WITHIN-TREE VARIATION IN CORTICAL MONOTERPENES OF SLASH PINE

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Abstract.--Cortical monoterpene composition, bud diameter, and length of the current flush from all buds (545) on a 10- to 12-year-old grafted, high gum-yielding Pinus elliottii Engelm. (slash pine), were analysed to determine within-tree variability. Approximately equal numbers of high-, medium- and low-vigor buds were sampled from the upper, middle, and lower crown position from the north and south aspects in the spring, summer, fall, and winter. No north or south differences were found. Beta-pinene content was significantly greater in the spring than in the other seasons and the inverse was true for a-pinene and ß-phellandrene. Alpha-pinene content was significantly greater in buds from the lower part of the crown. Beta-pinene and ß- phellandrene did not vary with crown position. Alpha-pinene content decreased from low- to high-vigor buds and the opposite was true for ß-phellandrene. Beta-pinene content was highest in low-vigor buds. Bud diameters and lengths of the flushes decreased progressively in size from the upper to lower crown, and from high- to low-vigor buds. Bud diameters were similar among seasons, and lengths of the current flushes were slightly longer in the spring than summer.

Additional keywords: terpene, oleoresin, gum, clone, ramet, seed orchard, a-pinene, ß-pinene, ß-phellandrene, limonene, myrcene, a-phellandrene, Pinus elliottii.

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INTRODUCTION

Analyses of the composition of monoterpenes in conifers has been a useful tool for identifying the geographic source of seed in plantations, identifying hybrids and inbreeding, and for identifying ramets in seed orchards (Squillace 1976; Kossuth and McCall 1984). Damage to trees from grazing animals and resistance to insects and disease have also been linked to the monoterpene composition of trees (Squillace 1976). In an early study it was suggested that before large-scale studies of monoterpene composition are undertaken, the best place to sample on a tree should be determined so that repeated sampling would give consistent results (Hanover 1966) for individual tree phenotypes. The objectives of this study were to determine the effects of season, aspect, position in crown, and bud vigor on monoterpene composition of one slash pine (Pinus elliottii Engelm.) ramet.

METHODS

One 10- to 12-year-old grafted ramet of high gum yielding slash pine clone number 335 in a seed orchard at the USDA Forest Service, Southeastern Forest Experiment Station, Olustee, Florida, was used for the study. It was 12.9 m tall, and had a live crown height of 9.8 m.

The ramet was divided into north and south aspects, and these were subdivided into three equal lengths of crown for upper, middle, and lower position. In the spring, all the buds within each of the **six** sections on the ramet were classified as high, medium, or low vigor. A count was made of the number of buds in each vigor class by section and ramet. One-fourth of the buds from each section on the tree were sampled for cortical monoterpenes in the spring (5/5/82), summer (7/12/82), fall (10/6/82) and winter (1/12/83). All 545 buds were sampled.

Samples were taken by removing approximately the terminal centimeter of the bud, collecting the cortical oleoresin that flowed out, and immediately placing it in vials containing pentane. If the quantity of oleoresin was low, the bud--with bud scales removed--was extracted in the pentane. Gas-liquid chromatographic analysis was conducted according to the method of Kossuth and Munson (1981) by using a 5840-A Hewlett-Packard gas chromatograph with an automatic sample injector and programmable integrator. The amount of each monoterpene is presented as a percentage of the total monoterpenes since this method has been shown to have the least variation (Powell and Adams 1973). At the time of sampling, each bud diameter was measured. The length of the current flush for the bud sampled was measured in the spring and summer only.

The percentage data was subjected to an arcsin square root transformation and analysed by analysis of variance (ANOVA) and Duncan's multiple range test. If significant interactions **were** present, then additional ANOVA was performed on each level of each factor involved to further investigate main effects. Higher order interaction mean squares were used to approximate associated error terms for each analysis.

