Monoterpene persistence in the sapwood and heartwood of longleaf pine stumps: assessment of differences in composition and stability under field conditions

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Abstract: Monoterpenes in exudates, phloem, and sapwood have received considerable attention relative to the active defenses of pine trees. However, little is known about the composition and function of the heartwood monoterpenes. To address this deficiency, monoterpene contents and relative compositions were determined for sapwood and heartwood samples from longleaf pine (*Pinus palustris* Mill.) stumps monitored in the field for a 1 year period postharvest. Gas chromatography – mass spectrometry analysis of sapwood and heartwood sample extracts showed the total monoterpene contents for both declined at essentially the same rate. For sapwood, α-pinene continued to comprise about 75% of all compounds detected, while the proportion of β-pinene declined with time (15%–7%). For the heartwood, lower proportions of both α- and β-pinenes (64% and 10%, respectively) were offset by higher proportions of other monoterpenes (e.g., limonene, α-terpineol, borneol). The low proportion of β-pinene in very old turpentine and lightwood stump samples further suggested the lower stability of this particular monoterpene. While it has not been specifically demonstrated that the monoterpenes have an active role in the decay resistance of longleaf pine stump heartwood, these compounds do persist for a very long time and thus are available should they serve this function.

Résumé : Les monoterpènes présents dans les exsudats, le phloème et le bois d'aubier ont fait l'objet de beaucoup d'études en lien avec les défenses actives chez les pins. Cependant, on sait peu de choses de la composition et de la fonction des monoterpènes présents dans le bois de cœur. Pour combler cette lacune, nous avons déterminé le contenu en monoterpènes et leur composition relative dans des échantillons de souches de pin des marais (*Pinus palustris* Mill.) qui ont été suivies sur le terrain pendant une période d'un an après la récolte. L'analyse des extraits des échantillons de bois d'aubier et de bois de cœur par chromatographie en phase gazeuse couplée à la spectrométrie de masse a montré que le contenu total en monoterpènes a diminué essentiellement au même rythme dans les deux cas. Dans le bois d'aubier, l'α-pinène a continué à représenter environ 75 % de tous les composés qui ont été détectés tandis que la proportion de β-pinène a diminué avec le temps (15 % à 7 %). Dans le bois de cœur, de plus faibles proportions d' α- et de β-pinènes (respectivement 64 % et 10 %) étaient compensées par une plus forte proportion d'autres monoterpènes (p. ex., limonène, α-terpinéol, bornéol). La faible proportion de β-pinène dans les échantillons de très vieilles souches de gommier et de glutier est un autre indice de la moins grande stabilité de ce monoterpène. Bien qu'il n'ait pas été spécifiquement démontré que les monoterpènes jouent un rôle actif dans la résistance à la décomposition du bois de cœur dans les souches de pin des marais, ces composés persistent pendant très longtemps et sont par conséquent disponibles s'ils devaient remplir cette fonction.

[Traduit par la Rédaction]

Introduction

A common trait among conifers is the production and exudation of oleoresin, a fragrant, viscous, and sticky substance also known as pitch. The volatile component of oleoresin is turpentine, a complex mixture of monoterpenes (e.g., α-pinene, β-pinene, limonene) and lesser amounts of sesquiterpines (e.g., β-caryophyllene, α-humulene) and nonterpene compounds (e.g., methyl chavicol) (Trapp and Croteau 2001; Helmig et al. 2006). The seemingly nonvolatile component of oleoresin is rosin, a complex mixture rich in diterpene resin acids accompanied by lesser amounts (ca. 5%–10%) of neutral compounds, including diterpene alcohols, aldehydes, methyl esters, and hydrocarbons (Soltes and Zinkel 1989). Aside from being a solvent for rosin, turpentine serves an active defensive role through its toxicity to both insects and microbial agents (Raffa and Smalley 1995; Klepzig et al. 1996; Nerg et al. 2004). Subsequent evaporation of the turpentine in the oleoresin at a wound site leaves behind a physical barrier, and in some cases, results in the permanent encasement of the offending insect in rosin (Phillips and Croteau 1999; Franceschi et al. 2005). The most current developments in the study of terpene biosynthesis can be found in recent reviews by Keeling and Bohlmann (2006) and Bohlmann and Keeling (2008).

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In older pine trees, the oleoresin content of the wood increases upon the transformation of living sapwood into nonliving heartwood. The resistance of heartwood to fungal decay and wood-boring insects suggests some sort of defensive role for heartwood formation in the living tree. While studies have shown resin acids to exhibit toxicity towards wood-destroying fungi under some experimental conditions, the hydrophobicity they impart may be the determining factor (Eberhardt et al. 1994; Gref et al. 1999). Given the inherent decay resistance of pine heartwood, especially in the southern pines, the wood treatment (preservation) industry does not generally require heartwood penetration for the preservative mixture.

