GEOGRAPHIC ORIGIN OF COTTONWOOD FROM THE SOUTHEAST AFFECTS *MELAMPSORA* INFECTION IN 3-YEAR-OLD CLONAL TRIALS

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Abstract—Open-pollinated seeds were collected separately by mother tree from several trees in each of 64 natural stands of eastern cottonwood (*Populus deltoides* J. Bartram ex Marsh. var. *deltoides*) in the Southeastern United States. Rooted cuttings from the seedlings were grown at four sites (North Carolina, Florida, Alabama, and Missouri). Measurements were taken during September-October in each of the first 3 years after planting for *Melampsora medusae* Thuem. f.sp. *deltoidae* leaf rust infection. Average severity of infection over the 3 years varied with latitude and longitude of the stand where the seeds were collected, with subregion of origin, and with individual stand of origin within subregion. However, differences among river-system groups or topographic positions (upland or bottomland) for stand origins within subregions were not significant. Some of the natural stands were comparable in resistance (less infection) to a set of 12 "check" clones in the trials that came from former tree improvement programs in the lower Mississippi River Valley and Texas.

INTRODUCTION

The poplars (*Populus* genus) are important worldwide for pulp, lumber, and biofuel. That importance is based on their fast growth, but leaf rusts caused by *Melampsora* species can seriously reduce this growth. *Melapmsora medusae* is native to eastern North America, and eastern cottonwood is a natural host. This rust can reduce growth by 20 to 35 percent as a result of premature defoliation during the growing season (Chastagner and Hudak 1999, Ostry and others 1988). Early defoliation also predisposes the tree to damage from other diseases and environmental stresses, and mortality has been observed in nursery stool beds from severe infections during the preceding fall (Newcombe and others 1994).

A major gene has been discovered by Newcombe and others (1996) for resistance to *Melampsora medusae*. However, there are different races of the pathogen, inheritance of resistance varies by race (suggesting that more than one gene may be involved), and environmental factors modify the degree of susceptibility (Prakash and Heather 1986). Those authors recommend that clones be tested over time and space to sample rust races and environmental factors, thereby ensuring "durability" of resistance.

Since eastern cottonwood is a natural host of *Melampsora medusae* and is also one of the parents in many of the poplar hybrids used throughout the world, information is needed for the species on how leaf rust resistance varies across the range of the natural population. That information will help in traditional breeding programs and in the search for additional resistance genes. A short-term nursery study at two sites in Mississippi suggested that southern sources were more resistant than northern sources in the Southern United States (Friend 1981). The present paper reports results from a more intensive sample of the natural population in the Southeast, and it provides assessment of resistance for 3 years over four widely distributed test sites in the region. The objective is to determine if specific geographic areas can be identified where the natural population has high resistance.

PROCEDURES

Open-pollinated seeds were collected by Mississippi State University and industrial cooperators from up to 10 eastern cottonwood trees per stand in 72 natural stands throughout the southeastern United States (fig. 1) (Land and others 2001). This region was subdivided into three subregions: the Southeast Atlantic (SA) from North Carolina to eastern Georgia, the East Gulf (EG) from northwest Florida and west Georgia through the southern 80 percent of Alabama into eastern Mississippi, and the East Central (EC) from Tennessee and the northern 20 percent of Alabama to western Kentucky and southeast Missouri. Each stand's origin was identified by latitude, longitude, river, and either floodplain (bottomland) or terrace/upland (upland) topographic position. Seeds were

