Graft Incompatibility in Red Oak: Theory and Practice

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According to a theory of graft incompatibility proposed by the senior author, successful long-term graft unions could only be developed in red oak (*Quercus rubra* L.) when stock and scion produced identical cambial isoperoxidase enzyme band patterns. Analyses of peroxidases in the seedling rootstocks and scions of 73 "failing" intraspecific grafts of red oak revealed that 82.2% (60/73) of those grafts could have been predicted as potentially incompatible on the basis of enzyme phenotypes. On the other hand, only 13 of 32 (40%) of grafts that had not shown incompatibility symptoms for 4 or 5 years had the same enzyme bands in stock and scion, while 60% (19/32) did not match. The use of seedlings from any particular tree as rootstocks for scions of that tree might not result in any greater enzyme-matching frequency. Future work to further test the theory and reproduce select clones for a seed orchard is briefly discussed.

INTRODUCTION

The paucity of oak (*Quercus*) cultivars currently available in the American nursery trade is certainly a reflection of the difficulties of vegetative propagation of select trees in this genus. This is especially true with regard to species in the subgenus *Erythrobalanus* (Spach) Oersted, the red or black oaks. Grafting would appear to be a reasonable method of multiplying mature trees with superior form, autumn leaf color, or other characteristics but there have been major problems with graft incompatibility.

Santamour (1988a) proposed an hypothesis that graft incompatibility could be related to variation in cambial isoperoxidase enzymes in stock and scion in certain genera. Peroxidases mediate the polymerization of cinnamic alcohols into lignin and the reconstitution of a working vascular system across the graft interface occurred only when the stock and scion produced the same peroxidase isozymes. This work was followed by detailed studies (Santamour, 1988b; 1988c) to "test" this hypothesis in Chinese chestnut (*Castanea mollissima*) and northern red oak (*Q. rubra*). Anagnostakis (1991) analyzed the progenies from controlled crosses in *Castanea* and concluded that the genes coding for the two major anodal peroxidases were allelic. More recently, however, Huang et al. (1994) studied various chestnut grafts and stated that "Comparisons of cambial isoperoxidase isozymes between successful and unsuccessful grafts did not support the hypothesis that peroxidase isozymes are indicators of rootstock-scion compatibility". S.L. Anagnostakis (pers. comm.,

1995) attempted to duplicate the work of Huang et al. (1994) and found that the major enzyme bands ("A" and "B") studied by her (Anagnostakis, 1991) and Santamour (1988 b) were not the same as those of Huang et al. (1994). Obviously, there are problems here that must be resolved.

Still, over the past several years, more than 500 chestnuts have been enzyme-typed in the senior author's laboratory for nurserymen and scientists, and the results have supported the peroxidase enzyme hypothesis.

Since 1984, the junior author has directed a tree improvement program for red oak in Indiana, and the background and philosophy of the project were presented by Coggeshall and Beineke (1986). Essentially, 180 superior trees were selected on the basis of growth rate, stem form, and apical dominance from native stands in Indiana and from provenance test plantations in Indiana and Ohio. These select "plus-trees" were spring bench grafted to seedling understocks and outplanted according to a prescribed design to serve as a clonal seed orchard that would be utilized to produce superior seedlings over several generations of selection. Grafting "success", the formation of a cohesive union between stock and scion, has averaged about 92% over the years. However, symptoms of graft incompatibility became apparent in many plants 4 to 5 years after grafting, and included vigorous suckering from the rootstock, swelling at the graft union, precocious flowering, and reduction of scion vigor. Death of the scions in these incompatible combinations may occur from 5 to 8 years after grafting.

Thus, we were drawn together by our mutual interests in oak propagation and the genetic improvement of trees. Furthermore, the earlier work that supported the enzyme hypothesis was based on "bark-ring" grafts (Santamour, 1988b; 1988c) that were not intended for propagation. The research program in Indiana presented a real-world propagation situation that demanded a solution if it were to fulfill its goal to improve the growth and form potential of the nearly 250,000 red oak seedlings produced annually by State nurseries in Indiana.

From 1992 through 1995, we have cooperated, as time and the availability of plant materials have permitted, in studies designed to help explain these grafting problems and to offer possible solutions for continuation of the Indiana program.

MATERIALS AND METHODS

All of the plant material was collected from the nursery areas and test plantings of the junior author in Indiana. All enzyme typing was performed at the U.S. National Arboretum and included, in the following sequence: (1) 66 select clones, (2) 73 seedling rootstock sprouts arising from below the failing graft unions of 47 of those clones, (3) 132 seedlings derived from open-pollination of six clones of known enzyme constitution, and (4) the roots of 32 grafts that had shown no symptoms of incompatibility after having been grafted for 4 or 5 years. Details of sample preparation and starch gel electrophoresis of enzymes have been published (Santamour, et al., 1986) and the various enzyme bands have been illustrated (Santamour, 1983; 1988c).

