

Monoterpene Content of Healthy Sand Pine and Sand Pine with Root Disease

SUSAN V. KOSSUTH

E. L. BARNARD

ABSTRACT. Graft combinations with slash pine (*Pinus elliottii* Engelm.) alone and with sand pine (*P. clausa* var. *immuginata* D. B. Ward, Choctawhatchee sand pine) were studied to determine if monoterpene content of rootstocks of healthy and diseased grafts is related to susceptibility to root infection. The amount and composition of monoterpenes in branches, scions, rootstocks (stem of the rootstock) and roots were determined for the following graft (scion/rootstock) combinations: healthy and diseased sand/sand, sand/slash, and healthy slash/slash grafts. Buds from the same grafts were analysed for monoterpene composition only. Similar analyses were made of xylem tissue at dbh of healthy and diseased Choctawhatchee and Ocala sand pines (*P. clausa* var. *clausa* [Chapm.] Vasey) growing in natural stands.

In seed orchard samples, the quantity of monoterpenes was higher in roots and rootstocks of diseased grafts than of healthy grafts. However, monoterpene composition in healthy and diseased grafts was similar within the sand/sand grafts and within the sand/slash grafts. No consistent differences in monoterpene composition were found between these two graft combinations. There was a significantly lower amount of β -phellandrene and a higher amount of β -pinene in roots and rootstocks of healthy and diseased sand/slash grafts as compared to healthy slash/slash grafts. The roots and rootstocks of slash/slash grafts were not infected by the root disease that is affecting sand pine. Of the trees sampled, sand and slash grafts with low β -phellandrene content in the roots and rootstocks were susceptible to root infection. Slash pine grafts with high β -phellandrene were not infected. In stem xylem samples from natural stands, no monoterpene differences were detected between healthy and diseased Ocala sand pine. Between varieties, the amounts of several monoterpenes found in both Choctawhatchee and Ocala sand pines differed very little and inconsistently. FOREST SCI. 29:791-797.

ADDITIONAL KEY WORDS. Graft, rootstock, scion, α -pinene, β -pinene, turpentine, slash pine.

SAND PINE ROOT DISEASE is a serious problem throughout the natural range of Ocala sand pine in the Florida Peninsula and of concern with natural stands of Choctawhatchee sand pine in the Florida Panhandle and in Florida plantations. In the Florida Division of Forestry seed orchard at Munson, Florida, 8- to 15-year-old Choctawhatchee sand pines (*P. clausa* var. *immuginata* D. B. Ward) are showing high mortality due to a root disease involving at least *Phytophthora cinnamomi* Rands (Barnard, unpublished data) and *Inonotus circinatus* (G. M. Blakeslee, University of Florida, Gainesville, personal communication). This orchard was established by grafting scions of selected Choctawhatchee sand pine onto rootstocks of Choctawhatchee sand pine, or Ocala sand pine (*P. clausa* [Chapm. ex. Engelm.] Vasey ex Sarg., race uncertain), and onto slash pine. Slash pine rootstocks were used in an attempt to confer off-site adaptability to the sand

The authors are with the USDA Forest Service, Southeastern Forest Experiment Station, School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611, and the Florida Department of Agriculture and Consumer Services, Division of Forestry and Plant Industry, Gainesville, FL 32602. This article was written and prepared by U.S. Government employees on official time, and it is therefore in the public domain. Mention of a trade name is for the convenience of the reader and does not constitute an endorsement by the U.S. Department of Agriculture or the Forest Service. Manuscript received 30 November 1981.

