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SLASH PINE ROOTSTOCKS WITH LOW β-PHELLANDRENE SHOW HIGH SUSCEPTIBILITY TO SAND PINE ROOT DISEASE

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Abstract.--Monoterpene composition of various graft combinnations of slash pine (Pinus elliottii Engelm.) and sand pine (P. clausa var. immuginata D. B. Ward), Choctawhatchee sand pine was studied to determine any effect of a root disease complex on diseased trees. Quantitative and qualitative determinations of scion, rootstock stem and root monoterpenes were made for healthy and diseased sand/sand (scion/rootstock), sand/slash and healthy slash/slash pines in the Florida Division of Forestry seed orchard at Munson. Slash pines had a greater quantity of monoterpenes. Diseased trees had a greater quantity but the same percent composition of monoterpenes as healthy trees of the same graft combination. The rootstock and roots of sand/slash trees had significantly lower β-phellandrene and higher β-pinene than slash/slash trees. Slash/slash trees had more β-phellandrene and less β-pinene than sand/sand and sand/slash trees and were not infected by the root disease complex. The data suggest that sand and slash pines with low β-phellandrene are ~~resistant~~ to the root disease complex.

Additional keywords: turpentine, monoterpenes, scion, α-pinene, β-pinene.

Southern pine seed orchards are established by grafting scions from superior trees onto juvenile rootstock of the same or compatible rootstock. Special attention has not been given to developing rootstock sources which may affect size, form, insect or disease resistance, and cone production. Schmidtling (1973) observed that clonal loblolly pine (Pinus taeda L.) scions grafted to spruce pine (P. glabra Walt.) grew more slowly and produced cones earlier than when grafted to other rootstocks, but survival was poorer. Hollis et al. (1979) found slash pine (P. elliottii var elliottii Engelm.) rootstocks could impart susceptibility, not resistance, to fusiform rust.

In the Florida Division of Forestry seed orchard at Munson, 8- to 15-year-old Choctawhatchee sand pines (P. clausa var immuginata D. B. Ward) are showing high mortality due to a root disease complex involving at least Phytophthora cinnamomi Rands (Barnard, unpublished) and Inonotus circinatus (G. M. Blakeslee, University of Florida, Gainesville, personal communication).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 or Ocala sand pine (P. clausa [Chapm. ex. Engelm] Vasey ex. Sarg., race uncertain). Slash pine rootstocks were used in an attempt to confer off-site adaptability to the sand pine which require a much drier site for optimum development. Choctawhatchee

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sand pine grafted to sand or slash pine rootstocks appear to be equally susceptible to the root disease complex. An adjacent 15-year-old slash/slash pine seed orchard has not developed any symptoms of root disease.

The study described here was initiated to examine relative amounts of monoterpenes in the scions, rootstock stem and roots of the graft combinations of both healthy and diseased trees.

METHODS

In the Munson seed orchard 15 trees each of healthy and diseased sand/sand and sand/slash&healthy slash/slash pine graft combinations were sampled as well as 10 healthy Choctawhatchee sand/Choctawhatchee sand pine seed orchard trees in Eglin Air Force Base 18 km away from the Munson orchard. Diseased sand pine trees were identified as those with aboveground symtoms of root disease including extreme crown thinning and foliar discoloration. Some diseased trees also had resin exudation through the bark near ground line. Healthy sand and slash pine were free of any symptoms and no diseased slash/slash pine were observed for sampling.

Increment cores 10 cm x 4 mm were collected from scions approximately 0.3 m above the graft rootstock stems, approximately 0.1 m below the graft and roots 0.4 m or more below the graft and placed in calibrated extraction tubes containing pesticide-grade pentane. All samples were kept on dry ice until delivery to the laboratory. A volumetric analysis was made of all wood samples (scion, rootstock stem, and root) to obtain quantitative estimates (mg/cc) of monoterpenes on a dry-weight basis of the wood. The percent age of each monoterpene in the turpentine fraction 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). The column was programmed for an initial temperature of 110°C for 10 min., then increased to 180°C at the rate 3°C/min. Data were analyzed by ANOVA after arc sine transformation of percentages.

RESULTS

Significant within-tree variation was found for α-pinene, β-pinene, and β-phellandrene in all graft combinations (Table 1). In the sand/sand grafts from both locations monoterpene composition of scion, rootstock stem and roots was similar. More variation among these tissues was found in the slash/slash trees.

The quantity of monoterpenes was significantly greater in the rootstock stem and root of diseased trees than in the healthy trees of the same graft combination (Table 1). The quantity of monoterpenes was also greater in the scion of the diseased sand/sand trees than in the healthy sand/sand trees. The diseased sand/slash rootstock stems and roots had a greater quantity of monoterpenes than the diseased sand/sand trees. Slash/slash trees contained a greater quantity of monoterpenes than the healthy sand/sand trees from the same location. The percent composition of monoterpenes was similar in healthy and diseased trees of the same graft combination.

