THE QUEST FOR METHODS TO IDENTIFY LONGLEAF PINE STUMP RELICTS IN SOUTHEASTERN VIRGINIA

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Abstract--The discovery of lightwood and turpentine stumps in southeastern Virginia raised questions about the true historical range for longleaf pine (*Pinus palustris* Mill.). Several investigative studies were therefore carried out to develop a method to determine the taxa of these relicts. Chemical approaches included the use of near infrared (NIR) spectroscopy coupled with principal component analysis and characterization of the monterpenes in stump wood extracts by gas chromatography-mass spectrometry (GC-MS). More recent efforts led to the revivification of a method involving measurements of pith and second annual ring diameters. Development of this method is still ongoing through the exploration of alternative measurements and the expansion of the data set. Results gathered thus far have been consistent with the putative range for longleaf pine in Virginia.

INTRODUCTION

Prior to colonial settlement, the vast longleaf pine (*Pinus palustris* Mill.) forest covered over 37 million ha (Croker 1987, Frost 1993, Outcalt and Sheffield 1996), ranging from southeastern Virginia to eastern Texas (Koch 1972, Wahlenberg 1946) as shown in figure 1 (inset). In Virginia, less than 323 ha remain of the estimated 607,000 ha of longleaf pine forests estimated to be present prior to colonial settlement (Frost 2006, Sheridan and others 1999). Provenance studies in Virginia have since involved the planting of longleaf pine within (Saucier and Taras 1966) and outside its putative range (Johnsen 2013).

Relicts from the harvesting of the original longleaf forest can still be found throughout the southeastern United States and have been used for tree-ring dating and the study of historical land use patterns (Grissino-Mayer and others 2001, van de Gevel and others 2009). The discovery of lightwood and turpentine stumps outside the putative range of longleaf pine in Virginia were of particular interest as possible physical evidence for justifying a northern extension of the historical range for longleaf pine (Eberhardt and Sheridan 2005). However, the presence of a stump relict alone does not provide sufficient evidence of longleaf pine. Since it is not possible to differentiate among the southern yellow pine species on the basis of anatomy (Panshin and de Zeeuw 1980), alternative methods of identification were sought. Limited utility was gleaned from spectroscopic and chemotaxonomic approaches (Eberhardt and others 2007, 2009a, 2010). Persistence in this endeavor led us to revive a method whereby measurements of the pith and the second annual ring diameters of tree disks allows longleaf pine to be distinguished from the other southern pines (Eberhardt and others 2009b, 2011). Here we provide a review and an update of our quest to develop methods that identify stump relicts, in the hope that they can provide physical evidence of the historical range of longleaf pine in Virginia.

MATERIALS AND METHODS

Method details for the characterization of stump-derived samples/specimens can be found in the publications cited in this section. Briefly, chemical analyses utilized stump relict specimens from Caroline, Prince George, Southampton, and Sussex counties in eastern Virginia. Longleaf, shortleaf (*P. echinata* Mill.) and loblolly pine (*P. taeda* L.) wood samples were included as controls. After grinding, samples were analyzed by near infrared (NIR) spectroscopy coupled with principal component analysis (PCA) (Eberhardt and others 2007) and/or GC-MS, the latter to determine the monoterpene compositions (Eberhardt and others 2009a, 2010); one of the relict stumps