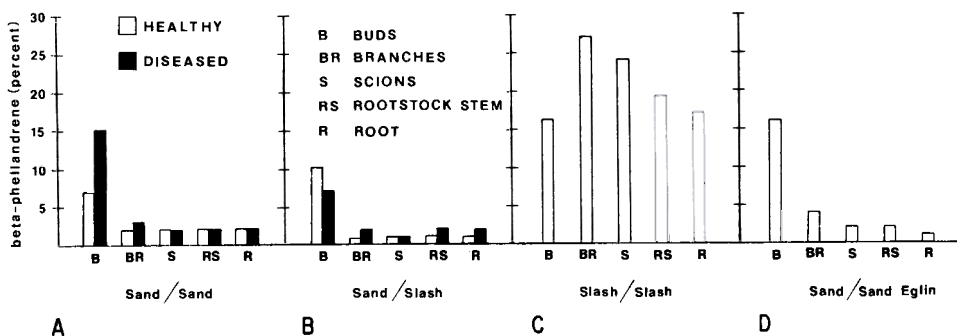


FIGURE 1. Percentage of the monoterpenes made up of β -phellandrene in healthy and diseased samples from pine grafts.

pine which require a much drier site for optimum development. Choctawhatchee sand pines grafted to sand or slash pine rootstocks appear to be equally susceptible to root infection. However, an adjacent 15-year-old slash/slash pine seed orchard has not developed symptoms of root disease.

Previous work in the Munson seed orchard (Kossuth and others 1981) showed that the percentage of β -phellandrene in the rootstock monoterpene fraction was significantly lower and β -pinene significantly higher in slash pine rootstocks grafted with sand pine scions as opposed to slash pine scions. The current study examined relative amounts of monoterpenes in five tissues of sand pine and slash pine graft combinations and related monoterpenes to susceptibility to root disease. The xylem monoterpenes of healthy and diseased Choctawhatchee and Ocala sand pines growing in natural stands were also compared.

METHODS

In November 1980, 15 grafts each of healthy and diseased Choctawhatchee sand/sand and Choctawhatchee sand/slash and healthy slash/slash pine combinations were sampled in the Munson seed orchard. Ten healthy Choctawhatchee sand/Choctawhatchee sand pine grafts in the seed orchard on Eglin Air Force Base 18 km from the Munson orchard were also sampled. Diseased sand pine grafts were identified as those with aboveground symptoms of root disease including extreme crown thinning, foliar discoloration, and sometimes resin exudation through the bark near ground line. Healthy sand and slash grafts were free of the foregoing symptoms. No trees were excavated to verify root disease or freedom from the root disease complex.

Xylem monoterpene content of trees in natural stands was determined for samples from a Florida Division of Forestry statewide survey on sand pine root disease. Increment cores, 10 cm $\times$ 4 mm taken at dbh, were collected from 3 healthy and 3 diseased trees in each of 9 stands of Choctawhatchee and Ocala sand pines. From seed orchard trees, 10 cm $\times$ 4 mm increment cores were extracted from scions approximately 0.3 m above the graft union, rootstock stems approximately 0.1 m below the graft, and roots 0.4 m or more below the graft union. Intact buds were collected and branch samples approximately 2 mm thick were peeled to the xylem. All increment cores were placed in calibrated extraction tubes containing pesticide-grade pentane. Buds and branch samples were placed in calibrated vials in pentane. All samples were kept in dry ice until delivery to the laboratory within 48 hours.