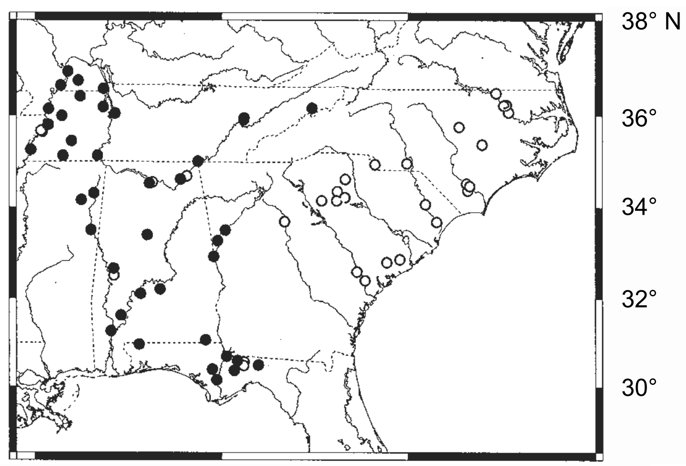

Figure 1—Map of 72 natural *Populus deltoides* stands from which seeds were collected to produce seedling cuttings for a southwide clonal trial. Stands in North Carolina, South Carolina, and east Georgia (open circles) represent the SA subregion; stands in west Georgia, southern 80 percent of Alabama, northwest Florida, and east Mississippi (filled and open circles) indicate the EG subregion; and stands in Tennessee, west Kentucky, and north 20 percent of Alabama (filled and open circles) depict the EC subregion.

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germinated at the University of Florida, and container-grown rooted cuttings were produced from seedlings for 64 stands.

Cuttings from stool beds of 12 “check” clones were rooted in containers at the same time and place as the seedling cuttings. Eleven of these check clones came from former tree improvement research by the United States Forest Service at Stoneville, MS, and their origins were in the lower Mississippi River Valley from Memphis, TN, to Baton Rouge, LA. Clone identities from the delta of northwest Mississippi were 110226, 110531, 110804, ST-121, 111101, and ST-71; clones from southwest Mississippi were 111733, 112127, 112620, and 112830; and the clone from near Baton Rouge, LA was 3324. The other check clone (S7C1) came from the Brazos River bottom near College Station, TX, and was selected by the Western Gulf Tree Improvement Program.

The rooted cuttings from seedlings and checks were planted at four locations during the period from June 1999 to March 2000, and the trees were scored for severity of *Melampsora* leaf rust infection in both September and October of each of the 3 years 2000-2002. The four test locations were a drip-irrigated upland field in northwest Florida (FL1), a drip-irrigated field on a Mississippi River alluvial site in southeast Missouri (MO1), a recently-harvested hardwood plantation site on the Tombigbee River floodplain in southwest Alabama (AL1), and a recently-harvested hardwood plantation site on a terrace of the Roanoke River in northeast North Carolina (NC1). The field design at each test location was a randomized complete block with three replications. Seedling clones were arranged by origin in subregion split plots within each replication. The 12 checks were placed in each subregion split plot. Each clone (seedling clones and check clones) was represented by a single-tree plot in each replication. Severity of leaf rust infection was scored as follows: 1 = no rust (no yellow urediospores on any leaves); 2 = little rust (isolated spores on a few leaves, but no “crinkled” leaves); 3 = medium rust (many spores, some coalesced necrotic spots, often has “crinkled” leaves and some defoliation in interior of crown); and 4 = heavy rust (most of crown defoliated, with only young leaves at branch tips). Mean score for the 3 years of observations was calculated for each tree, and stand plot means were used in analyses.

A data set with the checks excluded was used for (1) regression of leaf rust severity on latitude and longitude of stand origin and (2) analysis of variance for effects of subregions, differences between two river groups within subregions, and differences between the bottomland and upland origins within subregions on rust severity. PROC GLM in SAS (1999) provided the analyses. A mixed-effects model for the analysis of variance was assumed, with test locations, subregions, river groups, and topographic positions treated as fixed effects. A similar analysis of variance was conducted on a data set that included the checks, and the difference in “selection types” (checks versus wild stands) within subregions was treated as a fixed effect. Tukey-Kramer tests of ranked means were conducted to determine which fixed effects were significantly different.

