PONDERING THE MONOTERPENE COMPOSITION OF *PINUS SEROTINA* MICHX.:CAN LIMONENE BE USED AS A CHEMOTAXONOMIC MARKER FOR THE IDENTIFICATION OF OLD TURPENTINE STUMPS?

Thomas L. Eberhardt, Jolie M. Mahfouz, and Philip M. Sheridan[1]

Abstract—Wood samples from old turpentine stumps in Virginia were analyzed by GC-MS to determine if the monoterpene compositions could be used for species identification. Given that limonene is reported to be the predominant monoterpene for pond pine (*Pinus serotina* Michx.), low relative proportions of limonene in these samples appeared to suggest that these stumps were not from pond pine. Unexpectedly, analysis of wood samples from live trees identified as pond pine did not consistently confirm a high relative proportion of limonene. Further sampling of half-sib pond pine trees suggested that limonene may be an unreliable chemotaxonomic marker for pond pine hybrids.

INTRODUCTION

Longleaf pine (*Pinus palustris* Mill.) has a well established history in naval stores production from early turpentining operations to the subsequent processing of old resinous stumps (Gardner 1989). We were interested in comparing turpentine stumps from central and southeastern Virginia to determine their taxonomic identity and so establish the true range and specific sites that longleaf pine occupied in Virginia. Data reported for the fresh oleoresin from most southern pines (e.g., *P. palustris, P. taeda, P. echinata, P. elliottii*) have shown that α-pinene constitutes 50-80 percent of the monoterpenes detected (Hodges and others 1979, Strom and others 2002). The second most abundant monoterpene, β-pinene, accounts for 20-40 percent of the monoterpenes detected. Pond pine is the exception with limonene accounting for as much as 90 percent of the detected monoterpenes (Mirov 1961). We hypothesized that comparisons of the monoterpene compositions from the stump wood samples with wood samples from known sources, along with data from the literature, may allow the identification of said stump wood samples. Given the unique monoterpene composition reported for pond pine, we also speculated that high levels of limonene (>90 percent) could be used to positively identify stump samples as pond pine.

MATERIALS AND METHODS

Highly weathered wood samples were collected from old turpentine stumps located in Caroline, Prince George, Southampton, and Sussex counties in Virginia. An electric drill was used to collect shavings from the interior of wood samples with minimal heat generation. A drill was also used to sample longleaf pine stumps in an experimental forest in the Calcasieu Ranger District of the Kisatchie National Forest in Louisiana. A razor blade was used to cut heartwood and sapwood shavings from a pond pine branch that had been collected in southeastern Virginia. Wood cores from live pond pine trees were collected near Saucier, MS. Shavings were cut from these cores with a razor blade.

For the GC-MS analyses, wood shavings (1 g) were steeped in methylene chloride (5 ml). The resultant extracts were analyzed on a Hewlett Packard 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass selective detector and an HP-INNOWax column (0.25 mm ID × 60 m length × 25 µm film thickness). The column was programmed to hold for 1 min at 40 °C, increase to 80 °C at a rate of 16 °C/ min, and then to 240 °C at a rate of 7 °C/ min, with the final temperature being held for 10 minutes. The temperatures for the injector inlet and mass detector were maintained at 200 °C and 225 °C, respectively. Peaks were identified by spectral match with NIST 98 (NIST, Gaithersburg, MD) and in-house chemical libraries.

RESULTS AND DISCUSSION

Monoterpenes Present in Stump Wood Samples

GC-MS analysis of the stump wood samples showed that α-pinene was the most abundant monoterpene in 4 out of 6 cases (table 1). The second most abundant compound was the oxidized monoterpene, α-terpineol. This latter result was not surprising since wood naval stores (i.e., those from old pine stumps) have been reported to contain high amounts (50-60 percent) of α-terpineol (Buchanan 1963). Other oxidized monoterpenes (e.g., camphor, fenchyl alcohol, and borneol) were also present in significant amounts. Similarities in the monoterpene compositions for most southern pines prevented us from demonstrating that the stumps were specifically from longleaf pine; however, the absence of high relative proportions of limonene suggested that the turpentine stumps were not from pond pine. Samples of pond pine were thus sought to confirm the predominance of limonene among the monoterpenes present in the wood for this species.

Effect of Sample Handling on Limonene Levels

Heartwood and sapwood shavings from a pond pine branch were analyzed, and this confirmed that limonene made up a high proportion of the monoterpenes detected by GC-MS. Shavings of sapwood were also kept in open containers for various periods of time to determine whether low limonene levels could have resulted from sample handling. Results showed that the exposure periods did not seem to alter the relative amounts of limonene (table 2); the relative amounts

[1]Research Scientist, Biological Science Technician, U.S. Forest Service, Southern Research Station, Pineville, LA; Director, Meadowview Biological Research Station, Woodford, VA, respectively.