The myriad of products that can derive from oleoresin led to the development of the naval stores industry involving the very deliberate wounding of pine trees for oleoresin collection. Longleaf pine (*Pinus palustris* Mill.) and to a lesser extent slash pine (*Pinus elliottii* Engelm.) were widely used in the southeastern United States (US) for turpentining operations. A report dating back to 1884 indicated that loblolly pine was also turpentined but without commercial success (Wahlenberg 1960). Prior to colonial settlement, longleaf pine was the predominant pine species in this region (Croker 1987), with a range spanning from southeastern Virginia to eastern Texas. As trees suitable for turpentining became scarce in the late nineteenth and early twentieth centuries, the naval stores industry turned to the extraction of the residual stumps to produce wood turpentine (Gardner 1989). Compared with gum turpentine obtained from oleoresin, wood turpentine contains higher relative amounts of limonene, α-terpineol, and oxygenated monoterpenes (Buchanan 1963; Palmer 1921).

Today, there are few longleaf pine ecosystems in existence. Of the original 1.5×10^6 acres of longleaf pine forest estimated to exist in Virginia prior to colonial settlement, only 800 acres remain (Sheridan et al. 1999). The recent discovery of very old lightwood stumps outside the commonly accepted range in Virginia raises the question of whether the original range for longleaf pine extended farther north. Correctly determining the identity of these stumps would assist conservation biologists with longleaf pine ecosystem restoration efforts. The persistence of these stumps in nature is also of particular interest as an extreme demonstration of the inherent decay resistance of southern pine heartwood and, perhaps in this case, specifically that of longleaf pine.

Unfortunately, identifying these stumps as belonging to longleaf pine is not a simple task. Wood anatomy does not permit the identification of individual southern pine species (Panshin and de Zeeuw 1980). While a technique involving measurements of pith and the second annual growth ring diameters provides a reliable means to separate longleaf pine from the other southern pines (Koehler 1932), it requires the presence of intact pith. As for chemical characterizations, monoterpene compositions have been used in chemotaxonomic approaches to differentiate among various conifer species and genetic groupings (Wolff et al. 1997; Fäldt et al. 2001; Silvestrini et al. 2004). Analyses of southern pine oleoresins indicate that the monoterpene compositions, while variable, are predominated by α-pinene and β-pinene (Franklin and Snyder 1971; Hodges et al. 1979; Strom et al. 2002). The exception is pond pine (*Pinus serotina* Michx.) for which the predominant monoterpene appears to be limonene (Mirov 1961). If the monoterpene composition of a lightwood stump is representative of living trees, then it may be possible to identify the stump taxon (e.g., predominance of limonene characterizes *P. serotina*).

In preliminary studies, the fragrant nature of selected turpentine and lightwood stump wood specimens was noted and suggested the presence of monoterpenes. Analyses were carried out to verify that monoterpenes were indeed present and whether they were sufficiently representative for the purpose of stump identification. Also of specific interest was the persistence of monoterpenes in heartwood should they impart any contribution to the decay resistance exhibited by these stumps. Differences in the monoterpene compositions between these specimens and those reported for oleoresins of selected southern pines were noted. Unfortunately, more appropriate comparisons could not be made with heartwood of any southern pine given the absence of these data in the literature. Thus, the objective of this study was to determine both the amounts and the relative compositions of the monoterpenes in longleaf pine heartwood. The sapwood was also analyzed for comparison. Subsequent periodic sampling of the longleaf pine stumps was carried out with the purpose of detecting changes to the relative monoterpene compositions that may occur over time under conditions found in nature.

Materials and methods

The 12 longleaf pine stumps used in this study were scattered throughout a 40 acre longleaf pine plantation located in the Kisatchie National Forest near Alexandria, Louisiana, USA. All trees were 70 years old at the time of the harvest. Tree characteristics were recorded after felling. It should be noted that the logging operations were carefully conducted to prevent any physical damage to the stumps. One sample of wood shavings was collected from each of the 12 stumps at each of the four collection periods, those being at 1, 5, 30, and 57 weeks postharvest. Six weathered lightwood stumps (with one showing evidence of turpentining) located in Caroline, Prince George, Southampton, and Sussex counties in eastern Virginia, USA, were sampled once each by removing thick wood specimens (ca. 500–1500 g) with a saw. These specimens were sealed in plastic bags, shipped by overnight courier to the laboratory, and stored in a freezer until sampled.

