USE OF EXCESSIVE WATERLOGGING AND PHYSIOLOGICAL RESPONSES TO MEASURE GENETIC VARIATION IN LOBLOLLY PINE WATERLOGGING TOLERANCE

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Abstract.-- Eleven sources of loblolly pine were studied for genetic variation in waterlogging tolerance and physiological responses to waterlogging. There were source differences in both though there were no correlations between them.

Additional keywords: *Pinus taeda*, progeny tests, flooding, anaerobic metabolism

The purposes of this study were to determine: a) if there was interspecific genetic variation in waterlogging tolerance among several sources of loblolly pine (*Pinus* taeda L.); b) if this variation could be determined earlier under controlled waterlogging stress than in field plantings, and; c) if some of the physiological bases of this variation could be determined. The rationale and significance of these investigations have been discussed earlier (see Hook and Shear in these proceedings) and will not be reiterated here.

MATERIALS AND METHODS

Eleven sources of loblolly pine were studied, including:

1. Seven half-sibling families from two commercial seed orchards. The five families from the Federal Paperboard Co. orchard in Lumberton, NC (designated 6-13, 504, 512, 529, and 530) were originally selected as good performers on wet sites. The two families from the Westvaco seed orchard (11-9 and 7-56) in Ravenel, SC grow well throughout the coastal plain of South Carolina.

2. Three half-sibling families from single isolated trees growing in cypress-tupelo swamps in the coastal plain of South Carolina (HB, BB, and RS). Individual trees or small groups of loblolly pine often occur in these areas where they grow very well.

3. The general seed source used by the South Carolina Forestry Commission nursery in St. George, SC (STG). This source is a combination of many open-pollinated half-sibling families.

Seedlings from each source were planted on three field sites of varying wetness on the Francis Marion National Forest in SC in June, 1984. The design of each test is slightly different, though each consists of seven-tree row plots in varying numbers of replications and blocks, as described in Table 1.

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Table 1. Description of wet sites and test designs. Each replication contains a seven-tree row plot of each source.

Site Name	Mean Water Table Depth cm. Winter 1985-6	Summer 1986*	Test Design
Yellow Jacket Road (wet)	11.1	23.1	2 blocks, 4 reps each
Horse Island Road (wetter)	+0.6**	24.4	3 blocks, 2 reps each
Farewell Corner (wettest)	+13.1	14.2	1 block, 5 row plots of each source

* one of the driest seasons on record
** values preceded by a + indicate height of water above the soil surface

Seedlings were also planted at the same time in six soil tanks in which the water table level could be controlled. The tanks were filled with a Bethera series soil from the Francis Marion National Forest that will not support good pine growth when waterlogged without phosphorus fertilization. 50 ppm of phosphorus as monocalcium phosphate was added to the soil and a twelve-tree row plot of each source was planted in each tank. Three tanks were continuously waterlogged with the water table at the soil surface. The other tanks were waterlogged from November to April, but the water table was held at about 30 cm below the soil surface during the remainder of the year.

Growth measures were taken on all trees at the end of each growing season. At the end of the third growing season (1986), the seedlings in the soil tanks were harvested, oven-dried, and weighed. Fresh root tips were collected from most trees and for analyzed for: a) alcohol dehydrogenase (ADH) activity by the method of Denslow and Hook (1986), and b) anaerobic carbon dioxide (CO_2) production and resultant ethanol production by the methods of Hook and Denslow (1986). There were few viable root tips from each seedling, so either ADH activity or anaerobic metabolism (CO_2 and ethanol production) were measured, but seldom both.

Finally, standard regression and analysis of variance techniques were used to examine and compare variations in growth and physiological measures.

RESULTS AND DISCUSSION

After three growing seasons, differences in growth were developing at the wettest field site (Table 2). However, there were no significant differences in growth among sources at the other two field sites. Within family variations were very large, however, while all sources had many small trees, all did not have tall trees. The smaller trees have been dying and if they continue to do so, significant differences among sources should develop. The sources changed rank after every growing season. However, there was some stabilization and some sources were consistently good performers in all tests while others were consistently poor. Ranks in the three wet sites and the periodically waterlogged test were correlated, however, the results of the continuously waterlogged tests were poorly correlated with the other tests. Generally, the Federal Paperboard sources ranked higher when continuously waterlogged while the Westvaco and swamp sources ranked lower. FP 512 had the largest height growth under continuous waterlogging, but grew poorly in all other tests. We will continue to measure the field tests annually to determine if long term field performance is related to short term performance under controlled waterlogging.