RESULTS AND DISCUSSION

Effects of Latitude and Longitude of Stand Origin

Rust severity increased as origin of stand moved north and west within the southeast region, with latitude having the greatest influence (figs. 2 and 3). The latitude effect was significant at the Missouri, Alabama, and Florida locations, but the longitude effect was only significant at the Alabama location (table 1). These stand-origin effects were greatest at the Missouri site, where the rust severity was greatest. Friend (1981) also found an increase in susceptibility as latitude of stand origin increased in the south, but his study only involved

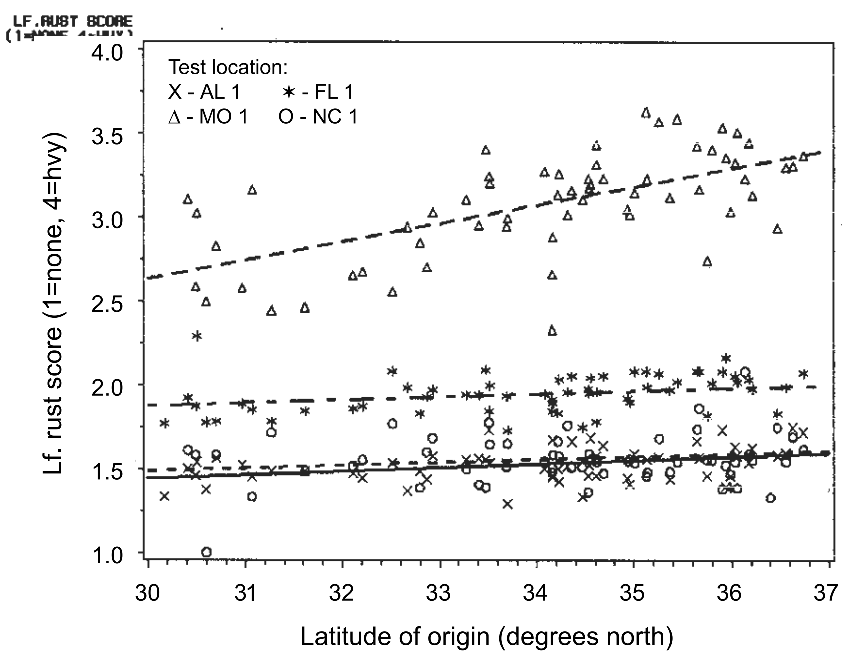

Figure 2—Effect of latitude of seed origin on *Melampsora* leaf rust infection in a 3-year-old southwide cottonwood clonal trial planted at four locations. Plotted points are stand averages at a location. A stand average is the mean of all clones derived from that stand. Higher leaf rust scores represent greater infection.

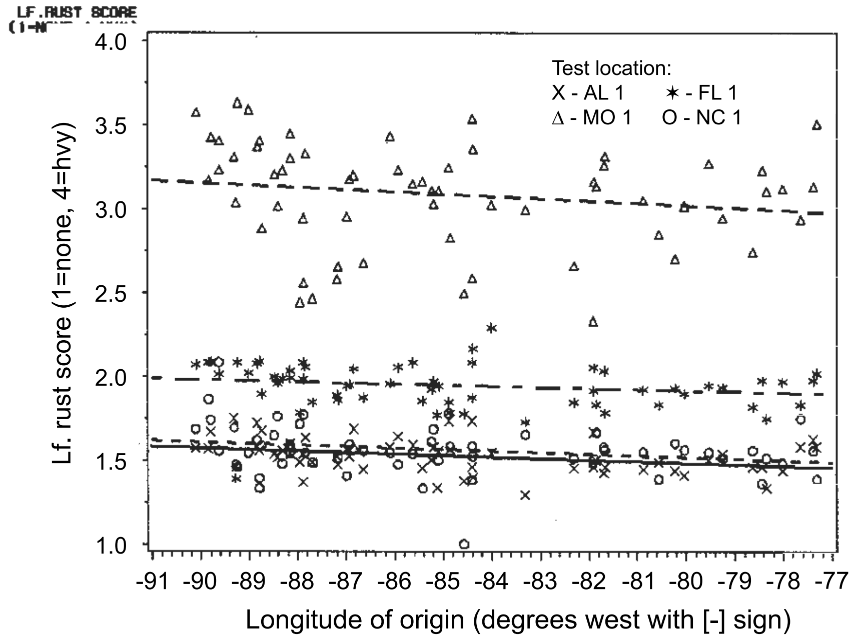

Figure 3—Effect of longitude of seed origin on *Melampsora* leaf rust infection in a 3-year-old southwide cottonwood clonal trial planted at four locations. Plotted points are stand averages at a location.