Sampling of stumps and stump specimens

A variable-speed drill equipped with a razor-sharp drill accessory (ca. 10 mm diameter) was used to cut a targeted 1–2 cm deep hole downward into a given longleaf pine stump. The heartwood was sampled midway between the pith and the heartwood–sapwood transition zone in a northern radial direction. The sapwood was sampled midway between the heartwood–sapwood transition zone and the cambium in the same direction. Subsequent sampling was carried out in a clockwise direction, leaving a 3 cm distance between sampling points. Extreme care was taken to apply a slow speed and moderate applied pressure to the tool to prevent heat generation. After clearing away the coarse surface shavings, the depth of the hole was extended to about 6–

Table 1. Characteristics of 70-year-old longleaf pine trees and stumps used in study.

Tree No.	Diameter at breast height (cm)	Total height (m)	Height to live crown (m)	Bark thickness at breast height (mm)	Diameter at stump surface (cm)	Height of stump (m)	Bark thickness at stump surface (mm)
1	47.9	25.5	11.8	14.5	54.7	0.15	16.8
2	31.6	27.3	17.9	9.8	46.0	0.12	27.3
3	37.4	28.5	18.5	6.8	48.4	0.18	20.8
4	35.5	26.3	15.4	11.5	42.1	0.20	13.5
5	35.7	27.3	15.8	8.0	42.2	0.26	13.0
6	34.0	29.1	21.8	12.5	42.4	0.15	22.8
7	14.0	17.6	14.0	4.0	16.4	0.15	12.5
8	41.0	26.6	14.8	14.2	49.7	0.15	26.5
9	39.3	26.3	18.4	10.8	49.0	0.20	28.3
10	34.3	24.4	14.9	10.0	39.0	0.20	29.8
11	25.2	27.5	20.0	5.3	29.0	0.20	9.5
12	31.2	22.9	13.1	7.0	43.2	0.24	20.0

7 cm from the surface of the stump, stopping periodically to collect shavings into tightly capped glass vials (20 mL). Vials containing shavings were immediately placed over ice and transported to the laboratory, where sample aliquots (1 g) were transferred to tightly capped amber-glass vials (7 mL) and steeped in methylene chloride (5 mL). Wood shavings were collected from turpentine and lightwood stump specimens by a similar process as above, discarding surface shavings to obtain a representative sample.

Monoterpene analysis by GC-MS

Methylene chloride extracts were subjected to gas chromatography – mass spectrometry (GC–MS) analysis on a Hewlett Packard 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass selective detector and a HP-INNOWax column (0.25 mm ID × 60 m length × 25 μm film thickness). The temperature regimen of the column was programmed to hold for 1 min at 40 °C, increase to 80 °C at a rate of 16 °C·min^{-1}, and then to 240 °C at a rate of 7 °C·min^{-1}, with the final temperature being held for 10 min. The temperatures for the injector inlet and mass detector were maintained at 200 and 225 °C, respectively. Peaks were identified by retention time and spectral match with NIST 98 (National Institute for Standards and Technology, Gaithersburg, Maryland) and in-house chemical libraries. Relative compositions of monoterpenes and methyl chavicol (also known as estragole, *p*-allylanisole, and chavicol methyl ether) represent peak areas as a percentage of the total peak area for all detected compounds. Concentrations were based on an assumption of similar response factors and four-point calibration curves for α-pinene, β-pinene, and limonene standards (Aldrich) prepared in methylene chloride. Moisture contents, determined by drying sample aliquots in an oven at 100 °C, were used to calculate total monoterpene contents on a dry mass basis.

Data analysis

Differences in the relative percentage of each compound between the sampling periods were analyzed by ANOVA (SAS Version 9.1, General Linear Model procedure, α = 0.05), with the dependent variable being the relative percentage of a given compound and the independent variable being the sampling period. A value of zero was used in those instances when a given compound was not detected for the sampling period. Variability in the data is shown by values for mean square error. The significance of the differences in the relative percentages between the sampling periods was determined using the Tukey's Studentized range Test option in SAS. For each compound, relative percentages showing the same letter were not significantly different between sampling periods. The ANOVA assumptions were tested by plotting the residuals and using the UNIVARIATE (Kolmogorov–Smirnov option) and CORR (Spearman correlation coefficient option) procedures in SAS; all assumptions were met for those compounds that were abundant (e.g., α-pinene, β-pinene). The possible relationships between the values for total monoterpene contents and tree characteristics with diameter at breast height (DBH) were assessed by simple linear regression. This and a *t* test were carried out using Excel 2003.