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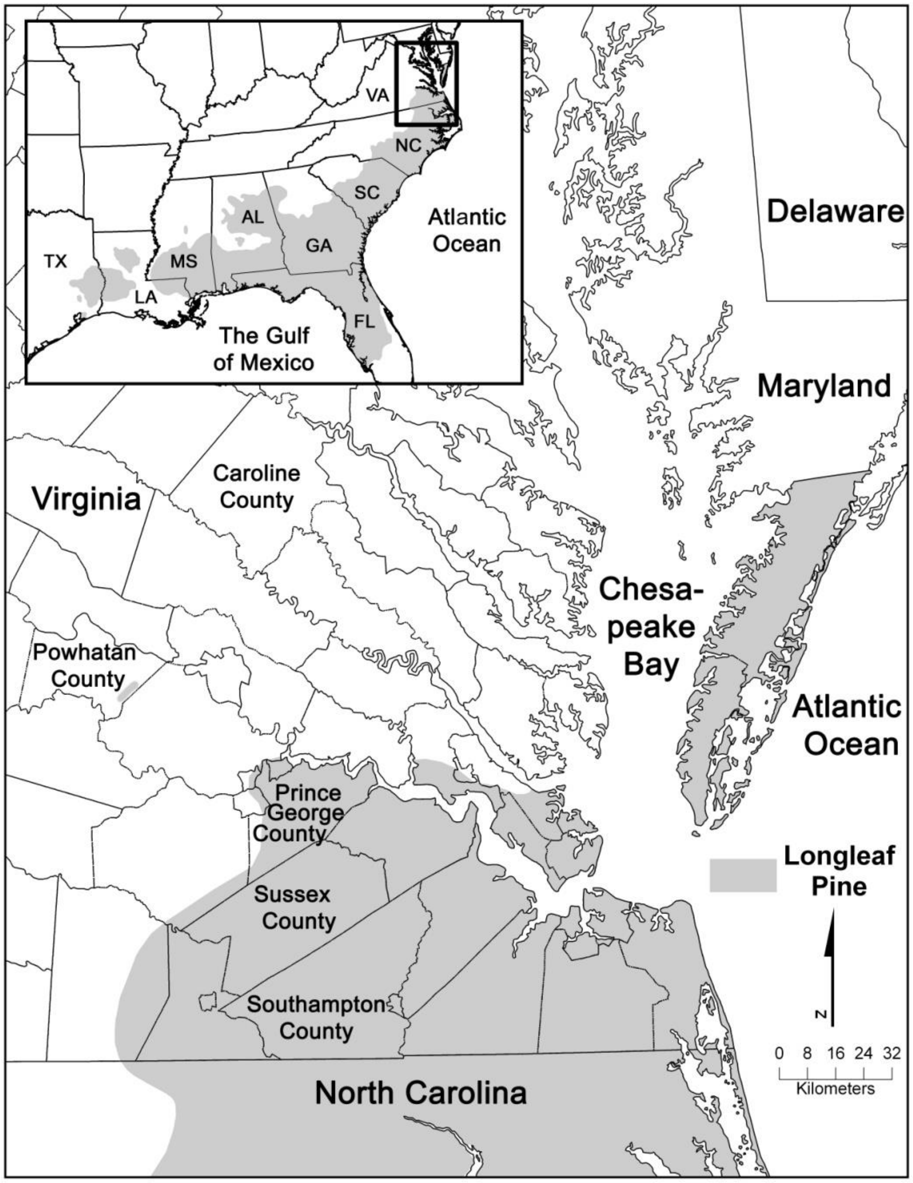

Figure 1--Map of longleaf pine ranges in Virginia and the southeastern United States (inset).

(Southampton County) subjected to gas chromatography-mass spectrometry (GC-MS) showed signs of turpentining and was therefore labeled as a turpentine stump. Validation of Koehler's method (1932) utilized southern pine tree cross-section disks from recent harvests throughout the southeastern United States. Among the relict lightwood stumps from eastern Virginia (Sussex, Prince George, Powhatan, and Caroline Counties), one from Caroline County was also labeled as being a turpentine stump. Included with the relicts was a snag from an old-growth longleaf pine tree struck by lightning (Suffolk, VA). Pith and second annual ring diameters were measured and plotted along with Koehler's (1932) delineating curve to identify those stumps belonging to longleaf pine (Eberhardt and others 2009b, 2011).