Citation for proceedings: Stanturf, John A., ed. 2010. Proceedings of the 14th biennial southern silvicultural research conference. Gen. Tech. Rep. SRS-121. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 614 p.

Table 1—Percentage compositions of monoterpenes and methyl chavicol in stump wood samples from selected counties in Virginia

Monoterpenes	Stump Wood Samples					
	Caroline (Scholl)[a]	Caroline (Pines)	Prince George	Southampton[b]	Sussex (John Hancock)	Sussex (Joseph Pines)
				percent		
α-Pinene	47.37	48.59	18.06	58.22	12.07	45.30
α-Fenchene	0.80	0.42	3.14	0.58	5.60	0.74
Camsphene	3.59	0.24	5.46	3.10	7.58	2.99
β-Pinene	1.55	2.41	-	1.25	-	2.75
Myrcene	1.29	1.88	-	0.03	-	0.19
α-Phellandrene	-	3.23	0.41	-	-	-
α-Terpinene	-	1.27	1.33	-	-	-
Limonene	10.96	8.80	1.63	9.29	0.42	4.61
β-Phellandrene	-	6.58	-	-	-	0.31
p-Cymene	0.74	0.11	47.97	0.28	19.14	1.40
Terpinolene	1.26	2.23	1.89	1.67		1.11
Fenchone	0.36	-	2.88	0.26	13.89	2.32
Camphor	1.10	-	6.58	0.82	19.95	4.36
Fenchyl Alcohol	2.83	2.78	1.69	1.92	0.15	0.89
Terpinen-4-ol	1.62		1.97	0.93	11.22	3.64
Methyl Chavicol	0.20	0.63	-	2.55	0.52	6.89
α-Terpineol	23.54	17.04	4.72	16.18	7.27	21.58
Borneol	2.78	3.26	2.27	2.91	2.18	0.92

[a]putative loblolly pine; specific site indicated in parentheses.
[b]putative longleaf pine.
Source: Eberhardt and others (2007).

of limonene in sapwood and heartwood samples were generally similar.

Assessment of Limonene Predominance in Pond Pine

Analysis of pond pine samples from a variety of sources failed to consistently demonstrate that limonene was the predominant monoterpene among those detected by GC-MS. Given the possibility of inaccurate species identification during sample collection, additional samples were obtained from three 30 year old half-sib pond pine trees near Saucier, MS. Since α-pinene was found to be the predominant monoterpene in one of these trees, it was speculated that a hybrid had been sampled. Eighteen additional cores were collected and it was found that α-pinene was predominant in 4 of the 16 trees with detectable monoterpene levels (fig. 1). Three of the cores were from grafted trees (15, 16, and 17). It is of particular interest to determine whether cores 2, 5, and 10 (half-sibs from seed) are indeed hybrids of pond pine. Although pond pine does hybridize with loblolly pine (*Pinus taeda* L.), this does not often occur in the natural range because of differences in flowering dates (Bramlett 1990). We attribute the discrepancies in monoterpene composition of the pond pine trees we sampled to hybridization. These trees, raised from seed from open-pollinated pond pine parents, were likely contaminated with loblolly pine pollen in the seed orchard.

Aging of Longleaf Pine Stumps under Field Conditions

In an effort to determine the changes to the monoterpene compositions in stumps under field conditions, samples of heartwood and sapwood from longleaf pine stumps were collected 1 week, 1 month, 6 months, and 1 year after harvesting. Relative monoterpene composition of the heartwood and of the sapwood did not appear to change over the initial 6-month period; samples collected 1 year after harvesting are still being analyzed. Average values for the heartwood and sapwood monoterpenes were calculated and are shown in table 3. Both the total yield of

Table 2—Selected monoterpenes in pond pine samples allowed to stand under ambient conditions

Sample type	Exposure Period	α-Pinene	β-Phellandrene	Limonene
	days	*percent*	*percent*	*percent*
Sapwood	0	0.3	0.3	97.3
	1	4.8	4.8	91.4
	2	5.9	5.9	90.5
	4	3.1	3.1	94.2
Heartwood	0	1.7	1.7	94.4