Volumetric analysis of increment cores and branches estimated the amount of

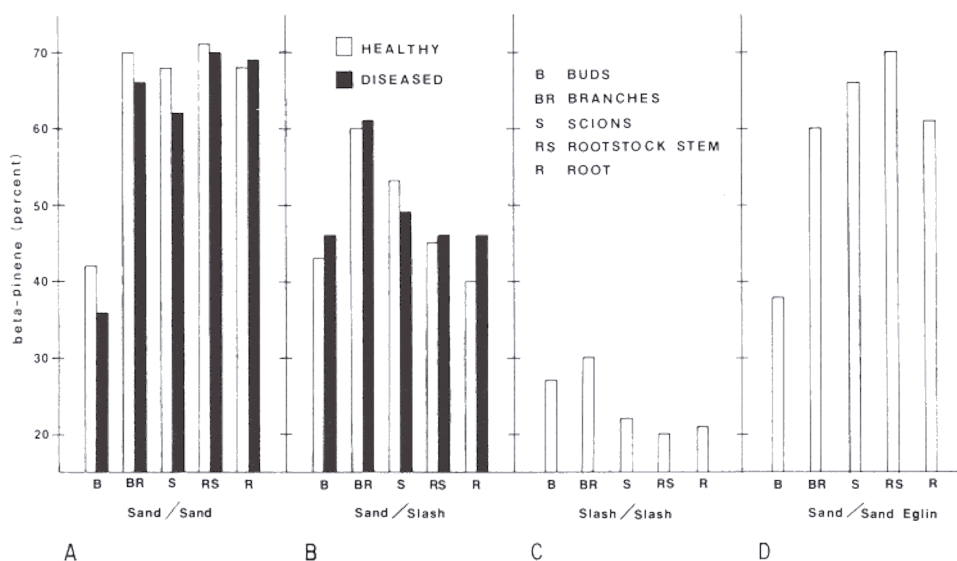


FIGURE 2. Percentage of the monoterpenes made up of β -pinene in healthy and diseased samples from pine grafts.

turpentine per dry-weight of wood. The percentage of each monoterpene in the turpentine fraction from increment cores, branches, and buds was determined by gas-liquid chromatography with a 5840 Hewlett Packard gas chromatograph and a 5-m 15-percent Carbowax 20M Column (Kossuth and Munson 1981). Each sample was injected once and one sample of each tissue per tree was used. The column was programmed for an initial temperature of 110°C for 10 min, and then increased to 180°C at the rate of 3°C/min. Data were analyzed by ANOVA after arc sine transformation of percentages.

RESULTS

Significantly less β -phellandrene and more β -pinene was found in the rootstock and roots of sand/sand and sand/slash trees in comparison with slash/slash grafts for both healthy and diseased trees (Figs. 1 and 2). Sand/sand grafts had significantly more β -pinene than the sand/slash grafts in the rootstock and root (compare Fig. 2A and 2B). Significantly less α -pinene was found in the rootstock and roots of sand/sand grafts than in the sand/slash and slash/slash grafts (compare Fig. 3A with 3B and 3C). Percent α -pinene was not significantly different in slash pine rootstocks regardless of scion (compare Fig. 3B and 3C).

Significant variation between different tissues in a tree was found for α -pinene, β -pinene, and β -phellandrene in all graft combinations (Figs. 1–3). In the sand/sand grafts from both locations the xylem monoterpene composition (branches, stem, rootstock, and root) tended to be similar. Bud monoterpenes differed from the xylem monoterpenes (Figs. 1A, 1D, 2A, 2D, 3A, and 3D). In the slash/slash grafts more variation in monoterpene composition was found among the xylary tissues (Figs. 1C, 2C, 3C).

The quantity of monoterpenes was significantly greater in the rootstock and root of diseased grafts than in the healthy trees of the same graft combination (Fig. 4A, 4B). In the sand/sand grafts there was a 2-, 8-, and 15-fold increase in the quantity of monoterpenes in the scion, rootstock, and root, respectively, of