RESULTS AND DISCUSSION

Spectroscopic Identification

Our quest began after acknowledging that the stump relict samples collected in Virginia could not be identified as belonging to longleaf pine on the basis of simple wood anatomy. This led us to inquire whether our in-house expertise on the characterization of wood chemical and physical properties by NIR spectroscopy, coupled with multivariate analysis, could be applied. Stump relict samples were analyzed along with known samples of longleaf and loblolly pine wood. Application of PCA to the spectroscopic data gave the groupings shown in figure 2; a weakness in this analysis was the lack of stump relict specimens for which the species were known. Several groupings were observed with the stump relict specimens generally being separate from the longleaf and loblolly wood samples. Overall, the groupings were not sufficiently distinct to conclude that all of the stump relict specimens were of the same species or that any of them specifically belonged to longleaf pine. With these preliminary results it was concluded that even with a much larger sample set, this technique would not allow us to identify the stump relict specimens as belonging to longleaf pine.

Chemotaxonomic Identification by Monoterpene Analyses

While handling the stump relict specimens during the spectroscopic analyses, their fragrant nature suggested the presences of volatile compounds, with many likely being monoterpenes. Given the heritable nature of the monoterpenes, it seemed plausible that their characterization provided an opportunity for chemotaxonomic identification (Fäldt and others 2001, Silvestrini and others 2004, Wolff and others 1997). The most abundant monterpene among the southern pines is α-pinene, comprising 50 to 80 percent of the detected monoterpenes in oleoresin from longleaf, shortleaf, loblolly and slash pines (Hodges and others 1979, Strom and others 2002); the second most abundant monoterpene, β-pinene, typically ranges from 20 to 40 percent. Along with the pinenes, much smaller amounts of camphene, myrcene, and limonene are also often reported (Hodges and others 1979, Strom and others 2002). Whereas the most abundant monoterpenes would appear to be of little utility, it was envisioned that the minor monoterpenes might afford patterns leading to identification.

Analyses of the stump relict samples by GS-MS showed the monoterpene contents to range from 14.2 to 58.3 mg/g (Eberhardt and others 2007). The turpentine stump sampled in Southampton County was of particular interest having the highest probability of belonging to longleaf pine on the basis of a box cut, as used during turpentining operations, and historical records of the presence of longleaf pine in that particular county and at the site (Harvill and others 1986, Sheridan and others 1999). Analysis of this specimen (table 1) gave a relative amount of α-pinene (58.22 percent) that was similar to the heartwood of longleaf pine stumps (60.82 percent) (Eberhardt and others 2009a). Interestingly, the mean value for α-pinene among the remaining lightwood stump relict samples was lower (34.28 percent), offset by the presence of more oxidized monoterpenes (e.g., terpinolene, terpinen-4-ol, fenchyl alcohol). Even with access to lightwood samples for which the species were known, it became readily apparent from these data that it would be difficult to account for the changes to the monoterpene compositions over time. The only feasible utility of the technique was that it eliminated pond pine (*P. serotina* Michx.) for which limonene comprises as much as 90 percent of the detected monoterpenes of trees within the species' native range (Eberhardt and others 2010).

Measurement of Pith and Second Annual Ring Diameters

Koehler's method (1932), involving the measurement and plotting of pith and second annual ring diameters, was first validated with longleaf, shortleaf, and loblolly pine specimens taken at stump height (approximately 0.5 m); points above the delineating curve are assigned as belonging to longleaf pine. All longleaf pine specimens gave data points that when plotted could be readily assigned to longleaf pine (fig. 3). Almost all data points for loblolly and shortleaf pines were indicative of southern pines other than longleaf pine. Thus, there was no reason to suspect a dramatically different rate of false positives for currently standing timber than the second-growth timbers likely available to Koehler some 70 years ago. Given the data points shown in figure 3, it seemed that the values for the second annual ring diameter were lower than those for the other southern pines; whereas the very rapid growth of loblolly pine is manifest in the measurements of second annual ring diameters reaching 54.88 mm, the largest value for longleaf pine was 40 mm. Identification and measurement of second annual ring diameters was therefore suggested as an adaptation to the method for tentative evidence in situations where the pith is missing or decayed away (Eberhardt and others 2011).