Results and discussion

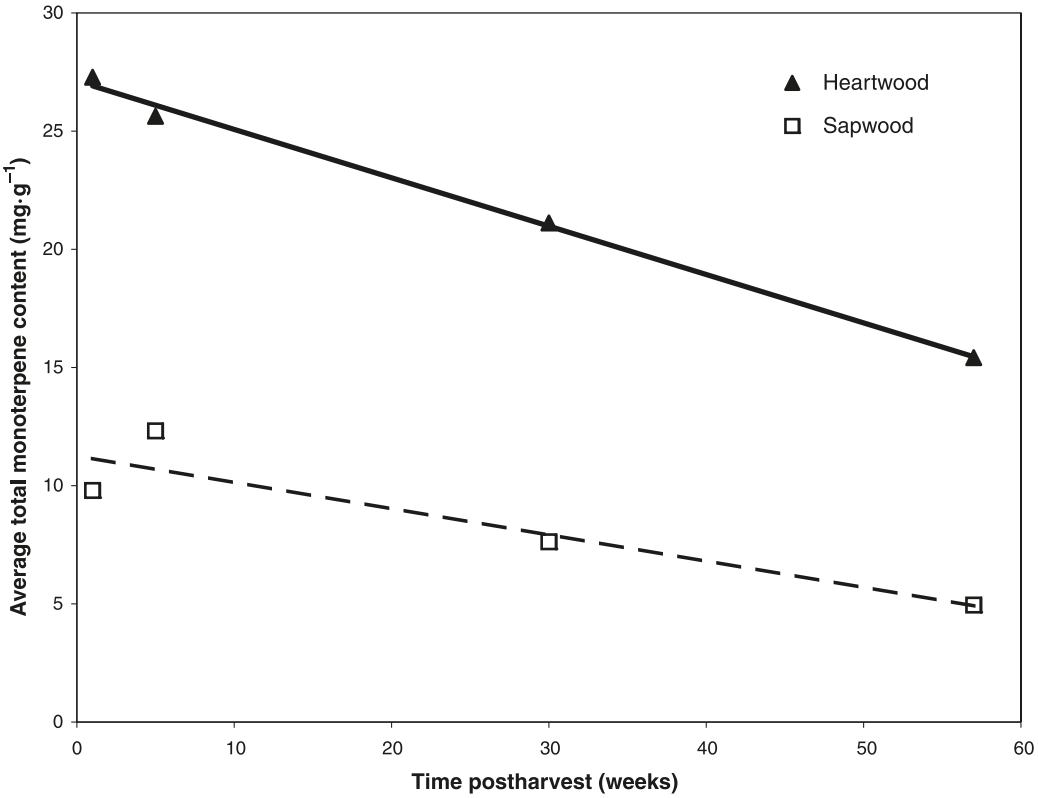
Tree characteristics

Variability in the tree characteristics reflect different growth rates resulting from the application of various silvicultural treatments (i.e., spacing, thinning, pruning) at the site for the 70-year-old longleaf pine (*P. palustris*) trees used in this study (Table 1). As expected, greater values for DBH coincided with thicker bark at breast height (R^2 = 0.65), greater stump diameter (R^2 = 0.90), greater total height (R^2 = 0.30), and larger crown size (i.e., total height minus height to live crown; R^2 = 0.74). DBH values did not correlate with values for height to live crown nor for bark thickness at stump height. A preliminary assessment of the impact, if any, of tree growth rates on the total monoterpene contents was made using the values for DBH.

Total monoterpene contents

Heartwood consistently maintained a significant (*t* stat 6.077, *t* critical 2.011, α = 0.05) threefold greater total monoterpene content than sapwood on a wet mass basis. The average total monoterpene contents for the heartwood and sapwood samples over the duration of the study were 18.4 ± 13.1 and 6.2 ± 6.0 mg·g^{-1}, respectively. To account

Fig. 1. Change in average total monoterpene content over time for heartwood and sapwood samples from longleaf pine stumps in Louisiana (n = 12 for each point).



for moisture, aliquots of the heartwood and sapwood samples were set aside for moisture content determinations. Overall, the moisture contents for the sapwood samples at each sampling were nearly twice those for the respective heartwood samples. The average sapwood moisture content declined slightly from 30.90% ± 5.53% at the first sampling to 28.33% ± 9.43% at the final sampling. The respective values for the heartwood were 19.36 ± 4.30% and 15.42% ± 2.06%. Adjusting for moisture gave average total monoterpene contents for the heartwood and sapwood, over the duration of the study, of 22.36 ± 13.37 and 8.67 ± 4.57 mg·g^{-1}, respectively. Plotting the average total monoterpene contents for each sampling period, on a dry mass basis, shows a gradual decrease for both the sapwood and heartwood (Fig. 1). The average total monoterpene contents for the heartwood and sapwood declined about 43% and 49%, respectively, over the 1 year sampling period. The similarity in these values was somewhat surprising given that sapwood showed signs of stain and decay fungi at the latter sampling periods whereas the heartwood did not.

