# Potential Causes of Graft Incompatibility

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There is probably no universal cause of graft incompatibility in woody plants. Anatomical, physiological, and biochemical factors may all play a part in this phenomenon. Scientific explanations of incompatibility in certain stock-scion combinations may have no relevance to other combinations. While intergeneric and interspecific graft incompatibilities may be more common and suggest that major differences based on phylogenetic evolution (and subsequent taxonomic classification) may be operable, graft incompatibilities between individual plants of the same species indicate that there are also more subtle forces at work. In this paper, the author presents and discusses some experimental data bearing on graft incompatibility as well as some suggestions for future research in this field.

## INTRODUCTION

There may not be a definition of graft incompatibility that is widely accepted by scientists working on such problems. However, I rather subscribe to the thoughts expressed by Barbara Mosse (1962). She has written that "the only certain criterion of incompatibility is the characteristic interruption in cambial and vascular continuity which leads to the spectacular smooth breaks at the point of union", and further that "at the point of union no normal vascular tissue develops. The gap thus formed is filled in by proliferating ray tissue which does not lignify normally". I am interested in long-term graft compatibility that will allow a landscape tree to flourish for 50 years or more.

## ENZYMES AND LIGNIFICATION

My own research over the past decade or so has concentrated on intraspecific graft incompatibilities as they may be influenced by variation in the peroxidase isozymes in the cambial tissue of stock and scion. The theory underlying this work was first fully explained by Santamour (1988a) and this was followed by several papers (Santamour 1988b, 1988c, 1989) to "prove" the theory in Chinese chestnut (*Castanea mollissima* Blume), red oak *(Quercus rubra* L.), and red maple *(Acer rubrum* L.). The reason for selecting these three species for intensive research was that nurserymen had reported significant incompatibility problems even when selected cultivars were grafted on seedlings of the same species.

What is the function of peroxidase enzymes and how do they relate to graft compatibility? Peroxidases are the only enzymes involved in the polymerization of p-coumaryl alcohols to form lignin. Lignin is the second most common organic compound in the world (cellulose is the most common) and most botanically oriented scientists may think of lignin as only the material deposited as (or in) secondary cell walls to give them "strength". Indeed, the evolution of lignin formation allowed the development of large, exceedingly long-lived, perennial plants called trees. It must

be stressed here, however, that lignin is not a single compound and no chemical formula exists. Actually, there may be several different types of lignin in the same plant or even in the same cell (Santamour, 1988a).

Lignins are also a major component in the middle lamella between cells. It would then follow that the middle lamella in cells of trees that had different cambial isoperoxidase enzymes might produce structurally different lignins and perhaps different bonding patterns between lignins and carbohydrates. Thus, in a graft, adjacent cells of the stock and scion might function in their genetically prescribed biochemical mode and produce different kinds of lignin. Such activity could interfere with the production of matching pits and primary pit fields, perforation plates of xylem vessel members, and sieve plates of sieve-tube members in the phloem. There would then be a disruption in normal cell-to-cell connections, a breakdown in cell development, and a failure to re-establish a functional vascular system across the graft union.

In our work with the chestnut, oak, and maple species mentioned earlier, we found at least two, and sometimes three anodal peroxidase isozymes that appeared to be involved in lignification and graft incompatibility. These enzyme bands, separated by starch-gel electrophoresis and visualized by specific stains, were, for convenience, labeled "A", "B", and "C", with band "A" moving the furthest from the origin toward the positive pole. Basically, we found that trees which differed in their cambial isoperoxidase patterns could not form a lasting union and no vascular continuity was established across the graft interface. Thus, trees having only the "A" band did not successfully graft with "B" trees, and neither "A" nor "B" trees formed successful unions with trees having an "AB" constitution. Our conclusion was that long-lasting graft unions could be achieved only when both stock and scion contained identical isoperoxidase isozymes. It is of some interest that we never found any variation in anodal peroxidases in *Acer platanoides* L. or *A. saccharum* Marsh. and nurserymen have not reported any problems with intraspecific grafting in these species.

Could this theory be a universal explanation of graft incompatibility? I doubt it. In our studies of presumed graft incompatibility between *Cornus florida* L. and *C. kousa* F. Buerger ex Hance, we found that neither species produced any strong and consistent anodal isoperoxidase bands. This work was not entirely in vain however, since we were able to utilize the cathodal isoperoxidases (that moved toward the negative pole) to show that a new evergreen species from China was not a variety of *C. kousa* but a distinct and separate species (Dudley and Santamour, 1994).