Table 1—Regression of *Melampsora* leaf rust score on latitude and longitude of seed origin (stand) at each of four test locations in a 3-year-old southwide clonal trial of *Populus deltoides*; higher scores represent greater leaf rust infection; test locations are in Choctaw County, AL (AL1), Gadsden County, FL (FL1), Scott County, MO (MO1), and Bertie County, NC (NC1)

Test location	Number of stands	Regression equation[a]	R-Square	Parameter	Pr>|t|[b]
AL1	59	RUST = .7784 + .0221 LAT	0.154	LAT	0.002*
		RUST = .7732 + .0089 LONG	0.105	LONG	0.012*
		RUST = -.1284 + .0239 LAT + .0100 LONG	0.285	LAT &	0.001**
				LONG	0.002*
FL1	61	RUST = 1.3308 + .0180 LAT	0.059	LAT	0.059
		RUST = 1.3911 + .0065 LONG	0.034	LONG	0.155
		RUST = .6921 + .0190 LAT + .0071 LONG	0.100	LAT &	0.044*
				LONG	0.111
MO1	62	RUST = -.6687 + .1100 LAT	0.395	LAT	0.001**
		RUST = 1.9269 + .0136 LONG	0.029	LONG	0.189
		RUST = -1.8972 + .1104 LAT + .0143 LONG	0.427	LAT &	0.001**
				LONG	0.077
NC1	60	RUST = .9676 + .0172 LAT	0.038	LAT	0.136
		RUST = .7857 + .0091 LONG	0.056	LONG	0.068
		RUST = .2325 + .0166 LAT + .0089 LONG	0.092	LAT &	0.142
				LONG	0.072

[a] RUST = average rust score for all clones from stand; LAT=latitude and LONG=longitude of stand.
[b] Asterisks denote level of significance: * = 0.001< Pr < 0.050, **= Pr ≤ 0.001.

two planting sites in Mississippi. The combined results from Friend's study and the current study indicate that clones from more southerly origins have more rust resistance than clones from further north within this southeast region.

Effects of Test Location

The Missouri location had the most severe leaf rust infection, followed by the Florida location (tables 2 and 3). The North Carolina and Alabama locations had the lowest incidence of infection and were not significantly different from each other. The Missouri and Florida locations had drip irrigation, while the Alabama and North Carolina locations did not. Perhaps the *Melampsora* urediospores are hindered in germination and leaf infection by low humidity, dry leaf surfaces in the early morning, and semi-senescent leaves during late summer on the non-irrigated sites. The high frequency of cottonwood trees along the Mississippi River in the vicinity of the Missouri location (and not at the other locations) may also allow a buildup

Table 2—Analysis of variance for effects[a] of test locations, subregions of origin, classification groups within subregions, and stands on 3-year average *Melampsora* rust score for a southwide trial of *Populus deltoids*

Source of variation	DF	Mean square #	F-test MS#/#	Classification groups: Rivers MS	Rivers F-test[b]	Bottom-vs-upland MS	Bottom-vs-upland F-test	Check-vs-wild MS	Check-vs-wild F-test[b]
Locations [=L]	3	#9	9/5	75.949	1315.00**	77.452	1330.00**	83.617	751.00**
Reps in L [=R(L)]	8	#8	8/5	0.056	0.96ns	0.052	0.90ns	0.093	0.83ns
Subregions [=S]	2	#7	(7+1) / (5+3)	1.216	8.58**	1.437	9.96**	0.572	2.31ns
S x L	6	#6	6/5	0.448	7.75**	0.502	8.62**	0.249	2.24ns
S x R(L)	16	#5	5/1	0.058	1.97*	0.058	2.01*	0.111	2.93**
Classif.in S [=C(S)]	3	#4	4/3	0.045	0.51ns	0.094	1.06ns	0.818	5.35*
Stands in C in S	58	#3	3/1	0.087	2.99**	0.089	3.07**	0.153	4.03**
L x C(S)	9	#2	2/1	0.072	2.48*	0.088	3.03**	0.264	6.97**
Pool error	517	#1		0.029		0.029		0.038	