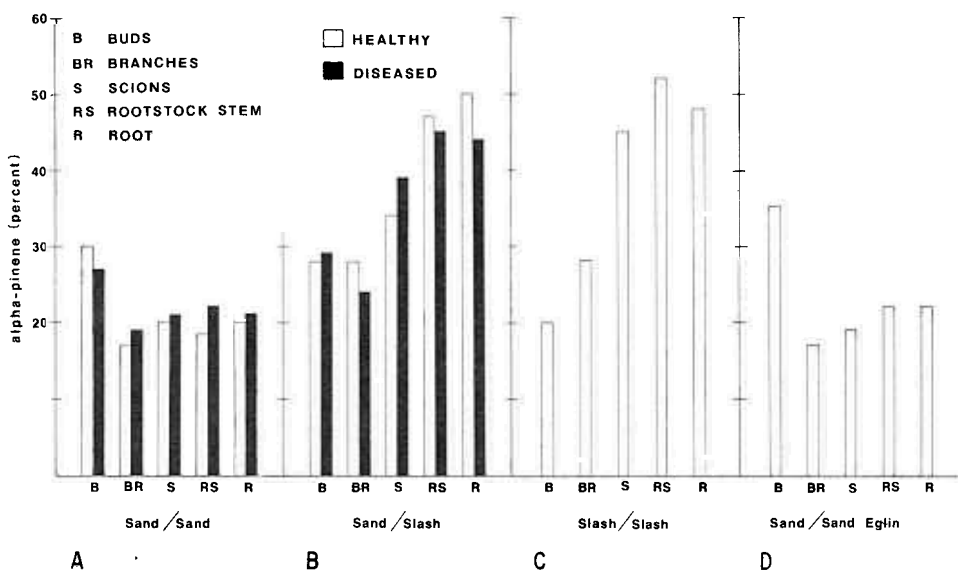


FIGURE 3. Percentage of the monoterpenes made up of α -pinene in healthy and diseased samples from pine grafts.

diseased tissues over that found in healthy tissues (Fig. 4A). The sand/slash rootstock and root of healthy and diseased grafts had a greater quantity of monoterpenes than the same tissue in the sand/sand grafts (compare Fig. 4B and 4A). However, diseased grafts had the same percent composition of monoterpenes as their respective healthy grafts (Fig. 4A, 4B). The xylary tissue in slash/slash grafts contained a greater quantity of monoterpenes than the sand/sand grafts (compare Fig. 4A and 4C).

β -phellandrene ranged from 1 to 4 percent in all sand pine stems and branches but was 24 and 27 percent in slash pine stems and branches, respectively (compare Fig. 1A and 1B with 1C). In the buds of sand pine β -phellandrene was significantly higher than in all xylem tissues regardless of rootstock (Fig. 1A, 1B). It was much higher in diseased than in healthy sand/sand grafts but not in diseased sand/slash grafts (compare Fig. 1A and 1B). The higher level was also found in healthy buds of sand/sand Eglin grafts which were on a Choctawhatchee sand pine rootstock (Fig. 1D).

Other monoterpenes occurred in amounts less than 2 percent except limonene, which was found at 11, 4, and 3 percent in the buds, branches, and roots, respectively, of the slash/slash grafts.

In the 9 natural stands of each sand pine variety, no significant differences among the monoterpenes occurred between xylem of healthy and diseased Choctawhatchee sand pines (Table 1). Differences found between healthy and diseased Ocala sand pine xylem monoterpenes were small and probably of no biological significance (Table 1). β -pinene was lower in the diseased Ocala sand pine by 7 percent, which accounted for increases in limonene and α -terpineol (Table 1).

DISCUSSION

The primary differences in xylem monoterpene content between slash and sand grafts is that slash pines contain more α -pinene (11 to 34 percent composition) (compare Fig. 3A, 3B, 3D with 3C), more β -phellandrene (15 to 25 percent

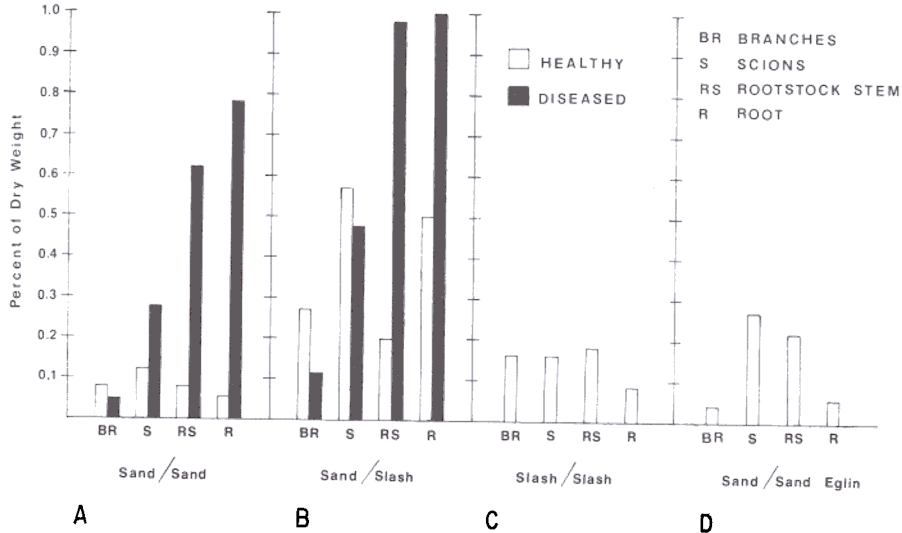


FIGURE 4. Monoterpenes as a percent of the dry weight of xylem samples of healthy and diseased pine grafts.