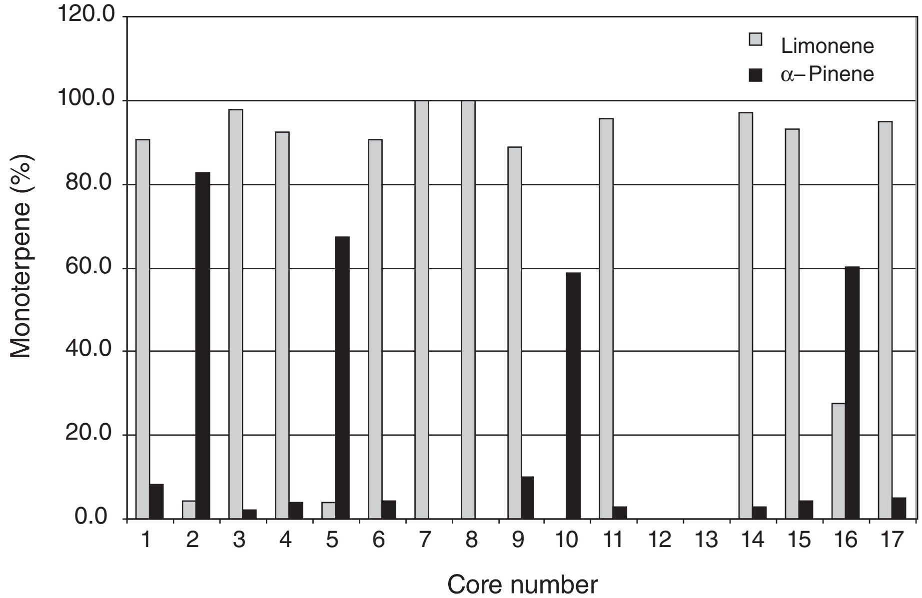

Figure 1—Limonene and α-pinene as percentages of the monoterpenes detected in cores from pond pine trees grown near Saucier, MS.

Table 3—Selected monoterpene compositions for longleaf pine stumps under field conditions

	α-Pinene	β-Pinene	α-Terpineol	Total Yield
	percent	*percent*	*percent*	*mg/g*
Sapwood	75.8 9.4	14.5 6.0	0.7 1.4	7.0 5.3
Heartwood	64.1 8.6	10.3 6.1	9.9 8.8	20.2 13.6

monoterpenes and the proportion of oxidized monoterpenes (e.g., α-terpineol) were higher in the heartwood (table 3). The former is indicative of the higher extractives contents typical of heartwood. The latter (high α-terpineol) suggests that the higher proportion of oxidized monoterpenes is not simply a function of post-harvest stump ageing. The presence of oxidized monoterpenes in pond pine stump heartwood remains to be determined.

CONCLUSIONS

Limonene is an unreliable chemotaxonomic marker for pond pines grown in environments conducive to hybridization. Since pond pine does not readily hybridize in its native range, it is unlikely that the old stump wood samples collected in Virginia were from pond pine.

ACKNOWLEDGMENTS

Larry Lott collected all fresh pond pine cores. James Scarborough and Jacob Floyd assisted with the sampling of longleaf pine stumps. Karen Reed assisted with sample preparation. Brian Strom and C. Dana Nelson participated in helpful discussions.

LITERATURE CITED

Bramlett, D.L. 1990. *Pinus Serotina* Michx. In: Burns, R.M.; Honkala, B.H. (tech. coords.) Silvics of North America, Volume 1, Conifers. Agriculture Handbook 654. U.S. Forest Service, Washington, DC: 470-475.

Buchanan, M.A. 1963. Extraneous components of wood. In: Browning, B.L. (ed.) The Chemistry of Wood. Interscience Publishers, New York: 313-367.

Eberhardt, T.L.; Sheridan, P.M.; Mahfouz, J.M. [and others]. 2007. Old resinous turpentine stumps as an indicator of the range of longleaf pine in southeastern Virginia. In: Estes, B.L.; Kush, J.S. (eds.) Seeing the forest through the trees. Proceedings of the sixth Longleaf Alliance regional conference. Report No. 10. Longleaf Alliance, Tifton, GA: 79-82.

Gardner, F.H., Jr. 1989. Wood naval stores. In: Zinkel, D.F., Russell, J. (eds.) Naval Stores: Production, Chemistry, Utilization. Pulp Chemicals Association, New York: 143-157.

Hodges, J.D.; Elam, W.W.; Watson, W.F. [and others]. 1979. Oleoresin characteristics and susceptibility of four southern pines to southern pine beetle (Coleoptera: Scolytidae) attacks. Canadian Entomologist. 111: 889-896.

Mirov, N.T. 1961. Composition of gum turpentines of pines. Tech. Bull. 1239. U.S. Forest Service, Washington, DC.

Strom, B.L.; Goyer, R.A.; Ingram, L.L., Jr. [and others]. 2002. Oleoresin characteristics of progeny of loblolly pines that escaped attack by the southern pine beetle. Forest Ecology Management. 158: 169-178.