[a] A mixed-effects model is used, where “Stands” and “Reps” are random effects and “Locations”, “Subregions”, and “Classification Groups” are fixed effects.
[b] Asterisks denote level of significance: * = 0.010 <Pr < 0.050, ** = Pr ≤ 0.010, and ns = not significant (Pr > 0.050).

Table 3—Tests[a] of differences among locations of trials, subregions of origin, and location-by-subregion combinations for mean leaf rust scores of cottonwood cuttings in clonal trials in the southeastern United States

Subregion of origin	Location of trial: AL1	NC1	FL1	MO1	Subregion differences
EC	1.59a	1.61a	2.01b	3.33d	2.14B
SA	1.48a	1.53a	1.92b	3.05c	2.00A
EG	1.52a	1.56a	1.91b	2.89c	1.97A
Location differences =	1.53X	1.57X	1.95Y	3.09Z	Overall = 2.08

[a] Tukey-Kramer tests were used. Means followed by the same letter and case were not significantly different at the 5 percent probability level.

of the rust population. If these hypotheses are correct, the greatest need for rust-resistant clones will be on irrigated sites in areas where cottonwood is prevalent in natural stands or other plantations. Conifers (pines, larches, or hemlocks) serve as alternate hosts for the disease and need to be nearby for rust infections to occur early in the year (Ostry and others 1988).

Subregion and Location-by-Subregion Effects

Stands from the East Central (EC) subregion had significantly more infection than stands from the Southeast Atlantic (SA) and East Gulf (EG) subregions (tables 2 and 3). There was a significant location-by-subregion effect for the data that excluded check clones (“Rivers” and “Bottom vs Upland” classification groups in table 2). The interaction was caused by a change in scale rather than a change in rank. The subregion means had similar ranks at all locations, but the differences were significant only at the Missouri location. The differences among subregions only become important when rust incidence is high. The practical application of this result is to avoid use of clones from natural stands in the EC subregion for plantings anywhere in the southeast until those clones have been tested for *Melampsora* resistance at a high-hazard location.

Effects of Rivers and Topographic Positions within Subregions

Stands from different river groups within subregions did not differ in rust susceptibility, nor did stands from different topographic positions within subregions (table 2). However, there were differences, primarily due to subregion effects, when the group-within-subregion mean rust scores for all subregions were compared (table 4). Combined results over all test locations indicated that the stands from rivers in the EG and southern half of the SA subregions had more resistance than stands from rivers in the EC subregion. River origins in the northern half of the SA subregion were not different from either the EG or EC rivers. Stands from the bottom topographic position in the EG subregion exhibited the greatest resistance, and stands from the bottom position in the EC subregion had the least.

There were significant location-by-group-within-subregion effects for both rivers and topographic positions (table 2). These interactions with locations were due to scale effects, because the differences were only significant at the Missouri location (table 4). Even then, they were only different between subregions (not within subregions). The origin with least rust at the Missouri location came from bottoms along the Tombigbee River in the EG subregion, and the origin with greatest rust at that location came from uplands along the central Mississippi River in the EC subregion.

Selected Checks versus Natural Stands

Overall, the check clones from the lower Mississippi River Valley and east Texas differed significantly from unselected stands within subregions for *Melampsora* infection (table 2). Specifically, however, the checks had significantly less rust than only the unselected stands from the EC subregion (combined results over all four test locations in right column of table 4). Checks and unselected stands did not differ for the SA and EG subregions.

There was an interaction with test locations (tables 2 and 4). The checks had less leaf rust than the unselected EC stands at all but the North Carolina location. However, they were better than the SA stands at only the Missouri location, and they were not better than the EG stands at any location.

