VARIATION IN CAMBIAL PEROXIDASE ISOZYMES IN *QUERCUS* SPECIES, PROVENANCES, AND PROGENIES [1/]

by Frank S. Santamour, Jr., research geneticist,
and Polly Demuth, biochemical technician,
U.S. National Arboretum, USDA, SEA, Washington, D.C. 20002

ABSTRACT. -- Dormant cambial tissue of 13 *Quercus* species, representing 37 individual mother-tree lots and diverse geographic origins, were tested for peroxidase isozymes. Twenty-three different peroxidase bands were found, although none appeared to be unique to a particular species. Fresh dormant cambium contained the greatest number of bands, and this number decreased with storage. Active cambium contained fewer bands than dormant cambium. Cambial peroxidase profiles developed from these trees will be used to plan and interpret the results of reciprocal grafting studies.

ASEXUAL REPRODUCTION of *Quercus* is difficult, especially from mature trees. Rooting of cuttings from young oaks may be possible, but by the time an oak tree has demonstrated its superior growth, form, and survival characteristics it is well beyond the age at which propagation from cuttings is feasible. Grafting of oaks has not proved to be much more successful. Two cultivars, 'Sovereign' and 'Crownright' of *palustris* Muenchh. (pin oak), have been propagated in the United States nursery trade since the late 1960's. Latent graft incompatibility has recently curtailed the production of these cultivars.

What is known about graft compatibility in general is that the closer the genetic relationship of stock and scion, the greater will be the chances of long-term successful grafting. However, for many fruit trees and ornamental plants the standard propagation technique involves grafting or budding on different species or even different genera. In other cases, grafting on the same species is not possible on a commercial scale. Therefore it is likely that there must be something other than a closeness of taxonomic classification or similarity of origin that determines graft compatibility.

[1/] This study was supported, in part, by a grant from the Horticultural Research Institute, Washington, D.C. The authors gratefully acknowledge the technical assistance of Elisa D. Ahmanson.

We are exploring the potential of utilizing cambial enzyme profiles in a number of tree genera, including Quercus, to predict or explain graft incompatibility. Buchloh (1960) suggested that lignification, necessary for the formation of a mechanically strong union, is essential for graft success. Harkin and Obst (1973) gave evidence that lignification is under the exclusive control of peroxidase enzymes. In a study of peroxidase banding patterns of acorns and seedlings of Q. alba L., Mayberry and Feret (1977) found considerable between-tree variation.

MATERIALS AND METHODS

The oak trees used in this study are part of the Michaux Quercetum planting at Longwood Gardens, Kennett Square, Pennsylvania, U.S.A. The Michaux Quercetum Project was begun in 1953 as a joint effort of the Morris Arboretum of the University of Pennsylvania and the Northeastern Forest Experiment Station of the U.S. Forest Service (Schramm and Schreiner, 1954). One of the purposes of the project was to provide "information on variation within oak species and some light on the existence and distribution of oak races".

For each native American species, cooperators were asked to collect acorns from each of two mother-trees in various localities throughout the species' natural range. The acorn collections were to be accompanied by a herbarium specimen and suspected interspecific hybrids were excluded from the study.

Acorns were sown in the autumn of 1953 and 1954 in specially prepared seedbeds. Observations and seedling growth data on many of the more important species were presented by Santamour and Schreiner (1961) and Schreiner and Santamour (1961).

The major outplanting in the Northeastern United States was made in 1957 and 1958 at Longwood Gardens, Kennett Square, Pennsylvania. The red oaks (subgenus Erythrobalanus Spach) and Q. macrocarpa Michx. were planted in 1957, and contained 2-tree plots in species replicates. The white oaks (subgenus Lepidobalanus Endl.) were planted in rows, in 1958, with no replication. Growth and survival of the 1957 planting at 10 years after outplanting was reported by Garrett and Kettlewood (1975). Santamour et al (1980) provided similar data at age 25.

For the present study, 185 trees were cut during January and February of 1979, to encourage clonal sprout clumps that could be used in graft compatibility tests. These included five trees from each of 13 Q. rubra L. seedlots, and from two seedlots each of Q. alba, Q. bicolor Willd., Q. coccinea Muenchh., Q. falcata Michx., Q. imbricaria Michx., Q. macrocarpa, Q. marilandica Muenchh., Q. nigra L., Q. palustris, Q. phellos L., Q. shumardii Budkl., and Q. velutina Lam.