## WOUND COMPARTMENTALIZATION

All forms of grafting involve wounding of both stock and scion, ranging from complete severance of both members to mere removal of some bark tissues. When woody plants are subjected to wounds that expose xylem tissue, at least one of the responses is the production of chemical compounds that are inhibitory to the growth and spread of microorganisms, thus walling off the injury and preventing wood discoloration and decay. This process has been termed "compartmentalization", and a model system, CODIT (compartmentalization of decay in trees) has been developed (Shigo and Marx, 1977). This model is not overly complicated, but it must be understood to be appreciated and a full exposition here is not possible. But it is important to know that the potential for any tree to successfully wall off or compartmentalize the cells killed by injury is under moderate to strong genetic control (Shigo et al., 1977; Santamour, 1979).

Of the four walls produced by the trees in response to wounding, the easiest to reproduce, the simplest to understand and possibly the most biologically meaningful is Wall 2. Wall 2 is formed interior to any incursion into the xylem and the amount and quite possibly the rate of production of various chemicals in the Wall 2 zone determines whether that tree is a "weak" or "strong" compartmentalizer. The formation of a strong Wall 2 prevents the inward spread of microorganism-caused discoloration toward the center of the tree. In trees that form only a weak Wall 2, such discoloration may extend even to the pith. The chemicals that form Wall 2 may be water-soluble or water-insoluble and are probably synthesized through the breakdown of carbohydrate reserves in ray parenchyma (Santamour, 1987). There are still many unknowns regarding the compartmentalization phenomenon, but some understanding may be achieved through reading some of the references cited in this paper.

What does wound compartmentalization have to do with graft compatibility? I'm not sure, but I can say that **every** cultivar I have tested, those that have traditionally been propagated by budding or grafting, has proved to be a strong compartmentalizer in that they produced a strong Wall 2 in response to a chisel driven into their trunk. These trees have included 33 cultivars of such genera as *Acer*, *Fraxinus*, *Ginkgo*, *Gleditsia*, and *Tilia*, (Santamour, 1984 a., 1986). When we tested some hybrid poplar (*Populus*) cultivars, traditionally reproduced by rooted cuttings, both strong and weak compartmentalizers were found (Santamour, 1986). Tests for the ability to compartmentalize trunk wounds can be made easily even on young trees (Santamour, 1984b). Both strong- and weak-compartmentalizing trees have been found in virtually all tree species we have studied, and it would be interesting to determine whether a weak-compartmentalizing tree could even be grafted to itself.

## GIRDLING

Girdling may be defined as the disruption of vertical continuity of phloem around the total circumference of a woody stem. In most forms of grafting, especially those involving complete severance of stock and scion, the resultant two-parted plant is girdled. The interruption of normal phloem activity may result in drastic alterations in cambial physiology (or in the formation of a new cambium) and these alterations may be dependent on the time of grafting and the distribution of vessels (ring porous vs. diffuse porous) in the species being grafted. There is little I can add to the brief discussion of this subject in Santamour (1988a), but it is apparent that investigations of the effects of girdling would require extremely detailed anatomical study.

## COUMARINS

Up to the present time, little attention has been given to the presence of coumarin compounds in the stem bark of woody plants. Yu and Carlson (1975) postulated that coumarins may play an important role in tree growth and (graft) compatibility. In their work, they were dealing only with seedlings of *Prunus avium* L. And *P. mahaleb* L., of which only the latter species contained the coumarin herniarin (7-methoxycoumarin). More recent work in our laboratory (Santamour and Riedel, 1994) found both scopoletin (6-methoxy-7-hydroxy coumarin) and its 7-glucoside (scopolin) in the bark of mature grafted trees of several *P. avium* cultivars. Strangely, no coumarins were found in *P. avium* seedlings. (Is there an effect of aging?). The distribution of scopolin and scopoletin varied both within and between

the *Prunus* taxa we studied and the inheritance patterns were highly erratic. Scopolin and scopoletin may also occur in the stem bark of other woody genera (unpublished data). While it has not yet been determined whether these coumarins actually could be related to certain graft incompatibility problems, their influence on IAA oxidase activity has been well documented (Andreae, 1952; Schaeffer el al., 1967). In fact, Imbert and Wilson (1970) considered scopoletin to be "the most potent, naturally occurring stimulator of IAA oxidase activity so far reported". The destruction and/or activity of IAA must have profound effects on the growth and development of tissues at the graft interface and the study of coumarins and graft incompatibility may prove to be a fertile field of endeavor.

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