RESULTS

The average monoterpene content for the tree was 30.8, 43.0, and 21.6 percent a-pinene, ß-pinene, and ß-phellandrene, respectively. Of the other constituents in the monoterpene fraction, camphene, myrcene, a-phellandrene and limonene each contributed less than 4 percent and were not analysed statistically. These low levels are probably of little physiological significance. Alpha-pinene, ß-pinene and ß-phellandrene contents ranged from 24.5-47.7, 18.2-54.5, 11.1-31.9 percent, respectively.

Overall, the content of the three major monoterpenes showed no significant differences for the north and south aspects (Table 1).

Table 1. Significance levels for monoterpene composition, bud diameter, and length of flush determined by analysis of variance, by source of variance.

Source of variance	α-pinene	β-pinene	β-phellandrene	Bud diam.	Flush length
Aspect					
Position	*			***	**
Season	**	**	**		
Aspect x season					
Position x season					
Vigor	****	***	***	****	****
Vigor x aspect					
Vigor x position				***	****
Vigor x season					

*, **, ***, **** indicate significance differences at the 0.05, 0.01, 0.001, and 0.0001 levels, respectively.

Alpha-pinene content increased progressively with decreasing bud vigor and the opposite was true for ß-phellandrene (Table 2). Beta-pinene content was significantly lower in the high- and medium-vigor buds than in the low vigor buds. Alpha-pinene and -phellandrene were significantly lower in the spring than in the other seasons, and the inverse was true for ß-pinene for high- medium- and low-vigor buds (Table 3).

Alpha-pinene content was higher in the lower part of the crown than in the middle and upper crown. Beta-pinene and ß-phellandrene content did not vary with crown position (Table 2).

Bud diameter and current flush length of the buds sampled decreased from high to medium to low-vigor buds, and from the upper to the lower crown (Table 2, 4,). Bud diameter and flush length varied significantly with position in the crown and bud vigor (Table 1). There was a significant interaction of bud vigor and crown position for both bud diameter and flush

TABLE 2. Cortical monoterpene composition, bud diameter, and current length of flush for buds collected from grafted high gum-yielding slash pine ramet number 335, by aspect, bud vigor, season, and position in crown.

Source	No.	α-pinene	β-pinene	β-phellandrene	Bud Dia.	Flush lgth.	No.
		.... Percent			mm	cm	
Overall	545	30.8± 2.4	43.0± 3.8	21.6± 3.7	6.8± 1.6	13.5± 8.8	259
Range		24.5-47.7	18.2-54.5	11.1-31.9	3.0-13.0	1.7-63.7	
Aspect							
North	280	31.0± 2.3a	43.4± 3.5a	21.2±3.7a	6.6± 1.6a	13.0± 9.1a	144
Range		25.2-40.2	34.2-54.0	11.2-31.9	3.0-13.0	1.7-63.7	
South	265	30.6± 2.5a	42.6± 4.0a	22.1± 3.8a	7.1± 1.7a	14.3± 8.5a	115
Range		24.5-47.7	18.2-54.5	11.1-30.5	4.0-13.0	3.1-52.2	
Vigor							
High	62	29.9± 2.2c	42.1± 3.7b	23.2± 3.5a	9.2± 1.6a	27.5±14.1a	24
Range		25.2-34.9	34.2-54.5	14.6-31.9	5.0-13.0	11.8-63.7	
Medium	335	30.6± 2.2b	42.6± 3.6b	22.3± 3.4a	7.2± 1.0b	14.5± 5.6b	94
Range		24.5-37.6	32.1-54.5	12.8-30.5	4.0-11.0	1.7-38.5	
Low	148	31.7± 2.8a	44.3± 4.0a	19.5± 3.8b	5.0± 0.9	6.5± 3.5c	75
Range		25.1-47.7	18.2-54.0	11.1-29.5	3.0- 8.0	2.7-26.3	
Season							
Spring	120	28.9± 2.4b	47.1± 2.7a	18.2± 2.1b	6.8± 1.4a	14.5± 7.4a	120
Range		24.5-36.3	40.2-54.5	11.1-24.8	4.0-10.0	3.1-47.6	
Summer	138	31.0± 2.7a	41.1± 3.1b	23.1± 4.4a	6.7± 1.8a	12.9± 9.8a	138
Range		26.1-40.2	34.2-51.2	11.9-31.9	3.0-13.0	1.7-63.7	
Fall	137	31.0± 1.5a	42.3± 3.2b	22.6± 3.2a	7.0± 1.9a	--	
Range		27.8-36.9	35.7-52.5	12.9-29.5	3.0-13.0	--	
Winter	150	31.9± 2.1a	42.0± 3.3b	22.1± 3.0a	6.7± 1.3a	--	
Range		29.1-47.7	18.2-50.2	11.8-28.4	4.0-12.0	--	
Position							
Top	171	30.5± 3.0b	42.7± 4.1a	22.2± 3.6a	7.8± 1.8a	20.5±12.0a	74
Range		24.5-47.7	18.2-54.5	12.9-30.5	4.0-13.0	1.7-63.7	
Middle	227	30.7± 2.0b	43.5± 3.6a	21.1± 3.8a	6.6± 1.4b	10.9± 5.1	113
Range		25.9-37.8	34.2-54.0	11.2-31.9	4.0-11.0	3.0-25.3	
Lower	147	31.2± 2.3a	42.6± 3.7a	21.7± 3.9a	6.0±1.3c	10.7±4.7c	72
Range		25.9-40.2	34.8-50.4	11.1-29.5	3.0-9.0	2.7-26.3	