Sections of dormant three-year-old branches were collected from the upper crowns of the trees and stored in plastic bags in a refrigerator at 2°C. Cambial tissue from these branch section was analyzed for peroxidase (and other) isozymes to provide additional information on genetic similarity or dissimilarity that would be useful in planning and interpreting the reciprocal grafting tests. In addition, active cambial tissue of several trees of many of the same seedlots was collected and analyzed in July, 1979.

The branches were scraped with a razor blade to remove all tissues external to the xylem. Immediately, 0.2 g of this tissue was weighed into a test tube and combined with 1.0 ml of extraction buffer (1 mM tris-maleic buffer, pH 7.0, containing 5 mM potassium metabisulphite, 10 mM cysteine, and 1% (v/v Tween 80). The samples were frozen overnight. After thawing, they were homogenized at 4°C, then centrifuged for 5 minutes at 400 x g. The supernatant was decanted and used for electrophoresis.

Twelve percent starch gel was prepared according to the method of Conkle (1972) except 0.14 M sucrose was added. Gels were run at 4°C using a 2.0 M lithium-borate running buffer, pH 8.0, at a constant voltage of 100 V until the bromophenol blue marker migrated 7 cm (about 4½ hours).

For urea gels, both the extraction buffer and the gels were made 8 m in urea. The gels were run overnight at a constant voltage of 25 V.

Gels were sliced and stained for peroxidase with 3-amino-9-ethylcarbazole (Shaw and Prasad, 1970) and 0.6 ml guaiacol and 0.5 ml 5% hydrogen peroxide in 100 ml 0.2 m acetate buffer, pH 4.0. Rf values for both anodal and cathodal bands were calculated on the basis of the anodal movement of the borate front.

Following our initial collections and electrophoresis runs in January, 1979, there occurred a breakdown in apparatus that could not be remedied until late February. Thus, the majority of the dormant cambium patterns were determined on stored branches. Actually, this "problem" led to some interesting results, as discussed below.

RESULTS AND DISCUSSION

Of the 185 trees felled for examination, eight proved to be dead or in such a stage of decline that no usable cambial tissue was available. Thus, our data on isozymes in dormant cambium were restricted to 177 trees.

A total of 23 different peroxidase bands were found in the cambial tissue of the 13 oak species tested (Tables 1, 2, and 3). None of the bands appeared to be unique to a particular species although Band Q was found only in one tree of Q. macrocarpa. Band S1 of the red oaks and Band S2 of the white oaks gave identical Rf values in standard preparations but were shown to be different when run on urea gels.

As mentioned above, only a few seedlots, mostly of Q rubra, were analyzed for dormant cambial peroxidases within a week after collection. These data are given in Table 1. Of the 30 trees in this sample, only two trees of MQ-471 showed identical banding patterns. Thus the (fresh) dormant cambial peroxidase profile could serve as a "fingerprint" for the majority of the trees. The uppermost bands (A1, A2, A3) in this material were quite distinct, with individual trees showing only A1 A2 or A2 A3.

After the dormant branches had been stored for one or two months, the banding patterns changed markedly. The "A" bands seemed to coalesce and stained less intensely. A number of the weak bands disappeared entirely. Comparisons can be made between the fresh dormant cambium of the seedlots in Table 1 and the stored dormant cambium of the same seedlots in Table 2.

The loss of bands after prolonged storage of dormant cambial tissue was somewhat distressing at first, until it was discovered that the enzyme patterns of stored dormant cambium were very similar to those of active cambium taken in July, 1979 (Table 3). As with the change in banding patterns between fresh and stored dormant cambium, the major differences between fresh dormant cambium and fresh active cambium seemed to involve the loss of bands that stained only weakly in the former tissue.

Using the peroxidase band profiles of stored cambial tissue (Table 2) as a guide, it is apparent that there are significant differences among individual trees within seedlots and species in the "major" dark-staining bands (Table 2). Notable among these are the anodal bands G, H and I, and the cathodal bands, M, N, P, R, S1 and S2. Differences in peroxidase pattern among seedlings within seedlots was considerable, and in some cases appeared to be as great as those among provenances or species. Except for Q. rubra, however, the number of seedlots and provenances available for study within a species was limited. With wider sampling, some of the apparent differences among species or provenances might not be as marked.

We will use the peroxidase profiles to select individual trees to be used in reciprocal grafting tests involving trees of the same and different seedlots, provenance, species, and subgenus. With such a pre-selection criterion, we should be better able to determine which, if any, peroxidase isozymes play a role in graft incompatibility.