Table 4—Tests[a] of differences among river origins, topographic origins, and selection types (selected checks vs. unselected wild types) for mean leaf rust scores of cottonwood cuttings in clonal trials at four locations in the southeastern United States

Sub-region	Classification types within subregion	Location of trial AL1	NC1	FL1	MO1	Class type differences
	River Groups					
EC	Central MS River	1.60ab	1.68b	1.98c	3.35f	2.15B
	Tennessee River	1.57ab	1.55ab	2.04c	3.31f	2.12B
SA	NC & n.SC rivers	1.50ab	1.52ab	1.94c	3.11e	2.02AB
	s.SC&ne.GA rivers	1.46a	1.54ab	1.90c	3.00e	1.98A
EG	Chatahoochee River	1.51ab	1.53ab	1.90c	2.96de	1.97A
	Tombigbee River	1.52ab	1.58ab	1.93c	2.81d	1.96A
	Topographic Position					
EC	Bottom (floodplain)	1.63g	1.65g	2.09i	3.31m	2.17I
	Upland	1.54g	1.57g	1.91hi	3.36m	2.11HI
SA	Bottom	1.50g	1.51g	1.93hi	3.13l	2.02GH
	Upland	1.46g	1.53g	1.91hi	2.99kl	1.97GH
EG	Bottom	1.50g	1.60g	1.90h	2.79j	1.95G
	Upland	1.53g	1.50g	1.93hi	2.94k	1.98GH
	Selection Type					
EC	Selected LM checks	1.40n	1.65opq	1.77pqr	2.92tu	1.94N
	Unselected EC wild	1.59op	1.63opq	2.03s	3.34v	2.15O
SA	Selected LM checks	1.45no	1.62opq	1.86qrs	2.87t	1.95N
	Unselected SA wild	1.4 no	1.53nop	1.92rs	3.06u	1.99N
EG	Selected LM checks	1.45no	1.56nop	1.81pqrs	2.79t	1.90N
	Unselected EG wild	1.52no	1.55nop	1.91rs	2.87t	1.96N

[a] Tukey-Kramer tests were used. Means followed by same letter and case were not significantly different at the 5 percent probability level.

In summary, superiority of the check clones for *Melampsora* resistance appears to be a result of the latitude-of-origin effect more than any selection effect in the former tree improvement programs. Equally high resistance may be found in natural stands from the EG and southern SA subregions.

Best Stands and Check Clones for Resistance

Variation among stands within river groups, within topographic positions, and within checks and wild stands was highly significant for leaf-rust severity (table 2). This result indicates that the most important gains in *Melampsora* resistance will come from clone selection within the checks and from stand selection (regardless of river group or topographic position) within the EG and southern SA subregions. Such selection can only be accomplished after clonal testing for rust resistance at multiple locations over multiple years.

The best nine clones or stands for *Melampsora* resistance in the present study included four check clones, two natural stands from the southern part of the EG subregion, and three natural stands from the southern part of the SA subregion (table 5). These were chosen because they performed very well at two or more test locations. Several general observations can be made: (1) No natural stands from the EC subregion were selected in the top nine, and none of the selections came from latitudes north of the southernmost EC stand. (2) The selected checks came from southwest Mississippi (south of the delta region in Mississippi) and from east Texas. (3) The first four stands or clones (ranks #1 through #4) exhibited consistently high resistance over most locations. (4) The next three stands or clones (ranks #5 through #7) had high resistance at the two southern locations (AL1 and FL1), but only average resistance at the two northern locations (NC1 and MO1). (5) The last two stands (ranks #8 and #9) indicated high resistance at the northernmost location (MO1) and were in the top third at the other locations. (6) There was no pattern of many selected stands coming from one river system or topographic position within the EG and SA subregions, but the selections did come from the southern halves of those two subregions.