composition) (compare Fig. 1A, 1B, 1D with 1C), less β -pinene (40 to 51 percent composition) (compare Fig. 2A, 2B, 2D with 2C), and a greater quantity of monoterpenes (compare Fig. 4A, 4B, 4D with 4C). In sand/slash grafts, rootstock and root monoterpenes in slash pine differed from those in slash/slash grafts: β -phellandrene was lower and β -pinene higher in both healthy and diseased sand/slash grafts than in slash/slash grafts (compare Figs. 1A and 1B with 1C; 2A and 2B with 2C). The monoterpene content of sand/slash grafts was similar to sand/sand grafts (compare Figs. 1A, 2A, 3A with 1B, 2B, 3B).

Previous research has implicated monoterpenes in tree disease resistance. Cobb and others (1968) demonstrated that growth of *Fomes annosus* (Fr.) Karst. (*Heterbasidion annosum* (Fr.) Bref.) as well as several *Ceratocystis* spp. was inhibited *in vitro* by volatile components of xylem oleoresin from *Pinus ponderosa*. A 2- to 8-fold increase in monoterpenes and sesquiterpenes has been measured following infection of *Pinus sylvestris* roots by mycorrhizal fungus (Krupa and Fries 1971), and it is believed that these compounds may mediate the growth of mycorrhizal fungi (Melin and Krupa 1971) as well as confer disease resistance to pine roots (Krupa and Nylund 1972). Δ -3-carene and β -phellandrene increased 40- and 30-fold, respectively, in feeder roots of *Pinus echinata* after infection by ectomycorrhizal fungi, and both of these monoterpenes inhibited growth of mycorrhizal and pathogenic fungi *in vitro* (Krupa and others 1973). Low and high β -phellandrene in cortical tissues of *Pinus taeda* and slash pines, respectively, were sometimes associated with resistance to fusiform rust, suggesting that resistance to this disease may be positively related in some manner to β -phellandrene even though this monoterpene *per se* was apparently not the cause of resistance (Rockwood 1973, 1974). Franklin and Snyder (1971) sampled stem oleoresin near the base of 58 longleaf pines and consistently found less than 1 percent β -phellandrene. If the composition were the same in roots, β -phellandrene may not be important in resistance to root disease in longleaf pine.

Several monoterpenes, including β -phellandrene, are known to inhibit the *in vitro* growth of *Phytophthora cinnamomi* (Krupa and Nylund 1972, Krupa and

TABLE 1. Xylem monoterpene comparison of 3 healthy and 3 diseased trees from each of 9 natural stands of Choctawhatchee and Ocala sand pine.

Component	Choctawhatchee		Ocala	
	Healthy	Diseased	Healthy	Diseased
Turpentine (Percent of dry wood)		0.047	0.027	
	Percent of total monoterpenes			
α -pinene	16	17	17	16
β -pinene	42	42	42	35
limonene	6	6	6	7
β -phellandrene	2	2	3	3
fenchol	6	4	4	5
terpinene-4-ol	5	4	4	5
methyl chavicol	4	3	3	4
α -terpineol	7	9	6	9
borneol	4	3	3	2

others 1973). The data in the present study show that slash pine grafts, with high β -phellandrene, are not affected by root disease in the Munson seed orchard (Fig. 1C). The slash pine rootstocks and roots with sand pine scions were low in β -phellandrene and infected (Fig. 1B). The quantity of monoterpenes in sand/slash rootstocks did not increase (Fig. 4B), so infection cannot be attributed to a lesser amount of turpentine in the roots and rootstock. This suggests that the low level of β -phellandrene in the oleoresin may be an indicator for root disease susceptibility. The presently healthy trees, both sand/sand and sand/slash, all have low β -phellandrene in roots and rootstocks (compare Fig. 1A and 1B). It is likely that they too may become infected in a matter of time as the disease continues to spread.