Table 1. Frequency of peroxidase bands from dormant cambium of Quercus rubra seedlots -- extracted and run within five days after collection. Bands marked with an asterisk (*) are usually strong bands.

		Seedlot Number and State of Origin					
Band	R_f	MQ-018 NH	MQ-020 NH	MQ-058 KAN	MQ-416 ILL	MQ-418 ILL	MQ-471 ILL
A_1	54	5/5	5/5	1/5	1/5	5/5	5/5
A_2	53	5/5	5/5	5/5	5/5	5/5	5/5
A_3	51	--	--	4/5	4/5	--	--
B	47	--	--	2/5	1/5	4/5	3/5
C	44	4/5	5/5	2/5	4/5	1/5	--
D	41	--	2/5	3/5	2/5	1/5	5/5
E	38	5/5	5/5	2/5	3/5	1/5	--
F	34	5/5	5/5	5/5	5/5	4/5	2/5
G*	31	5/5	5/5	5/5	5/5	5/5	5/5
H*	27	2/5	1/5	5/5	5/5	5/5	5/5
I*	21	3/5	1/5	5/5	5/5	5/5	--
J	16	--	--	3/5	--	--	--
K	11	--	--	2/5	--	--	--
ORIGIN							
L	-01	--	--	--	--	--	--
M*	-07	--	--	--	--	--	--
N*	-10	--	--	4/5	--	--	--
O	-21	3/5	2/5	5/5	4/5	5/5	5/5
P*	-31	5/5	5/5	5/5	5/5	5/5	5/5
Q*	-33	--	--	--	--	--	--
R*	-36	5/5	5/5	5/5	5/5	5/5	5/5
S_1*	-40	3/5	3/5	5/5	5/5	4/5	5/5
S_2*	-40	--	--	--	--	--	--
T	-44	--	--	--	--	--	--

Table 2. Frequency of peroxidase bands from dormant cambium of Quercus species -- cambium stored two months before extraction. Bands marked with an asterisk (*) are usually strong.

		Species, Seedlot Number, and State of Origin								
		Q. rubra								
Band	R_f	MQ-017 NH	MQ-018 NH	MQ-019 NH	MQ-020 NH	MQ-052 KAN	MQ-058 KAN	MQ-210 NC	MQ-212 NC	MQ-377 PA
A	53	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
B	47	--	--	--	--	--	--	--	--	--
C	44	--	--	--	--	--	--	--	--	--
D	41	--	--	--	--	--	--	--	--	--
E	38	--	--	--	--	--	--	--	--	--
F	34	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
G*	31	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
H*	27	3/5	1/5	4/5	1/5	2/5	4/5	1/5	4/5	--
I*	21	--	2/5	1/5	1/5	--	2/5	1/5	3/5	4/5
J	16	--	--	--	--	--	--	--	--	--
K	11	--	--	--	--	--	--	--	--	--
ORIGIN										
L	-01	--	--	--	--	--	--	--	--	--
M*	-07	--	--	--	--	2/5	--	--	--	--
N*	-10	1/5	--	--	--	3/5	--	--	--	--
O	-21	--	3/5	4/5	2/5	--	5/5	4/5	--	5/5
P*	-31	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Q*	-33	--	--	--	--	--	--	--	--	--
R*	-36	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
S_1*	-40	2/5	3/5	2/5	3/5	--	2/5	4/5	3/5	3/5
S_2*	-40	--	--	--	--	--	--	--	--	--
T	-44	--	--	--	--	--	--	--	--	--