There were significant differences in ADH activity and ethanol production among sources in the periodically waterlogged test, but no differences in CO_2 production (Table 3). Conversely, in the continuously waterlogged test, levels of CO_2 production differed, but there were no differences in ADH activity or ethanol production. The levels of ADH activity were similar among treatments, but carbon dioxide and ethanol production were much higher under continuous waterlogging. This suggests that there are differences between the short term and long term responses to waterlogging. However, none of the physiological measures were related to growth or performance.

Collecting adequate numbers of physiological measures in a reasonable time is a difficult task. Only seven samples of fresh root tips could be tested in a single day for ethanol and CO_2 production using a Warburg gas measurement apparatus. As a result, the soil tanks were harvested over a two-month period. These physiological variables change with stage of development, growth phase, and many other factors, and surely changed over the harvest. Also, the unharvested trees continued to grow. The day of harvest was included in all models and was an important variable. However, we are currently developing gas chromatographic techniques that will allow us to process a greater number of smaller samples and reduce the variance introduced by an extended harvest. Also, by reducing the number of root tips in each sample, we will be able to measure a greater number of physiological parameters on each tree.

Since all progeny were from open-pollinated seeds, we could not control or measure the variation introduced by the paternal parent. While there were not significant differences in growth in all tests, all sources produced many small trees but only some of the sources made large trees.

While survival was good in both tank treatments, the poorest trees had died by the time of harvest. We do not know what they did or did not do that resulted in intolerance to waterlogging (or other causes of death).

Yet, despite these limitations, we have demonstrated genetic differences in both growth and physiological responses. We are currently conducting similar tests in growth chambers and with rooted cuttings in pots in greenhouses. We are confident that by taking more physiological measures in a shorter time, controlling sources of geographical and parental variation, and using techniques of plant growth analysis, we will begin to uncover the fundamental mechanisms of genetic variation in waterlogging tolerance.

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LITERATURE CITED

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Table 2. Means of growth and physiological parameters and ranks by source after three growing seasons. Means followed by the same letter were not determined to be different by Tukey's studentized range test (alpha=0.05; no letters are given for a test if there were no differences).

	--------Height (cm)--------					Total Weight (gm)		----------Per----------			---------Con---------		
Source	YJ *	HI	FC	Per	Con	Per	Con	ADH	CO2	EtOH	ADH	CO2	EtOH
BB	139	137	72 ab	118	46	80 b	19 ab	.3129 ab	.0391	.30 ab	.4298	.0617 ab	.47
HB	127	146	76 ab	126	47	72 b	12 a	.4702 ab	.0297	.35 ab	.4559	.1154 a	.51
RS	135	134	78 ab	129	46	91 ab	18 ab	.4036 ab	.0266	.37 ab	.3674	.0484 b	.51
STG	138	149	79 ab	130	51	120 a	22 b	.4615 ab	.0179	.24 b	.4546	.0651 ab	.53
FP6-13	120	122	69 b	113	54	74 b	22 b	.4856 ab	.0174	.37 ab	.3807	.0795 ab	.48
FP504	130	123	65 b	117	49	92 ab	22 b	.3605 ab	.0379	.48 a	.3191	.0052 b	.45
FP512	149	143	87 a	126	54	84 b	21 ab	.4972 b	.0429	.28 ab	.4246	.0568 ab	.41
FP529	136	130	78 ab	123	51	75 b	19 ab	.4687 ab	.0429	.36 ab	.3949	.0696 ab	.57
FP530	141	137	83 ab	117	51	86 ab	22 b	.3989 ab	.0322	.43 ab	.4054	.0685 ab	.55
WV11-9	140	134	80 ab	126	49	85 ab	21 b	.2838 a	.0389	.29 ab	.4100	.0619 ab	.46
WV7-56	142	149	90 a	129	49	88 ab	15 ab	.4103 ab	.0293	.43 ab	.4863	.0700 ab	.42
all	136	137	78	123	50	86	19	.4139	.0323	.35	.4117	.0638	.49