Significantly less β-phellandrene and more β-pinene was found in the rootstock and roots of sand/slash trees (Table 1). Sand/sand trees had significantly more β-pinene than the sand/slash trees in the rootstock and root. Significantly less α-pinene was found in the rootstock and roots of sand/sand trees than in the sand/slash and slash/slash trees. Percent α-pinene was similar in all slash pine rootstocks regardless of scion.

DISCUSSION

The lack of qualitative differences in monoterpenes between healthy and diseased tree graft combinations indicatesthat the quantitative increases in monoterpenes in diseased trees were a general response to infection. Hence no specific monoterpene was preferentially synthesized due to the root diseased complex. Resin soaking, which can be seen by cutting into the rootstock and root of diseased trees, is indicative of the quantitative increase in turpentine detected.

The major differences in monoterpene content between healthy slash and healthy sand pines was that slash pines contained more α-pinene and β-phellandrene and less β-pinene. Slash pines also contained a greater quantity of monoterpenes. When the sand/slash pine graft was made, the rootstock stem and root monoterpenes in slash pines were noticeably different from slash/slash pine grafts: β-phellandrene was lower and β-pinene higher in both healthy and diseased sand/slash pines and similar to the sand/sand trees.

In vivo and in vitro studies have implicated monoterpenes in tree root disease resistance. A 2- to 8-fold increase in monoterpenes and sesquiterpenes has been measured following infection of P. sylvestris roots by a mycorrhizal fungus (Krupa and Fries 1971). It is believed that these compounds may mediate to growth of mycorrhizal fungi (Melin and Krupa 1971) as well as confer resistance to disease to pine roots (Krupa and Nylund 1972). Delta-3-carene and β-phellandrene increased 40- and 30-fold, respectively in feeder roots of P. echinata after infection by ectomycorrhizal fungi, and both of these monoterpenes inhibited growth of mycorrhizal and pathogenic fungi in vitro (Krupa et al. 1973).

Several monoterpenes, including β-phellandrene are known to inhibit the in vitro growth of Phytophthora cinnamomi (Krupa and Nylund 1972; Krupa et al. 1973). The data in this study show that slash pine, with high β-phellandrene, is not affected by the root disease complex in the Munson seed orchard. The slash pine rootstocks and roots with sand pine scions were low in β-phellandrene and were infected. The quantity of monoterpenes in sand/slash rootstocks did not decrease so infection cannot be attributed to a lesser amount of turpentine in the roots and rootstock. This suggests that the low levels of β-phellandrene in the oleoresin may be an indicator for root disease susceptibility. That the healthy trees, both sand/sand and sand/slash all had low β-phellandrene in roots and rootstocks suggests that they too may become infected in matter of time because the disease is continuing to spread.

Further studies are 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. The only other study of which the authors are aware of a scion effect on rootstocks was reported by Mirov (1945) in which aldehyde content was altered when reciprocal grafts were made between Digger and ponderosa pine.

More research is also needed to clarify the role of monoterpenes in tree root disease resistance. If a positive role could be identified, geneticists could use monoterpene composition as a tool for screening and selecting for disease resistant genotypes.

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Table 1.--Quantitative and qualitative monoterpene composition of pine graft combinations.[1/]

Graft Combination	Rootstock Scion	Rootstock Stem	Rootstock Root
	MONOTERPENE COMPOSITION OF WOOD		
	----------%		
Sand/Sand Healthy	0.123	0.081	0.053
Sand/Sand Diseased	0.278	0.618	0.777
Sand/Slash Healthy	0.573	0.204	0.496
Sand/Slash Diseased	0.474	0.980	0.998
Slash/Slash Healthv	0.160	0.174	0.085
Sand/Sand Healthy (Eglin AFB)	0.267	0.215	0.057
	PERCENT OF TOTAL MONOTERPENES		
	-------% β-phellendrene-------		
Sand/Sand Helathy	2b	2b	2b
Sand/Sand Diseased	2b	2b	2b
Sand/Slash Healthy	1b	1b	1b
Sand/Slash Diseased	1b	2b	2b
Slash/Slash Healthy	24a	19a	17a
Sand/Sand Healthy (Eglin AFB)	2b	2b	1b
	·% β-pinene----------		
Sand/Sand Healthy	68a	71a	68a
Sand/Sand Diseased	62a	70a	69a
Sand/Slash Healthy	53a	45a	40a
Sand/Slash Diseased	49a	46a	46a
Slash/Slash Healthy	22c	20c	21c
Sand/Sand Healthy (Eglin AFB)	66a	70a	61a
	----------% α-pinene----------		
Sand/Sand Healthy	20b	18b	20b
Sand/Sand Diseased	21b	22b	21b
Sand/Slash Healthy	34a	47a	50a
Sand/Slash Diseased	39a	45a	44a
Slash/Slash Healthy	45a	52a	48a
Sand/Sand Healthy (Eglin AFB)	19b	22b	22b

1/ Column numbers not followed by the same letter are significantly different at the 0.05 level.