Mean ± standard deviation. Column values within groups followed by different letters are significantly different at the 0.05 level.

TABLE 3. Cortical monoterpene composition, bud diameter, and length of flush for buds collected from one slash pine ramet, by bud vigor and season.

Bud vigor and season	No.	α-pinene	β-pinene	β-phellandrene	Bud Dia.	Flush lgth.
		. . . .	Percent	. . . .	mm	cm
High						
Spring	12	27.2± 1.5	47.3± 2.8	18.7± 1.9	9.1± 0.9	27.2± 9.6
Range		25.2-29.3	43.1-54.5	14.6-21.5	8.0-10.0	15.7-47.6
Summer	15	29.5± 2.1	39.2± 2.3	26.4± 2.4	9.5± 1.9	27.8±17.2
Range		26.1-33.9	34.2-42.0	23.1-31.9	7.0-13.0	11.8-63.7
Fall	16	30.0± 0.8	41.5± 3.3	24.2± 2.7	9.6± 2.1	--
Range		28.6-32.1	37.8-49.2	18.4-27.5	5.0-13.0	--
Winter	19	31.9± 1.5	41.7± 1.8	22.5± 2.1	8.6± 1.2	--
Range		29.8-34.9	39.4-45.7	19.3-26.3	7.0-12.0	--
Medium						
Spring	73	28.8± 2.2	46.8± 2.8	18.8± 1.7	7.1± 0.9	15.4± 4.7
Range		24.5-34.7	40.2-54.5	15.3-24.8	5.0-10.0	5.3-32.1
Summer	84	30.5± 2.4	40.8± 3.0	24.0± 3.9	7.1± 1.0	13.8± 6.2
Range		26.4-37.6	36.2-48.7	12.8-30.5	5.0-11.0	1.7-38.5
Fall	79	30.8± 1.3	42.0± 3.1	23.1± 2.8	7.6± 1.2	--
Range		27.8-30.0	35.7-52.5	14.9-28.5	5.0-10.0	--
Winter	99	31.7± 1.7	41.5± 2.2	22.8± 2.5	7.0± 0.8	--
Range		29.3-37.4	32.1-46.6	15.9-28.1	4.0-10.0	--
Low						
Spring	35	29.7± 2.6	47.6± 2.4	17.0± 2.3	5.4± 1.2	8.3± 4.1
Range		25.0-36.3	43.3-54.0	11.1-21.3	4.0- 8.0	3.1-26.3
Summer	39	32.7± 2.6	42.6± 3.1	20.1± 4.4	4.7± 0.8	5.1± 1.4
Range		27.9-40.2	35.5-51.2	11.9-27.7	3.0- 7.0	2.7- 8.5
Fall	42	31.7± 1.8	43.4± 3.1	20.9± 3.5	5.0± 0.8	--
Range		28.0-36.9	35.8-51.0	13.0-30.0	3.0- 7.0	--
Winter	32	32.4± 3.1	44.0± 5.4	19.7± 3.7	5.0± 0.6	--
Range		29.1-47.7	18.2-50.2	11.8-28.4	4.0- 6.0	--