In addition to currently growing southern pines, we were fortunate to gain access to an old-growth longleaf pine near Suffolk, VA that had been killed by lightning. Measurements from this

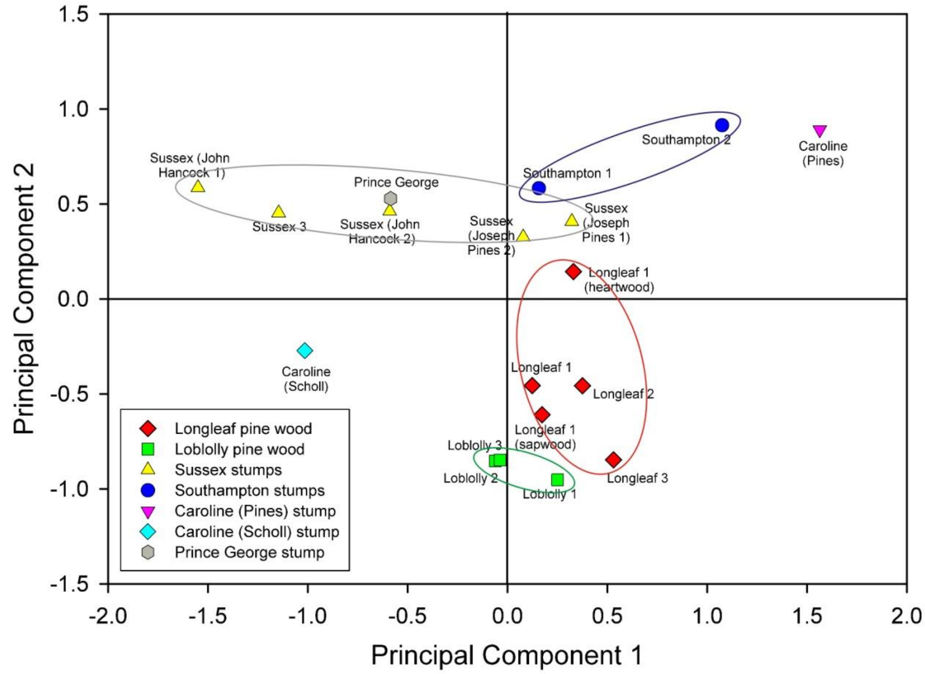

Figure 2--Principal component analysis of samples from stump relicts and controls (longleaf and loblolly pine wood). Figure reproduced from Eberhardt and others 2007.

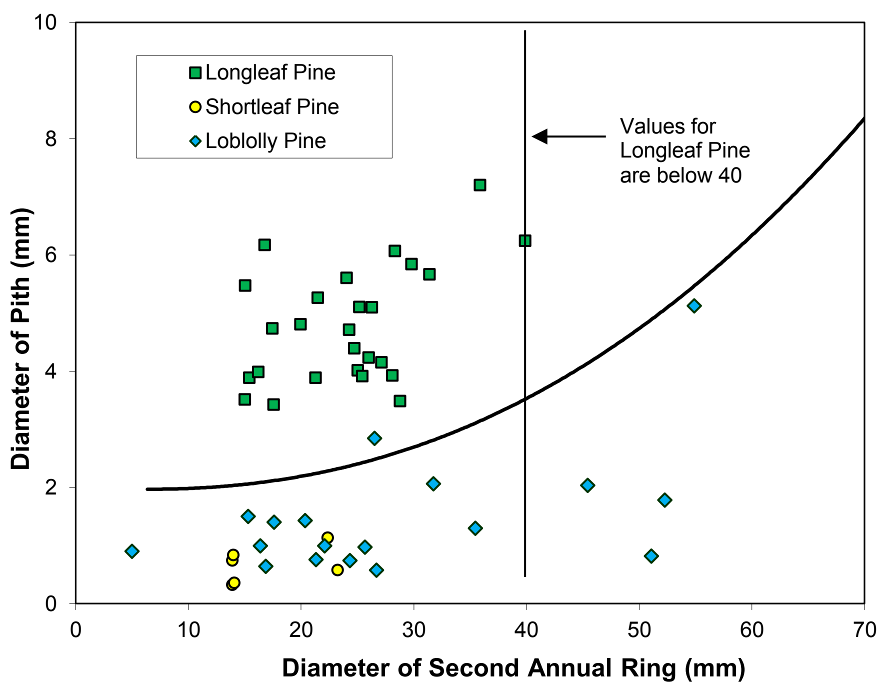

Figure 3--Plot of pith and second annual ring diameter measurements for longleaf, shortleaf and loblolly pine specimens. Figure adapted from Eberhardt and others 2011.

