				NL
	2	2	3	13	l	50	2		2	26			NL
	2(m)	11	4	13	2	14		13	2	28	l	15	NL
	l	4	2	60	l	t	4		l	24			NL
	l	9	l	57	2	28			3	t	t		NL
	l	32	2	57	2	5			l				MS
	l			55	l	2			t	42			NL
	l			50	l	l	33		2	11	l	2	NL
	l	20	l	29		45	4		l		l		NL
	l			21	2	5			2	70	t		NL
	l			17	3	45	2		2	30	l	t	NL
	l	14	2	11	l	3	9		4	54		t	NL
	l	8	4	6	2	t	t		4	76			NL
P. pseudostrobus	23	38	7	49	t	3	l		l	t	t		O,P,Mi
	9(m)			98	l	l			t	t			Cs,G
	6			61	t	37	l		t	t	t		Ca,Cs G, Mi, P
	6	27	6	47	t	18			l	t	t		O,P
	4	10	6	75	t	7	l		l		t	l	O,P
	4	36	5	26	3	5	l	t	l	23			P
	2	14	8	44	t	5	30		2	t	t	l	Mi,P
	2	20	3	26		16			l	34			P
	1(mg)			90	t	10							G
	l	42	10	25	t	23			l	t	t		T
	l	21	2	24		9	28		l	14			Mi
	l	47	7	22		4	18		l			l	P

[1]This approximate composition type has been reported as noted: m = Mirov (1961); w = Williams and Bannister (1962); s = Smith (1967); mg = Manjarrez and Guzman (1964).

[2]States in Mexico are Ca, Chihuahua; D, Durango; MS, Mexico State; NL, Nuevo Leon; O, Oaxaca; T, Tlaxcala; P, Pueblo; Mi, Michoacan; Cs, Chiapas. G is Guatemala.

[3]t = trace.

RESEARCH PROCEDURE STUDIES

Sample Plot Size

In an earlier report (Smith 1977), the relation of plot size to frequency distribution of the percentages of the five major monoterpenes of ponderosa pine—α-pinene, β-pinene, 3-carene, myrcene, and limonene—was examined. No appreciable change in distribution was found between a plot of 78 trees and one of 641 trees. The conclusion was that an 80-tree plot was adequate for determining the average level of occurrence for these components. Because the number of compositional types was slightly larger for the larger plot, however, additional data were gathered from three more plots to test the earlier conclusion. These plots, containing 366, 377, and 94 trees, were in the same general forest area near Adin Summit on the Modoc National Forest, California, as the two plots sampled earlier.

Chromatographic analysis was designed to allow comparison of results with the earlier work, and also to test the comparative accuracy of short-column analysis as opposed to the standard long-column method (see below). Resin samples from the 78-tree plot had been analyzed on the long column, and those from the 641-tree plot on the short column. Samples from the new plots were analyzed on the short column; in addition, the first 72, 79, and 76 samples from the new plots were analyzed on the long column as well. All analyses were expressed in code form, according to a system defined in the original study (Smith 1977).[1] The normalized percent frequency of each coded type was determined for each plot—that is, the number of trees of each compositional type was expressed as a percent of the total number of trees in the plot. Plots were compared by regression analysis of these normalized values.

The occurrence of composition types in the five plots is given in *figure 2*. All five are correlated with each other between 0.5 and 0.9 (r^2):

Number of trees in plot:	Coefficient of determination (r^2)				
	n = 78	n = 641	n = 366	n = 377	n = 94
78		0.69	0.62	0.55	0.48
641			0.64	0.54	0.55
366				0.89	0.49
377					0.58