The lack of compositional differences in monoterpenes between healthy and diseased tree graft combinations indicated that the increases in monoterpenes in diseased trees were a general response to infection. Resin soaking, which can be seen by cutting into the rootstock and root of diseased trees, is indicative of the increase detected (Fig. 4A, 4B). However, no specific monoterpene was preferentially synthesized as a result of root infection.

The sand pine trees sampled from natural stands in this study tended to have more than 2 percent composition for the five oxygenated monoterpenes, fenchol, terpinene-4-ol, methyl chavicol, terpineol, and borneol. As trees age, the older xylem tissue monoterpenes become oxygenated and the older age of these trees probably accounts for the differences observed.

Further research is needed *in vitro* to clarify physiological responses of identified pathogens to individual monoterpenes and other inhibiting substances present in tree roots. *In vivo* studies are needed to clarify responses of tree roots to known pathogens. If the composition of monoterpenes in tree roots could be consistently related to root disease resistance or susceptibility, tree breeders could use monoterpene composition as a tool for screening and selecting for disease resistant genotypes.

Further studies are also needed using reciprocal grafts of clonal scions and rootstocks of known monoterpene composition to determine if the differences in monoterpene composition observed in this study are indeed a scion effect on the rootstock. A scion effect on the rootstock was demonstrated by Mirov (1945) in which aldehyde content was altered when reciprocal grafts were made between Digger and ponderosa pines.

LITERATURE CITED

- COBB, F. W., JR., M. KRISTIC, E. ZAVARIN, and H. W. BARBER, JR. 1968. Inhibitory effects of volatile oleoresin components on *Fomes annosus* and four *Ceratocystis* species. *Phytopathology* 58:1337-1325.
- FRANKLIN, E. C., and E. B. SNYDER. 1971. Variation and inheritance of monoterpene composition in longleaf pine. *Forest Sci* 17:178-179.
- KOSSUTH, S. V., and J. W. MUNSON. 1981. An automated approach to terpene analysis using an internal standard. *Tappi* 64:174-175.
- KOSSUTH, S. V., E. L. BARNARD, A. E. SQUILLACE, and S. KRATKA. 1981. Sand pine scion changes monoterpene composition in slash pine rootstocks. *Can J Forest Res* 11:857-859.
- KRUPA, S., J. ANDERSON, and D. H. MARX. 1973. Studies on ectomycorrhizae of pine. IV. Volatile organic compounds in mycorrhizal and nonmycorrhizal root systems of *Pinus echinata* Mill. *Eur J Forest Pathol* 3:194-200.
- KRUPA, S., and N. FRIES. 1971. Studies on ectomycorrhizae of pine. I. Production of volatile organic compounds. *Can J Bot* 49:1425-1431.
- KRUPA, S., and J. E. NYLUND. 1972. Studies on ectomycorrhizae of pine. III. Growth inhibition of two root pathogenic fungi by volatile organic constituents of ectomycorrhizal root systems of *Pinus sylvestris* L. *Eur J Forest Pathol* 2:88-94.
- MELIN, E., and S. KRUPA. 1971. Studies on ectomycorrhizae of pine. II. Growth inhibition of mycorrhizal fungi by volatile organic constituents of *Pinus sylvestris* (Scots pine) roots. *Physiol Plant* 25:337-340.
- MIROV, N. T. 1945. Effect of the crown on the composition of oleoresin in pines. *J For* 4:345-348.
- ROCKWOOD, D. L. 1973. Monoterpene-fusiform rust relationships in loblolly pine. *Phytopathology* 63:551-553.
- ROCKWOOD, D. L. 1974. Cortical monoterpene and fusiform rust resistance relationships in slash pine. *Phytopathology* 64:976-979.