Table 1--Relative compositions of monoterpenes and methyl chavicol detected in stump wood samples: recently-harvested longleaf pine in central Louisiana; lightwood and turpentine stumps in eastern Virginia (ND = not detected)

Compound	Longleaf pine stumps	Unknown lightwood stumps	Southampton turpentine stump
	-------------------------*percent*----------------------------		
α-Pinene	60.82	34.28	58.22
α-Fenchene	0.21	2.14	0.58
Camphene	1.39	3.97	3.10
β-Pinene	9.79	1.34	1.25
Myrcene	0.92	0.67	0.03
α-Phellandrene	0.03	0.73	ND
α-Terpinene	0.08	0.52	ND
Limonene	3.30	5.29	9.29
β-Phellandrene	0.36	1.38	ND
p-Cymene	0.01	13.87	0.28
Terpinolene	1.55	1.20	1.67
Fenchone	ND	3.89	0.26
Camphor	ND	6.40	0.82
Fenchyl Alcohol	2.13	1.66	1.93
Terpinen-4-ol	0.90	3.80	0.93
Methyl Chavicol	5.03	1.65	2.55
α-Terpineol	11.84	14.83	16.18
Borneol	1.64	2.28	2.91
Total	100.00	100.00	100.00

tree were well within the limits for longleaf pine (fig. 4). Lightwood stump relicts located within (Powhatan, Prince George, and Sussex counties) and outside (Caroline County) the putative range of longleaf pine in eastern Virginia gave data points on the plot that excluded longleaf pine. A few loblolly pine trees in Virginia were also sampled and gave similar results. Turpentine scars were suggested by Koehler (1932) as an indicator of longleaf pine. Contradicting this indicator was a result that showed the turpentine stump in Caroline County was not longleaf pine. This result was particularly intriguing since it could substantiate the historical report that loblolly pine was subjected to turpentining operations but without commercial success (Wahlenberg 1960).

Finally, additional samples of longleaf, shortleaf, and loblolly pine were accessed from dendrochronological studies throughout the southeastern United States (Bhuta and others 2008, 2009). These samples were independently measured, and therefore demonstrate that the technique is indeed robust. No patterns were observed in this plot (fig. 5) to suggest that the pith and second annual ring measurements are influenced by the ecological region from which they were taken.

CONCLUSIONS

It is unlikely that NIR spectroscopy coupled with PCA will provide a means to identify stump relict specimens as belonging to longleaf pine, even with a much larger sample set. Given the difficulties in accounting for the changes to the monoterpene compositions over time, the chemotaxonomic identification of longleaf pine would be difficult to achieve; this technique may only allow the exclusion of pond pine for which limonene is the most abundant monoterpene. Among the methods that we tested, the only robust technique for the identification of stump relicts as belonging to longleaf pine involves the measurement of pith and second annual ring diameters. Results collected to date do not provide physical evidence for a northern range extension for the historical range of longleaf pine in Virginia.