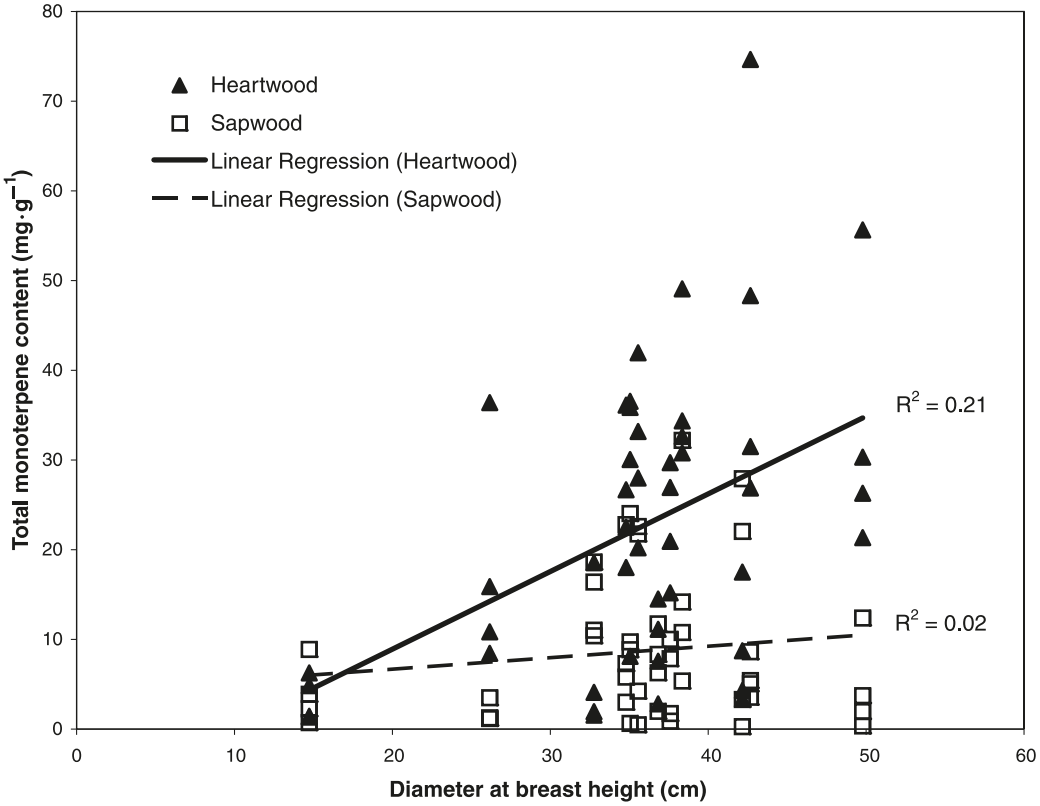
The average total monoterpene content for the sapwood did not correlate with DBH (R^2 = 0.02), whereas a very weak (R^2 = 0.21) relationship appeared possible for the heartwood (Fig. 2). The relative volume of heartwood in longleaf pine is inversely related to tree growth rate (Koch 1972), thus fast-growing trees have a higher proportion of sapwood. For the longleaf pine stumps, higher heartwood total monoterpene content coincided with faster growth, as indicated by the values for DBH (Fig. 2). Therefore, vigorous tree growth may boost interior defenses by producing heartwood, albeit a lesser relative volume, with higher total monoterpene content.

Prior studies by others have shown that higher sapwood resin flows impart resistance to attacking insects, such as the southern pine beetle, *Dendroctonus frontalis* Zimm. (Strom et al. 2002; Tisdale et al. 2003). Resin flows in *Pinus taeda* L. have been subdivided as being either constitutive (i.e., present as a reserve) or induced as a response to attack or wounding (Lombardero et al. 2000). Induced oleoresin flows, measured after the constitutive oleoresin reserves had been depleted, were the greatest in the fastest growing trees during periods of rapid growth (Lombardero et al. 2000). In the present study, harvesting represents an extreme case of physical wounding, since there are no longer photosynthetic resources available for a wound response. Thus, it could be argued that the oleoresin in the samples we collected, as well as that which accumulated on many of the cut stump surfaces, was solely of a constitutive nature.

Relative composition of sapwood monoterpenes

Reported analyses of fresh oleoresin from most southern pines (e.g., *P. palustris*, *P. taeda*, *Pinus echinata* Mill., *P. elliottii*) have shown α-pinene to comprise 50%–80% of the monoterpenes detected (Hodges et al. 1979; Strom et al. 2002). The second most abundant monoterpene, β-pinene, typically ranges from 20% to 40%. Along with the pinenes, much smaller amounts of camphene, myrcene, and limonene are also often reported. Pond pine (*P. serotina*) is the exception among the southern pines, with limonene comprising as

Fig. 2. Regression analysis of monoterpene content and diameter at breast height values for heartwood and sapwood samples from longleaf pine stumps in Louisiana.