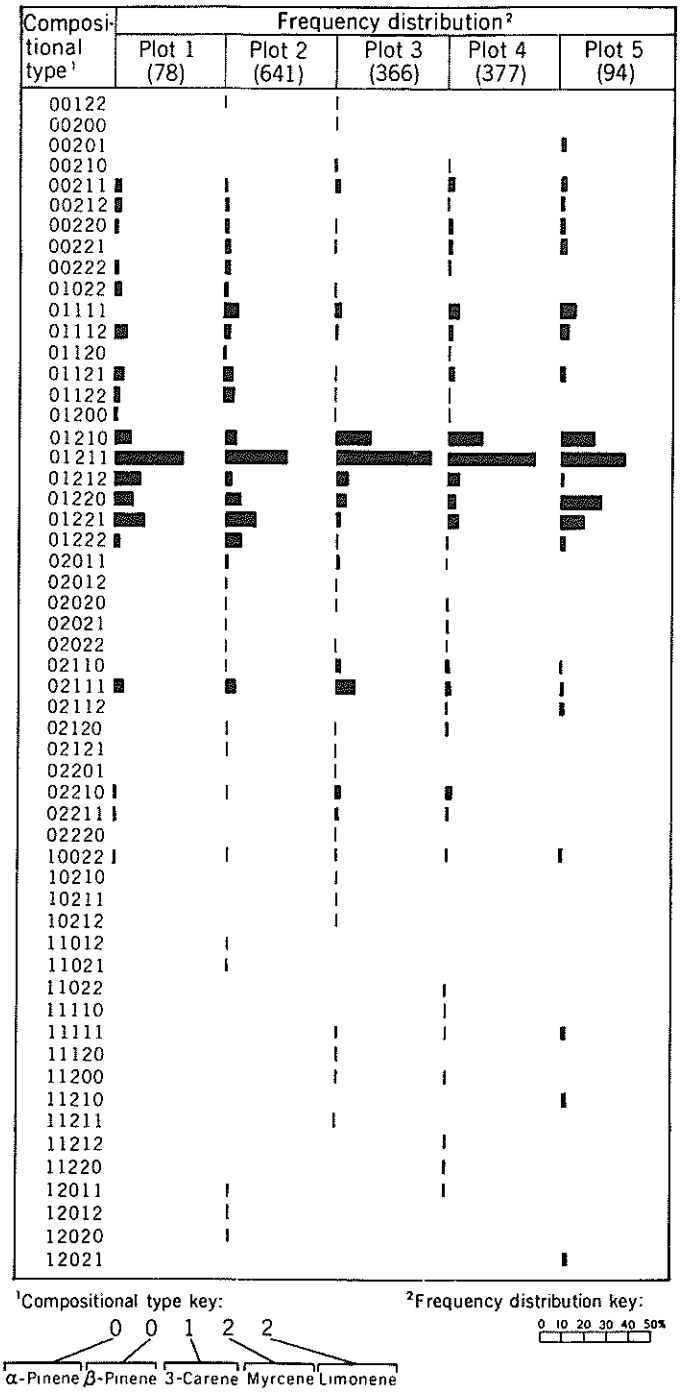

Figure 2—Normalized distribution of the compositional types of xylem monoterpenes of 1556 ponderosa pines in five plots near Adin Summit, Modoc National Forest. The sum of the bars for each plot equals 100 percent of the trees in that plot.

[1]Intervals for the code value of each component are as follows (Smith 1977): α-pinene, 0 = 0 to 17.4 percent, 1 = 17.5 to 64.4 percent, 2 = 64.5 to 100 percent; β-pinene, 0 = 0 to 4.4 percent, 1 = 4.5 to 35.4 percent, 2 = 35.5 to 100 percent; 3-carene, 0 = 0 to 15.4 percent, 1 = 15.5 to 35.4 percent, 2 = 35.5 to 100 percent; myrcene, 0 = 0 to 2.4 percent, 1 = 2.5 to 15.4 percent, 2 = 15.5 to 100 percent; limonene, 0 = 0 to 2.4 percent, 1 = 2.5 to 17.4 percent, 2 = 17.5 to 100 percent.

There is an evident increase in the types of composition with increase in the number of trees in a plot (*fig. 2*). However, the three largest plots had about the same number of types of composition—between 35 and 38. Thus, it appears that about 350 trees is the optimum size of a plot in this type of forest. But each of these three

plots has a slightly different array of the kinds and the frequency of tree types. This could be caused by accumulation of three small variations: actual composition in the tree, the gas chromatographic analysis, and the coding procedure.

In the course of the plot study, an effort was made to find trees having the apparently rare high limonene composition type. Three were found (coded as 10022 or 11022, *fig. 2*): one in the first original plot of 78 trees, one more in the second plot, containing 641 trees, and one more in the 366-tree plot. (None was found in the 377-tree plot.) The frequency is thus about one in 500.

Chromatographic Analysis on Short Column

In the plot-size investigation, the use of short column analysis (Smith and Greene 1971) was also tested for rapid surveys of large numbers of trees from the same area. In this testing, the code values derived from the long column analysis were considered the correct values.

The accuracy of the short column, with respect to the long column, was determined on two points: coding of individual component and coding of tree composition. Of the 1135 components (5 components for each of the 227 trees) coded from the short column analysis, 1103 or 97 percent were coded correctly. Of the 227 trees coded from the short column analysis, 195 or 86 percent were coded correctly (*table 4*). This slight inaccuracy is acceptable for rapid survey and classification of a large number of trees in an area. The savings in analysis time can be as much as 20 hours per hundred trees. The coefficient of determination (r^2) between the two types of analyses was greater than 0.9. Most of the incorrectly coded components were either limonene or myrcene. This is to be expected since the chi-square values obtained with previous work were lower for these two than for α-pinene, β-pinene, and 3-carene. The short-column results for two of the plots—about 98 percent correct for components, and about 90 percent correct for composition types—were noticeably better than the results for the third plot at 95 percent and 78 percent respectively.

An inspection of the incorrectly coded components showed all falling a percent or two outside the limit of a code class. Thus, a shift of about 2 percent in the short-column values would have made nearly all analyses correct. One might expect to get differences of somewhat similar magnitude between repeated analyses of the same sample on the same column.

Table 4—*Accuracy of short column analysis as compared to long column, in determining coded monoterpene values and composition types for ponderosa pine*

Trees in sample	Components[1]	Component values correct		Composition types correct	
		Number	Percent	Number	Percent
72	360	354	98	66	92
79	395	386	98	70	89
76	380	363	95	59	78
			$\bar{x}$ 97		$\bar{x}$ 86

[1]There were five monoterpene components for each sample.

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Monoterpene composition was studied in a number of hard pine species and results were compared with earlier work. (1) Intratree measurements showed strong constancy of composition in both single-stemmed and forked trees of ponderosa, Jeffrey, Coulter, and Jeffrey × ponderosa pines. In grafts of these and other pines, the scion influenced the root stock, but not the reverse. (2) Large intertree variation in composition was found in a small sample of seven hard pines native to Mexico; the value of monoterpenes in clarifying taxonomy of these pines remains uncertain. (3) An 80-tree plot of ponderosa is adequate to determine average monoterpene composition. For the best estimate of the kinds and abundance of types of composition, a 350-tree plot is needed. Short-column chromatographic analysis is acceptably accurate for rapid classification of a large number of samples.

Retrieval Terms: xylem monoterpenes, *Pinus ponderosa*, *P. coulteri*, *P. jeffreyi*, *P. torreyana*, *P. sabiniana*, *P. montezumae*, *P. hartwegii*, *P. rudis*, *P. pseudostrobus*, *P. durangensis*, *P. cooperi*, *P. engelmannii*