Mean ± standard deviation.

Table 4. Diameter and flush length of buds by bud-vigor class and position in crown.

Source of variance	Bud diam. (mm)	Flush length (cm)
VIGOR		
High x		
Upper	10.3a	39.1a
Middle	8.8b	20.7b
Lower	7.6b	16.1b
Medium x		
Upper	7.9a	19.8a
Middle	7.1b	13.2b
Lower	6.5b	11.3b
Low x		
Upper	5.8a	9.41a
Middle	4.9b	5.7a
Lower	4.5b	5.7a
POSITION		
Upper x		
High	10.3a	39.1a
Medium	7.9b	19.8b
Low	5.8c	9.4c
Middle x		
High	8.9a	20.7a
Medium	7.1b	13.2ab
Low	4.9c	5.7b
Lower x		
High	7.6a	16.1a
Medium	6.5b	11.4b
Low	4.5c	5.7c

Column values in each group followed by different letters are significantly different at the 0.05 level.

length (Table 1). Further analysis by bud vigor to determine position effects showed that any conclusions about these effects depended on bud vigor (Table 5). However, analyses by position to determine bud vigor

Table 5. Significance levels of bud diameter and flush length determined by analysis of variance of effects of bud-vigor class on position in crown, and of position effects on vigor.

Variable	Bud diam.	Flush length
Vigor effects on position		
Upper	****	*
Middle	****	*
Lower	****	**
Position effects on vigor		
High	**	**
Medium	*	*
Low	**	--

*, **, ***, **** indicates significantly different at the 0.05, 0.01, 0.001, and 0.0001 levels.

effects showed that conclusions about position effects can be made without regard to bud-vigor considerations. High-, medium- and low-vigor buds tended to be larger in the upper crown and decrease toward the lower crown (Table 4). Flush lengths followed the same general pattern.

DISCUSSION

The seasonal differences in monoterpene composition found in this study are consistent with those found in P. taeda L. (Rockwood 1973) and in Picea glauca (Moench) Voss, P. pungens Engelm., P. mariana (Mill.) B.S.P., and Abies balsamea (L.) Mill., when several samples from each tree were combined for each determination (vonRudloff 1972, 1975a, 1975b; vonRudloff and Granat 1982). Sampling in the spring seems to result in the most variation and should be avoided. Based on the standard deviations and ranges for the monoterpenes, ß-pinene and ß-phellandrene vary the most, and a-pinene is the most stable.

Because of the significant differences in monoterpene composition associated with bud vigor and the lack of any interaction with season, trees should be sampled by using buds of the same vigor. Because low- vigor buds are small and difficult to sample there is a greater chance of getting some xylem oleoresin, which tends to be lower in ß-phellandrene (Squillace and Fisher 1966) in the sample. Sampling is easiest from the lower crown where more low-vigor buds are found. Although ß-pinene was somewhat higher in content here, the differences were not large. Kossuth and Muse (1985) determined that combining five buds is adequate to ensure an error of, at most, 5 percent in phenotypic determinations of the three monoterpene concentrations with a 95 percent confidence limit. Based on the data in this study, it is recommended that sampling for determining individual tree phenotypes be done in the summer, fall, or winter from the lower crown from buds of similar vigor or size, preferably large buds.

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