much as 90% of the detected monoterpenes (Mirov 1961). Thus, the results for the sapwood samples collected shortly after harvesting were not especially surprising, with α- and β-pinene levels averaging 75.57% and 15.28%, respectively (Table 2). Since these values were similar to those reported for oleoresin (α-pinene, 75.64%; β-pinene, 18.68%) (Hodges et al. 1979), it is likely that the monoterpene compositions in stump wood parallel those farther up the bole of the tree. In addition to the monoterpenes, a significant amount of methyl chavicol was often present. This compound, formed via a phenylpropanoid biosynthetic pathway (Gang et al. 2001), has been suggested to be repellent to the southern pine beetle (Hayes et al. 1994). Since there was a high degree of variability in the data, what appeared to be a greater presence of methyl chavicol in the freshest (e.g., 1 week postharvest) samples was not statistically significant.

The relative amount of α-pinene in the sapwood samples did not change significantly over the sampling period. More than a year postharvest, the proportion of α-pinene (75.64%) was the same as that shortly after harvesting (75.57%). In contrast, the relative proportion of β-pinene declined at about 57 weeks to about one-half (7%) of the value shortly after harvesting (15%). The lower amounts of β-pinene relative to α-pinene we observed are consistent with the more rapid loss of β-pinene observed for decaying pine needle litter (Kainulainen and Holopainen 2002). Since β-pinene has a higher boiling point than α-pinene, attributing the more rapid loss of β-pinene to volatility is counterintuitive. Evidence for stain and decay fungi 30 days after harvesting implicated the possibility of greater susceptibility to microbial degradation.

Relative composition of heartwood monoterpenes

In contrast to the data for the sapwood, the relative monoterpene compositions for the heartwood samples showed fewer changes over the course of the study (Table 3), and thus the amounts for most of the monoterpenes present in the heartwood declined more or less at the same rate. At this juncture, it should be noted that the monoterpene compositions for the sapwood and heartwood were found to be distinctly different. The average relative compositions of α- and β-pinenes in the heartwood, over the duration of the study, were roughly 64% and 10%, respectively. The lower relative amount of α-pinene in the heartwood was offset by higher amounts of limonene, fenchyl alcohol, α-terpineol, and borneol. This was an important finding that demonstrated monoterpene compositions reported for oleoresin exudates and sapwood are not representative of those in heartwood.

Relative compositions of turpentine and lightwood stump monoterpenes

Despite many years of weathering under field conditions, turpentine and lightwood stump specimens, gathered within and outside the historical range for longleaf pine in Virginia, were shown to have significant monoterpene contents (14.2–58.3 mg·g^{-1}) in addition to anticipated high contents (10%–43%, *m*/*m*) of resinous nonvolatile extractives (Eberhardt et

Table 2. Relative compositions of monoterpenes and methyl chavicol detected in sapwood of longleaf pine stumps in Louisiana.

		Relative composition (%) after:			
Compound	Mean square error	1 week	5 weeks	30 weeks	57 weeks
α-Pinene	87.150	75.57a	78.01a	74.19a	75.64a
α-Fenchene	0.383	NDa	NDa	0.38ab	0.76b
Camphene	8.203	0.68a	0.85a	1.35a	4.69b
β-Pinene	32.788	15.28a	15.97a	12.32ab	7.02b
Myrcene	1.625	1.01a	0.83a	0.17a	0.66a
α-Phellandrene	0.019	NDa	NDa	0.08a	NDa
α-Terpinene	0.000	NDa	NDa	NDa	NDa
Limonene	1.943	0.44a	0.50a	1.45ab	2.56b
β-Phellandrene	0.055	0.43a	0.32ab	0.14b	0.11b
p-Cymene	0.496	NDa	NDa	0.16a	0.45a
Terpinolene	0.514	0.29a	0.07a	0.67a	0.67a
Fenchone	0.002	NDa	NDa	0.03a	NDa
Camphor	0.466	NDa	NDa	0.54a	0.57a
Fenchyl alcohol	0.014	0.02a	0.07a	NDa	NDa
Terpinen-4-ol	11.663	NDa	0.22a	3.63a	1.69a
Methyl chavicol	20.967	5.90a	2.90a	3.40a	1.31a
α-Terpineol	3.367	0.36a	0.26a	1.49a	3.87b
Borneol	0.001	0.02a	NDa	NDa	NDa
Total		100.00	100.00	100.00	100.00

Note: Each composition value represents the mean percent peak area among detected compounds (n = 12 for each sampling period). Comparisons for each compound were determined by ANOVA (Tukey's Studentized range Test), and values followed by the same letter are not significantly different (α = 0.05, error df = 44). ND, not detected.

Table 3. Relative compositions of monoterpenes and methyl chavicol detected in heartwood of longleaf pine stumps in Louisiana.