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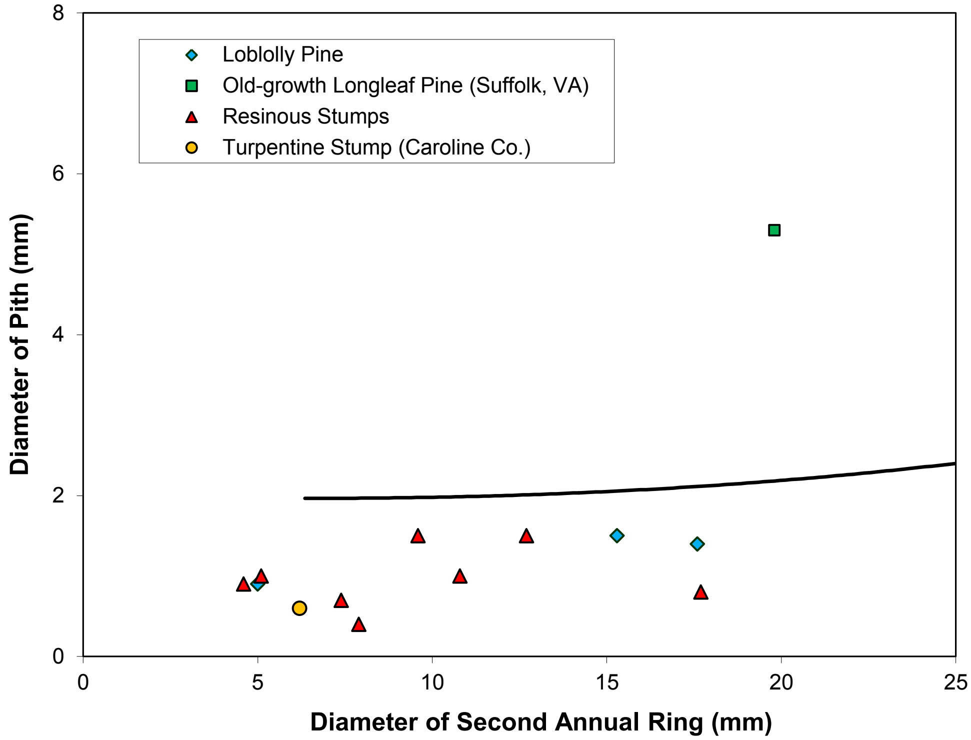

Figure 4--Plot of pith and second annual ring diameter measurements for loblolly pine tree and relict specimens collected in southeastern Virginia. Figure adapted from Eberhardt and others 2011.

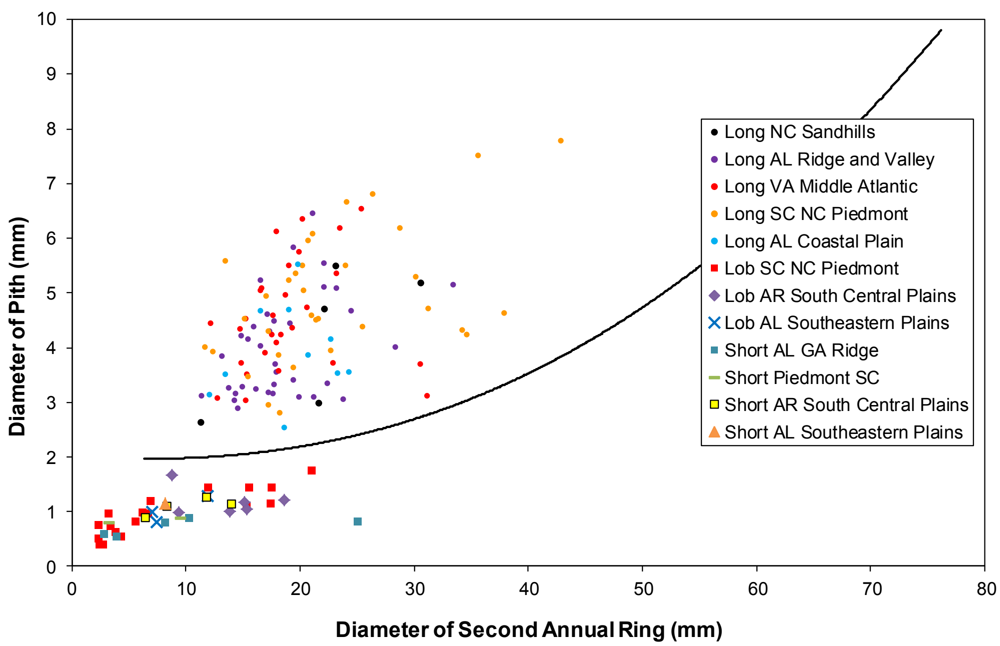

Figure 5--Plot of independently-collected pith and second annual ring diameter measurements for longleaf, shortleaf and loblolly pine specimens taken in various ecological regions across the southeastern U.S.

Jolie Mahfouz carried out all monoterpene analyses.

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