# Graft Incompatibility Related to Cambial Peroxidase Isozymes in Chinese Chestnut[1]

**Frank S. Santamour, Jr.**[2]
*U.S. National Arboretum*
*Agricultural Research Service*
*Washington, D.C. 20002*

## Abstract

Chinese chestnut (*Castanea mollissima* Blume) seedlings were ring-grafted with bark rings from seedlings of the same species whose cambial peroxidase isozyme banding pattern was similar to or different from that of the stock. When the isozyme phenotype of the donor matched that of the stock plant, a complete encircling cambium was formed and vascular xylem continuity was restored through the zone of grafted tissue. However, when the isozyme phenotype of the donor differed from that of the stock, vascular continuity was not restored. In some cases, the cambial cells of the donor plant died after producing a few new cells. When the donor cambium continued to function as a meristem, only parenchymatous cells were produced. Peroxidases are the only enzymes known that mediate the polymerization of cinnamic alcohols into lignin and the bonding of lignin to carbohydrates of the primary cell wall. These findings substantiate the hypothesis that plants differing in major peroxidases would not be graft compatible.

**Index Words:** *Castanea mollissima*, lignification, vascular continuity

## Introduction

The research and theory upon which the present study is based have been published in two earlier papers (5, 6). In the first paper (6), it was noted that graft incompatibility was common between different individuals of Chinese chestnut (*Castanea mollissima* Blume) and that the production of major cambial isoperoxidase enzymes in this species was highly variable. Individual trees could be "typed" phenotypically by the presence of various enzyme bands as: A, B, AB, and BC. The second paper (5) presented an expanded explanation of the earlier (4) hypothesis that graft compatibility between stock and scion could be related to the similarity or dissimilarity of their cambial isoperoxidase constitution. It was hypothesized that dissimilarity could result in abnormal lignification of adjacent cells at the graft union and the failure of establishment of vascular continuity between stock and scion.

Ring "grafts" have not been widely used in propagation. Depending on the extent of bark area (with bud) such propagation techniques are known variously as patch budding, flute budding, and ring budding (2). Obviously, a patch of bark without a bud would be useless in propagation. Thus, observations and experience with bark grafts have been limited.

[1]Received for publication August 14, 1987; in revised form January 7, 1988.

[2]Research Geneticist. This work was supported in part by a grant from the Horticultural Research Institute, Inc., 1250 I Street, N.W., Suite 500, Washington, DC 20005.



The *Journal of Environmental Horticulture* (USPS Publication No. 698-330) is published quarterly in March, June, September, and December by the Horticultural Research Institute. Subscription rate is $30.00 per year in USA; $45.00 per year for others. Second-class postage paid at Washington, D.C. and at additional mailing office. Send address changes to HRI, 1250 I Street, N.W., Suite 500, Washington, D.C. 20005

Sax (1, 7, 8, 9) and his co-workers used budless bark ring grafts to study the effects of phloem blockage on growth and flowering of apple trees. Using two cultivars (presumably graft compatible) that produced different colored wood and bark to facilitate observation, they found that wood regenerated at the seam was derived from the stock plant and that wood regenerated under the patch was derived from the donor plant. Complete reconstitution of vascular continuity (xylem and phloem) along the seam and through the patch area occurred when the bark ring was grafted in a normal polar orientation. However, when the bark ring was inverted before grafting, the cambial derivatives of the donor retained their normal polarity and did not form a continuous vascular connection with the stock system. Growth was thus retarded by the interruption in downward translocation of carbohydrates from shoot to root.

Thair and Steeves (10) repeated and confirmed these studies with other apples using normal, inverted, and transversely oriented bark patches. In addition, they self-grafted bark in various orientations on plants of *Cornus stolonifera* Michx. (=*C. sericea* L.), *Sorbus aucuparia* L., and *Thuja occidentalis* L.. Although the cambial derivatives retained their original polarity in all cases, bark inversion did not reduce growth in *Cornus* or *Thuja*, and resulted in only a slight growth reduction in *Sorbus*. Vessel members were shorter than normal in all disoriented xylem of *Cornus* and *Sorbus*, and in the transversely oriented xylem perforation plates occurred on radial walls. Of course, the conifer (*Thuja*) did not produce vessels, and the xylem under the disoriented patches appeared practically normal. It is of major interest that in none of the above research were there any attempts made to physiologically verify the continuity or non-continuity of xylem or phloem across the grafted patch.

Mosse (3) used budless ring grafts in peaches to demonstrate the translocation of an "incompatibility principle" across a bark ring mutually compatible to both graft members. It was not known whether cyanide compounds or viruses were involved and her data do not pertain to the present study.

This report gives the results of grafting between young Chinese chestnut seedlings with both similar and dissimilar cambial isoperoxidase enzyme patterns.

## Materials and Methods

In the spring of 1982, 100 seedlings (30 to 45cm; 12 to 18 in) were purchased from each of 2 different nurseries and potted in 1L (trade designation-#3) plastic containers. A small portion (about 5cm;2 in) of the dormant stem was cut from the tip of each seedling. Determinations of cambial isoperoxidase profiles for each seedling were made using the same starch gel electrophoresis methods as previously described (6). All 4 isozyme band phenotypes (Fig. 1) that were reported earlier were found in the seedlings. There was no significant observed variation in the distribution of these phenotypes between the 2 seedling populations. The enzyme profiles of a few selections and cultivars were also determined.