Rank in above test (1 = smallest, 11 = largest)

Source	YJ *	HI	FC	Per	Con	Per	Con	ADH	CO2	EtOH	ADH	CO2	EtOH
BB	2	9	4	6	3	4	4	2	9	4	8	4	5
HB	7	7	3	4	1	1	1	9	5	5	10	11	7
RS	4	4	6	9	2	9	3	5	3	8	2	1	8
STG	6	10	7	11	9	11	11	7	2	1	9	6	9
FP6-13	3	2	1	2	5	2	8	10	1	7	3	10	6
FP504	11	8	10	7	10	10	10	3	7	11	1	2	3
FP512	1	1	2	1	11	5	7	11	11	2	7	3	1
FP529	9	6	9	3	7	3	5	8	10	6	4	8	11
FP530	5	3	5	5	8	7	9	4	6	9	5	7	10
WV11-9	8	5	8	8	4	6	6	1	8	3	6	5	4
WV7-56	10	11	11	10	6	8	2	6	4	10	11	9	2

<u>Abbreviations:</u> YJ - Yellow Jacket Road test (wet site); HI – Horse Island Road test (wetter site); YJ – Corner test (wettest site); Per – periodically waterlogged soil tank test; Con – continuously waterlogged soil tank test; ADH – alcohol dehydrogenase activity ((umoles/mg root tip/minute)/10); CO2 – anaerobic carbon dioxide production ((umoles/mg root tip/hour)/100); EtOH – anaerobic ethanol production ((umoles/mg root tip/hour)/1000)

Table 3a. Correlation matrix of means of growth and physiological measures (eleven sources; r greater than .59 significant when alpha = 0.05).

	HI-HT*	FC-HT	PER-HT	C-HT	PER-WT	C-WT	P-ADH	P-CO	P-ETOH	C-ADH	C-CO	C-ETOH
YJ-HT	.54	.76	.47	.05	.30	.11	-.19	.51	-.30	.38	-.19	-.26
HI-HT		.72	.74	-.16	.37	-.49	.18	-.12	-.41	.89	.45	-.10
FC-HT			.63	.17	.14	-.25	.18	.12	-.20	.67	.29	-.16
PER-HT				-.26	.44	-.44	.11	-.05	-.41	.53	.17	-.07
C-HT					.02	.55	.59	-.11	-.12	-.05	.02	-.07
PER-WT						.38	-.05	-.39	-.27	.10	-.39	.05
C-WT							-.13	-.00	-.11	-.52	-.58	.04
P-ADH								-.34	-.09	.18	.40	.20
P-CO									.04	-.16	-.29	-.20
P-ETOH										-.43	-.32	-.02
C-ADH											.68	-.11
C-CO												.29

Table 3b. Correlation matrix of growth and physiological measures from soil tank tests.

------Periodic Waterlogging------

	TOTWT	ADH	CO	ETOH
HT	0.79	-0.01	-0.16	0.15
	0.00	0.87	0.29	0.27
	345	167	43	60
TOTWT		0.13	-0.09	0.04
		0.10	0.59	0.78
		158	41	58
ADH			**	**
CO				0.19
				0.23
				43

-----Continuous Waterlogging-----

	TOTWT	ADH	CO	ETOH
HT	0.78	-0.00	-0.06	-0.13
	0.00	1.00	0.65	0.30
	304	176	67	70
TOTWT		-0.11	0.03	0.07
		0.15	0.81	0.59
		170	62	64
ADH			0.05	-0.34
			0.80	0.07
			30	30
CO				0.04
				0.75
				67

* Abbreviations: YJ - Yellow Jacket Road test; HI - Horse Island Road test; FC - Farewell Corner test; PER - periodically waterlogged tanks; C - continuously waterlogged tanks; HT - height; WT - total weight; ADH - ADH activity; CO - anaerobic CO production; ETOH - anaerobic ethanol production;

** Not measured on the same trees.