SUMMARY AND CONCLUSIONS

Melampsora medusae leaf rust resistance declined as stand origin moved north and west in the southeastern region from the Atlantic coast to the Mississippi River. The greatest effect was latitude. This southeast region was subdivided into Southeast Atlantic (SA), East Gulf (EG), and East Central (EC) subregions, and a significantly greater resistance was observed for stands from the EG and SA subregions as a reflection of the latitude-of-origin effect. River groups and topographic positions (bottomland and upland) of stand origins within subregions did not exhibit significant differences in rust resistance. However, river-group-by-subregion and topographic-position-by-subregion means for leaf rust score were different across all subregions. Greatest resistance was observed for rivers in the EG and southern SA subregions. Bottomland stands from the EG subregion had the least rust, and bottomland stands from the EC subregion had the most.

Table 5—Stands and check clones with highest *Melampsora* resistance (lowest leaf score) over all locations, based on average of ranks at each location

		Geographic origin			Topographic	Test	At-location	
							Rust	
Rank	Stand or clone[a]	State	County	River	position	location	score	Rank
#1	CK-112830	MS	Wilkinson	Lower Miss.	Bottom	AL1	1.32	3
						FL1	—	—
						MO1	2.33	1
						NC1	1.38	5
#2	CK-111733	MS	Warren	Lower Miss.	Bottom	AL1	1.38	10
						FL1	1.65	3
						MO1	2.49	7
						NC1	1.40	10
#3	EG-1U1	FL	Gadsden	Ochlockonee	Upland	AL1	1.38	11
						FL1	1.78	10
						MO1	2.50	8
						NC1	1.00	1
#4	CK-S7C1	TX	Brazos	Brazos	Bottom	AL1	1.31	2
						FL1	1.64	2
						MO1	2.43	3
						NC1	1.79	69
#5	SA-5U2	NC	Bladen	Cape Fear	Upland	AL1	1.33	5
						FL1	1.75	7
						MO1	3.11	37
						NC1	1.51	24
#6	CK-112127	MS	Claiborne	Lower Miss.	Bottom	AL1	1.35	7
						FL1	1.75	6
						MO1	2.83	18
						NC1	1.58	46
#7	SA-7B1	GA	Greene	Oconee	Bottom	AL1	1.29	1
						FL1	1.73	5
						MO1	3.00	26
						NC1	1.65	56
#8	SA-4B1	SC	Greenwood	Saluda	Bottom	AL1	1.46	23
						FL1	1.92	29
						MO1	2.33	2
						NC1	1.48	18
#9	EG-2U6	AL	Clarke	Tombigbee	Upland	AL1	1.49	32
						FL1	1.85	18
						MO1	2.47	5
						NC1	1.49	20

[a] First two letters = subregion (EG, SA) or check (CK).

The tests reported here were planted at four locations: North Carolina, Florida, Alabama, and Missouri. The Missouri location exhibited the greatest rust, and the Florida location was next. Both of these locations had drip irrigation, so greater moisture and humidity on irrigated sites may be important in rust incidence. The other two locations were not irrigated and had the lowest amount of rust. There were interactions between origins and test locations for degree of resistance, but these interactions were usually a result of scale changes (changes in range and significance of differences from one location to another) rather than changes in ranks of origins. The Missouri location with its highest rust incidence provided the greatest number of significant differences among origins. Differences were often not significant at other locations, but ranks of means were similar to the Missouri location.

The selected check clones from the lower Mississippi River Valley and east Texas had less rust than the unselected stands from the EC subregion, but these checks were not significantly different from the stands that came from the EG and southern SA subregions. Thus, the improved resistance of the checks is apparently a result of the more southerly latitude-of-origin effect than the effect of selection in the original tree improvement program.

Significant variation among stands within origin classifications and among clones within checks was obtained for rust score, so large gains in resistance can be obtained from selection in clonal tests. The best nine clones or stands in the present study consisted of four check clones, three stands from the southern half of the SA subregion, and two stands from the southern half of the EG subregion. The four checks came from the southwestern half of Mississippi and east Texas.

There was no pattern indicating a majority of the best stands came from one river or topographic position. Additional gains may be possible from clone selection within the best stands.

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