		Relative composition (%) after:			
Compound	Mean square error	1 week	5 weeks	30 weeks	57 weeks
α-Pinene	72.661	60.82a	66.94a	64.95a	65.31a
α-Fenchene	0.058	0.21a	0.18a	0.08a	0.04a
Camphene	0.154	1.39a	1.22a	1.31a	1.52a
β-Pinene	34.301	9.79a	11.56a	9.58a	7.86a
Myrcene	0.698	0.92a	1.01a	0.84a	0.55a
α-Phellandrene	0.002	0.03a	0.03a	NDa	NDa
α-Terpinene	0.008	0.08a	0.06a	NDa	NDa
Limonene	6.153	3.30a	2.69a	2.75a	4.41a
β-Phellandrene	0.063	0.36a	0.42a	0.34a	0.04b
p-Cymene	0.005	0.01a	NDa	NDa	0.04a
Terpinolene	0.613	1.55a	1.27ab	1.09ab	0.59b
Fenchone	0.000	NDa	NDa	NDa	NDa
Camphor	0.258	NDa	NDa	0.10a	0.40a
Fenchyl alcohol	3.136	2.13a	0.89a	1.13a	1.14a
Terpinen-4-ol	3.120	0.90a	1.09a	0.71a	0.34a
Methyl chavicol	11.973	5.03ab	3.55ab	6.08a	2.14b
α-Terpineol	89.942	11.84a	8.03a	9.88a	13.76a
Borneol	1.768	1.64a	1.06a	1.16a	1.86a
Total		100.00	100.00	100.00	100.00

Note: Each composition value represents the mean percent peak area among detected compounds (n = 12 for each sampling period). Comparisons for each compound were determined by ANOVA (Tukey's Studentized range Test), and values followed by the same letter are not significantly different (α = 0.05, error df = 44). ND, not detected.

Table 4. Relative compositions of monoterpenes and methyl chavicol detected in lightwood and turpentine stumps located in eastern Virginia.

	Lightwood stumps (%)*	Turpentine stump (%)†
α-Pinene	34.28 (17.71)	58.22
α-Fenchene	2.14 (2.22)	0.58
Camphene	3.97 (2.75)	3.10
β-Pinene	1.34 (1.30)	1.25
Myrcene	0.67 (0.86)	0.03
α-Phellandrene	0.73 (1.41)	ND
α-Terpinene	0.52 (0.71)	ND
Limonene	5.29 (4.53)	9.29
β-Phellandrene	1.38 (2.91)	ND
p-Cymene	13.87 (20.66)	0.28
Terpinolene	1.30 (0.86)	1.67
Fenchone	3.89 (5.73)	0.26
Camphor	6.40 (8.01)	0.82
Fenchyl alcohol	1.66 (1.17)	1.93
Terpinen-4-ol	3.80 (4.29)	0.93
Methyl chavicol	1.65 (2.94)	2.55
α-Terpineol	14.83 (8.45)	16.18
Borneol	2.28 (0.88)	2.91
Total	100.00	100.00

Note: ND, not detected.

*Values are the mean percent peak area for identified compounds ($n = 5$) with standard deviation in parentheses.

†Analysis of turpentine stump is shown separately given its high likelihood of belonging to longleaf pine.

al. 2007). The turpentine stump sampled in Southampton County, Virginia, was of particular interest, having the highest probability of belonging to longleaf pine on the basis of a box cut and historical records of longleaf pine in that particular county and at the site (Harvill et al. 1986; Sheridan et al. 1999). The relative amount of α-pinene in this specimen (58.22%; Table 4) was similar to that reported for the known *P. palustris* stumps monitored in Louisiana (60.82%, Week 1; Table 3). In contrast, the relative amount of β-pinene was low by comparison (9.79% vs. 1.25%). This indicates that not only is β-pinene unstable in the sapwood, but it may also be unstable in the heartwood. In the absence of stain and decay fungi, such β-pinene losses are more appropriately attributed to isomerization and (or) oxidation as a function of time under conditions found in nature. By extension, the higher rate of β-pinene loss relative to α-pinene loss in the sapwood is likely a function of both preferential microbial degradation and chemical instability (isomerization and (or) oxidation) inherent to β-pinene.

Studies on fossilized wood and bark tissues have demonstrated changes that occur to the nonvolatile extractives (e.g., resin acids) over very long periods of time (Menchi et al. 1995; Staccioli et al. 1999, 2002). The presence of more highly oxidized monoterpenes (e.g., cymene, fenchyl alcohol, borneol, α-terpineol) was shown for an Arctic fossil bark sample that was more than 45 million years old (Staccioli et al. 2002). Although within much shorter timeframes (i.e., from decades up to centuries), it is nevertheless intriguing that the turpentine and lightwood stumps we sampled still retained relatively high monoterpene contents despite their constant exposure to the heat and humidity of the southern US. The average relative monoterpene composition for the lightwood stumps also shows higher proportions of oxidized monoterpenes.