Table 2. continued

Band	R_f	Species, Seedlot Number, and State of Origin									
		Q.rubra				Q.coccinea		Q.falcata		Q.imbricaria	
		416 ILL	418 ILL	426 ILL	471 ILL	108 VA	289 ALA	298 ARK	409 VA	350 OH	417 ILL
A	53	5/5	5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5	4/5
B	47	--	--	--	--	2/5	--	--	--	--	--
C	44	--	--	--	--	2/5	3/5	--	--	--	--
D	41	--	--	--	--	--	--	--	--	--	--
E	38	--	--	--	--	5/5	5/5	--	--	--	--
F	34	5/5	4/5	5/5	1/5	5/5	5/5	4/4	5/5	5/5	5/5
G*	31	5/5	5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5	5/5
H*	27	2/5	4/5	1/5	5/5	--	1/5	--	--	--	1/5
I*	21	--	1/5	1/5	--	--	--	--	--	--	--
J	16	--	--	--	--	--	--	--	--	--	--
K	11	--	--	--	--	--	--	--	--	--	--
L	-01	--	--	--	--	--	--	--	--	2/5	2/5
M*	-07	--	--	--	--	--	--	--	--	--	--
N*	-10	--	--	--	--	5/5	--	2/4	1/5	3/5	--
O	-21	4/5	5/5	4/5	5/5	5/5	5/5	3/4	4/5	2/5	3/5
P*	-31	5/5	5/5	5/5	5/5	5/5	5/5	3/4	5/5	3/5	5/5
Q*	-33	--	--	--	--	--	--	--	--	--	--
R*	-36	5/5	5/5	5/5	5/5	5/5	5/5	1/4	--	3/5	2/5
S_1*	-40	--	--	--	2/5	--	--	2/4	2/5	3/5	1/5
S_2*	-40	--	--	--	--	--	--	--	--	--	--
T	-44	--	--	--	--	--	--	--	--	--	--

Table 2. continued

Band	R_f	Species, Seedlot Number, and State of Origin									
		Q.marilandica		Q.nigra		Q.palustris		Q.phellos		Q.shumardii	
		057 KAN	299 ARK	286 MS	408 VA	349 OH	424 ILL	297 ARK	584 MD	332 FLA	420 ILL
A	53	5/5	3/3	4/4	5/5	5/5	5/5	5/5	5/5	3/3	5/5
B	47	--	--	--	4/5	--	--	--	--	--	1/5
C	44	---	--	--	--	--	--	--	--	--	2/5
D	41	--	--	--	--	--	--	--	--	--	--
E	38	--	--	4/5	4/5	--	--	--	--	--	3/5
F	34	2/5	2/3	4/4	5/5	5/5	5/5	5/5	5/5	--	4/5
G*	31	5/5	3/3	4/4	5/5	5/5	5/5	5/5	5/5	3/3	5/5
H*	27	--	--	--	--	1/5	1/5	--	1/5	1/3	3/5
I*	21	3/5	1/3	--	--	--	--	--	--	1/3	1/5
J	16	--	--	--	--	--	--	--	--	--	--
K	11	--	--	--	--	--	--	--	--	--	--
L	-01	--	--	--	--	--	--	--	--	--	--
M*	-07	1/5	--	--	--	--	--	--	--	--	--
N*	-10	3/5	--	1/4	4/5	--	--	--	--	3/3	5/5
O	-21	1/5	1/3	4/4	5/5	5/5	5/5	5/5	4/5	3/3	5/5
P*	-31	5/5	2/3	4/4	5/5	5/5	5/5	5/5	5/5	--	5/5
Q*	-33	--	--	--	--	--	--	--	--	--	--
R*	-36	--	2/3	3/4	--	--	--	--	2/5	--	1/5
S_1*	-40	3/5	1/3	--	--	--	--	--	--	2/3	2/5
S_2*	-40	--	--	--	--	--	--	--	--	--	--
T	-44	--	--	--	--	--	--	--	--	--	--

Table 2. continued

		Species, Seedlot Number and State of Origin							
		Q. velutina		Q. alba		Q. bicolor		Q. macrocarpa	
Band	R_f	MQ-372 NC	MQ-428 ILL	MQ-360 MI	MQ-533 VA	MQ-362 MI	MQ-757 ?	MQ-186 SD	MQ-402 KAN
A	53	4/4	5/5	5/5	5/5	5/5	5/5	5/5	4/4
B	47	--	--	--	--	--	--	--	--
C	44	--	--	1/5	--	1/5	--	--	--
D	41	--	--	4/5	4/5	5/5	5/5	--	--
E	38	--	--	3/5	--	4/5	--	--	--
F	34	4/4	5/5	--	--	--	--	--	--
G*	31	4/4	5/5	5/5	5/5	5/5	5/5	5/5	4/4
H*	27	--	5/5	--	--	--	--	2/5	--
I*	21	--	5/5	5/5	4/5	5/5	5/5	4/5	4/4
J	16	--	5/5	--	--	--	--	--	--
K	11	--	--	--	--	--	--	--	--
L	-01	--	--	--	--	--	--	--	--
M*	-07	2/4	1/5	--	--	--	--	--	--
N*	-10	3/4	3/5	--	--	--	--	--	--
O	-21	--	5/5	4/5	5/5	2/5	3/5	--	--
P*	-31	3/4	5/5	5/5	5/5	5/5	5/5	5/5	4/4
Q*	-33	--	--	--	--	--	--	1/5	--
R*	-36	3/4	3/5	1/5	--	--	--	--	--
S_1*	-40	--	4/5	--	--	--	--	--	--
S_2*	-40	--	--	5/5	5/5	5/5	5/5	4/5	4/4
T	-44	--	--	2/5	5/5	1/5	3/5	--	--

Table 3. Frequency of peroxidase bands from active cambium of Quercus species. Bands marked with an asterisk (*) are usually strong bands.