RESULTS AND DISCUSSION

This section will follow the same sequence of analyses presented in Materials and Methods. The 66 select clones were enzyme typed as follows: (A) 15, (AB) 28, (B) 12, (AC) 3, (BC) 7, and (C) 1. As in previous work, the majority of trees, 55 of 66 (83.3%), had enzyme phenotypes of A, AB, or B. Roughly 83% of the 47 grafted clones were also A, AB, or B. The 73 seedling understocks, typed from sprouts, were more variable: (A) 16, (AB) 26, (B) 12, (AC) 8, (ABC) 2, and (C) 1. All of the above data are given only to show the variation encountered in red oak. The most important finding from this study was the fact that 60/73 (82.2%) of the graft failures could have been predicted on the bases of enzyme typing, with stock and scion having different enzyme phenotypes. There are probably other biochemical, physiological, or biological reasons for the other 13 graft failures.

The enzyme phenotypes of the open-pollinated seedlings are given in Table 1. Since we did not know the inheritance patterns of the enzyme bands in red oak nor did we have any progenies from controlled crosses, the analyses of these seedlings might provide some insight on enzymes inheritance. It is obvious that the use of seedlings from any given tree as rootstocks for the grafting propagation of that tree might not result in reduced incompatibility levels compared to nursery-run seedlings. The limited variability among seedlings derived from mother trees with A, AB, or B phenotypes is indicative of the predominance of A and B pollen that would be expected from trees of similar enzyme constitution. A higher degree of variation would be expected, and was found, in progenies from trees with AC and BC phenotypes. The seedlings in those progenies that had a C phenotype probably resulted from self-pollination, but what about the ABC individual? If the expression of the band A, B, and C were codominant, and the genes coding for those enzyme bands were allelic, a maximum of two different bands should be present in any given plant of this diploid species. Santamour (1988c) reported that about 5% of the 463 trees he investigated had ABC phenotypes. McArdle and Santamour (1987) also found three isoperoxidase bands in some trees of *Koelreuteria paniculata* Laxm. in which the genes were thought to be allelic. They postulated that chromosomal crossing-over might be the cause of this phenomenon.

Table 1. Distribution of isoperoxidase phenotypes in seedlings derived from open-pollination of mother-tree clones having various phenotypes.

	Seedling phenotype							
Clone no. (phenotype)	A	AB	B	AC	BC	C	ABC	No.trees
43(A)	15	13	-	-	-	-	-	28
107(AB)	6	13	1	-	-	-	-	20
93(B)	-	13	7	-	-	-	-	20
123(AC)	4	12	-	5	2	2	-	25
116(BC)	-	3	2	8	1	1	1	16
94(C)	-	-	4!	2	3	9	5	23
								Total 132

We were certainly not prepared for the results of the analyses of the seedlings produced by clone 94, which had a C phenotype. It would appear fortunate that C-type trees are relatively rare: 1 of 463 (Santamour, 1988c) and 2 of 139 (this study). As would be expected from a C-type parent there were no progeny with A or AB phenotypes. However, there should also be no progeny with a B phenotype, but four seedlings were found with only this band (of the three major bands). All four B-type seedlings also had a strong-staining enzyme band that migrated slightly above the A band. This "above-A" band was also found in two of the nine seedlings with C phenotypes. Although further investigation of these anomalies might prove interesting, they may not be important in the general scheme for oak grafting and incompatibility.

Up to this point, we had not examined any supposedly successful grafts, those that had not exhibited any signs of incompatibility for 4 to 5 years following grafting. There were relatively few grafts available for study and the graft unions on many trees were so low that sampling the stem cambium of the stock plant would have been extremely difficult. Still, such material had to be analyzed in order to obtain a more balanced view of the situation. Fortunately, we found that the enzymes in the root cambium were exactly the same as those in the stem cambium, and it was an easy matter to obtain a few roots for analysis. We analyzed the enzymes in the rootstock roots of 32 such grafts and also double-checked the enzyme patterns in the grafted scions. Although we had expected a high proportion of enzyme "matches" in this material, with both stock and scion having identical enzyme patterns, such was not the case. Only 13 (40%) of the graft combinations exhibited matched enzyme patterns while 19 (60%) were definite mismatches.

THE FUTURE

Has the isoperoxidase enzyme theory of graft compatibility been proved in practice? No, but neither has it been disproved. The long-term survival of those grafted trees that have survived for 5 years can only be determined after many more years of observation. If the distribution of enzyme phenotypes in the general red oak population were similar to that found in the 463 trees analyzed by Santamour (1988c), the probability of achieving enzyme matches from random samples is only 30±5%. That level of grafting success is not acceptable in a program designed to establish a seed orchard for the production of superior seedlings. At the moment, the isoperoxidase enzyme theory may be worth exploring further.

As recommended by Santamour (1988c), the production of seedling rootstocks of the three major enzyme phenotypes (A, AB, B) could be accomplished by the creation of isolated seed orchards containing only parent trees having certain phenotypes. That sort of scheme is, of course, a long-term proposition. The junior author is currently planning a more direct approach to complete the requisite number of successful grafts needed in his project. Using the young seedlings that have already been enzyme typed, he will establish cutting stock blocks of plants having each of the seven enzyme phenotypes. These plants will then be "hedged" to produce juvenile shoots for cutting propagation of a range of rootstocks that should be compatible with all of the selected "plus trees".

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