Given their presence, and heritable nature, the monoterpenes seemed to provide an option for chemotaxonomic identification. The lack of baseline data for longleaf pine stump heartwood led, in part, to the current study. At this juncture, it should be noted that the only reliable method for identifying longleaf timbers as belonging to longleaf pine is that by Koehler (1932), by which measurements of the pith and second growth ring diameters can be used to differentiate longleaf pine from the other southern pines. Limiting our use of this method is the difficulty in finding turpentine and lightwood stumps with intact pith. Similarities in the relative monoterpene compositions between the Southampton County, Virginia, turpentine stump and the longleaf pine stumps we sampled suggest some utility as a chemotaxonomic identifier. Of course, positive identification for a turpentine stump as belonging to longleaf pine is not possible given similarities in the monoterpene compositions of longleaf pine and other southern pines (*P. taeda*, *P. echinata*, *P. elliottii*). However, herein we do have a limited chemotaxonomic tool in that the relative monoterpene compositions for the turpentine and lightwood stumps may allow us to eliminate pond pine (*P. serotina*) as a possibility given the predominance of limonene in this particular southern pine.

The processes involved in heartwood formation are not well understood. It would be rather speculative to suggest that differences in monoterpene compositions between sapwood and heartwood are the result of the specific deposition of more highly oxidized monoterpenes in the heartwood. Perhaps an oversimplified explanation would be that these differences are simply the product of monoterpene aging within the heartwood, a nonliving compartment in the living tree. Data collected in the current study suggest that the changes in heartwood monoterpene compositions are gradual. Under extreme conditions, such as during a forest fire, it is likely that oxidation and isomerization could be accelerated. It has been reported that monoterpene compositions can change upon exposure to heat and air during the processing of wood (McGraw et al. 1999) or turpentine processing (Derfer and Traynor 1989; Drew et al. 1971). Under less extreme conditions, for example with stored wood chips, the inherent acidity of wood has been suggested to promote the conversion of α-pinene to cymene (Drew et al. 1971). Given the high amount of α-terpineol in the representative turpentine stump, it would appear that the high amounts (50%–60%) of α-terpineol reported for wood naval stores (Buchanan 1963) is a product of both harsh processing conditions and the high levels of this monoterpene in the heartwood. With respect to the latter, the inherent acidity of pine woods (pH 4.9–6.0) (Fengel and Wegner 1984) may promote the conversion of α-pinene to α-terpineol in a manner analogous to the acid-catalyzed hydration process widely used in production of synthetic pine oil from turpentine, with α-terpineol being the primary terpene alcohol produced (Kelly and Rohl 1989; Pakdel et al. 2001).

The exact mechanisms (e.g., isomerization, oxidation) resulting in differences in the relative monoterpene compositions is beyond the scope of this study. However, it appears

plausible that further research on these differences could be used to develop models to predict elapsed time in nature. Of course, large sample sets would be necessary to address known sources of data variability (e.g., within a tree, between trees, between sites). Again, impeding this research is the lack of older heartwood samples for which the specific species is known as well as the age of the tree and the time postharvest.

Finally, the decay resistance of the southern pines is generally attributed to the deposition of nonvolatile extractives, such as the resin acids. While the data presented here do not specifically demonstrate that monoterpenes have an active role in the decay resistance of longleaf pine stump heartwood, they do demonstrate that the monoterpenes persist and thus are available should they serve any such function. Further study is warranted to specifically demonstrate whether volatile extractives, such as the monoterpenes, do indeed contribute, albeit likely in a transient manner, to the natural durability of southern pine heartwood.

Conclusions

The total monoterpene contents of the sapwood and heartwood of residual longleaf pine stumps decline at similar rates 1 year postharvest. While the relative proportion of α-pinene in the sapwood remains constant over time, the relative proportion of β-pinene declines significantly by mechanisms, which likely include aging phenomena (e.g., volatilization, oxidation, isomerization) and possibly preferential metabolism by colonizing stain and decay fungi. Differences in the sapwood and heartwood total monoterpene contents and relative compositions demonstrated that oleoresin collected from trees is not representative of the heartwood monoterpenes. While the total monoterpene content of stump heartwood also declines after harvesting, the relative monoterpene composition is little changed 1 year later. The relatively high total monoterpene contents and low relative proportion of β-pinene in turpentine and lightwood stumps provide further evidence for the apparent chemical instability of β-pinene. Despite the changes in composition over time, monoterpene compositions for turpentine and lightwood stumps provide a limited chemotaxonomic tool for identification purposes. Although a definite role for monoterpenes in the decay resistance of longleaf pine stump heartwood has not yet been shown, these compounds do persist for a very long time and thus may be a contributing factor.

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