		Species, Seedlot Number and State of Origin							
		Q. rubra			Q.alba	Q.bicolor	Q.coccinea	Q.nigra	Q.velutina
Band	R_f	MQ-058 KAN	MQ-418 ILL	MQ-471 ILL	MQ-533 VA	MQ-362 MI	MQ-108 VA	MQ-408 VA	MQ-428 ILL
A	53	2/3	3/3	3/3	3/3	3/3	2/3	2/3	3/3
B	47	--	--	--	--	--	--	--	--
C	44	--	--	--	--	--	--	--	--
D	41	--	--	--	--	--	--	--	--
E	38	--	--	--	--	--	--	--	--
F	34	3/3	3/3	3/3	--	--	3/3	3/3	3/3
G*	31	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
H*	27	3/3	2/3	2/3	--	--	--	--	1/3
I*	21	1/3	2/3	1/3	3/3	3/3	--	--	1/3
J	16	--	--	--	--	--	--	--	--
K	11	--	--	--	--	--	--	--	--
ORIGIN									
L	-01	--	--	--	--	--	--	--	--
M*	-07	--	--	--	--	--	--	--	--
N*	-10	1/3	--	--	--	--	1/3	1/3	1/3
O	-21	2/3	3/3	3/3	2/3	--	2/3	3/3	2/3
P*	-31	3/3	3/3	3/3	2/3	1/3	3/3	3/3	3/3
Q*	-33	--	--	--	--	--	--	--	--
R*	-36	3/3	3/3	3/3	--	--	3/3	--	3/3
S_1*	-40	--	--	--	--	--	--	--	--
S_2*	-40	--	--	--	3/3	3/3	--	--	--
T	-44	--	--	--	--	--	--	--	--

LITERATURE CITED

Buchloh, G.
1960. THE LIGNIFICATION IN STOCK-SCION JUNCTIONS AND ITS RELATION TO COMPATIBILITY. In Phenolics in Plants in Health and Disease, p. 67-71. Ed. J.B. Pridham, Pergamon.

Conkle, M. Thompson
1972. ANALYZING GENETIC DIVERSITY IN CONIFERS ... ISOZYME RESOLUTION BY STARCH GEL ELECTROPHORESIS. USDA Forest Service Res. Note PSW-264, 5 p.

Garrett, Peter W., and Harry C. Kettlewood
1975. VARIATIONS IN JUVENILE OAK. USDA Forest Service Res. Note NE-204, 4 p.

Harkin, John M., and John R. Obst
1973. LIGNIFICATION IN TREES: INDICATION OF EXCLUSIVE PEROXIDASE PARTICIPATION. Science 180: 296-298.

Mayberry, J.S. and P.P. Feret
1977. PEROXIDASES IN DEVELOPING ACORNS AND SEEDLINGS OF QUERCUS ALBA L. Silva Genet. 26: 218-223.

Santamour, Frank S., Jr. and Ernst J. Schreiner
1961. JUVENILE VARIATION IN FIVE WHITE OAK SPECIES. Univ. Penna. Morris Arb. Bull. 12: 37-46.

Santamour, Frank S., Jr., Peter W. Garrett, and David B. Paterson
1980. OAK PROVENANCE RESEARCH: THE MICHAUX QUERCETUM AFTER 25 YEARS. J. Arboric. 6: 156-160.

Schramm, J.R. and Ernst J. Schreiner
1954. THE MICHAUX QUERCETUM. Univ. Penna. Morris Arb. Bull. 5: 54-57.

Schreiner, Ernst J., and Frank S. Santamour, Jr.
1961. JUVENILE VARIATION IN FIVE RED OAK SPECIES. Univ.Penna. Morris Arb. Bull. 12: 65-70.

Shaw, Charles R., and Rupi Prasad
1970. STARCH GEL ELECTROPHORESIS OF ENZYMES - A COMPILATION OF RECIPES. Biochem. Genet. 4: 297-320.