The overall total numbers of seedlings for each enzyme phenotype was: A (5), AB (98), B (89), and BC (8). Thus, the majority of the seedlings (93.5%) were either B or AB types. These enzyme data were needed to plan our grafting experiments.

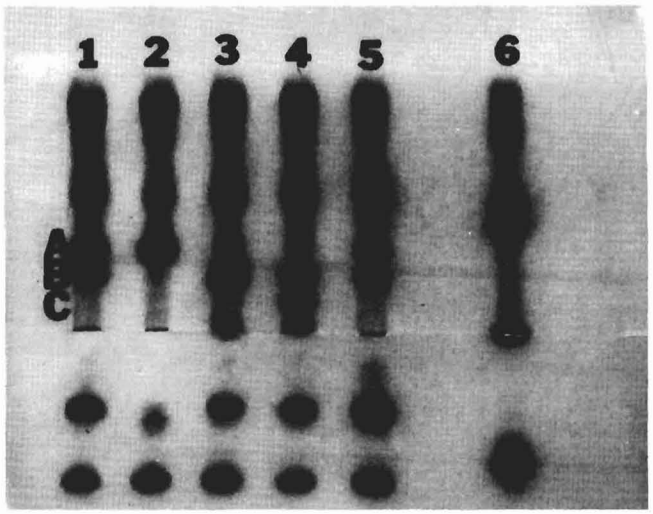



**Fig. 1.** **Cambial isoperoxidase patterns in *Castanea mollissima* (1, 2, 3, 4, 5) and *Quercus rubra* (6), showing the four isozyme phenotypes found in *C. mollissima* (AB, A, B, BC) contrasted with the AB type in *Q. rubra*.**

In late July, 1982 we made 116 bark-ring grafts in order to study the anatomy and physiology of the interface between cambia with similar and different peroxidase isozyme profiles. To make these grafts, a ring of bark, without a bud, was removed from the seedlings with a pecan budding tool, which consists essentially of 2 knives fastened together so their parallel blades are about 2.8cm (1.2 in) apart. The stem area from which the bark ring was removed on the donor seedling and that of the stock plant were of similar diameter. The ring grafts were tied with grafting rubbers, which were removed after about 3 weeks. The ring-grafting technique was selected because (1) the cambium interfaces would be oriented in a straight horizontal line and (2) the regeneration of cambium from the "seam" area of the stock plant would tend to keep the grafted plant alive even when incompatible situations were encountered.

Unfortunately, we could not use all of the seedlings in the grafting studies. Some plants died and others were too small. Fifty of the 187 seedlings with B and AB isozyme phenotypes were used in reciprocal grafts. Because of the scarcity of A and BC phenotypes, seedlings with these isozyme bands were used only as donors on stocks of B or AB types. In addition to grafts made between trees of different isozyme constitution, we also tested graft combinations between seedlings of the same type as well as self grafts, where a ring of bark was removed and immediately replaced on the same stem. Furthermore, a number of grafts of all types were made by placing the donor bark ring in an inverted orientation on the stock stem. It was thought that this bark inversion might enhance the development of any incompabilities.

About half of the successful grafts were harvested in the autumn of 1983. The graft zones were sawed through vertically and horizontally to give 4 blocks, and the wood blocks were fixed and embedded in paraffin for microtome sectioning. It was fortunate that some grafts were not harvested in 1983, because the sectioned material of 1-year-old grafts did not provide conclusive and graphic distinctions between compatible and incompatible combinations.

The remainder of the grafts were harvested during the growing season of 1986. Before harvest, however, each grafted tree was injected with a 1.5% aqueous solution of (red) safranin dye about 2 cm (0.75 in) below the lower boundary of the "seam" and "patch" areas (see below). This was done to determine the continuity of the xylem vascular system through the graft and along the seam of regenerated stock xylem. The grafted trees were cut about 2 to 3 hours after dye injection, and brought into the laboratory where the bark was peeled from the stem.

At the time of bark peeling, a section of "patch" or donor bark from grafts involving different isozyme combinations was saved for isozyme analysis. Portions of the tree stems illustrating the various donor-stock combinations were photographed.

The dry woody stems were sawed into blocks, softened in HF for 10 days, washed for 48 hours, dehydrated through 20, 40, and 60% ethanol, and stored in a mixture of equal volumes of ethanol, glycerine, and water. Sections were cut with a sliding microtome and stained with safranin—fast green.

## Results and Discussion

In retrospect it appeared that more care should have been taken to insure that the bark donor ring did not entirely encompass the stock stem so that a "seam" zone remained. Some early graft mortality probably occurred because the vascular continuity through regenerated stock tissue in the "seam" was lacking. As the stems of the living grafts increased in diameter, the "seam" tissue expanded and the donor "ring" (which did not expand in incompatible combinations) looked more like a "patch". This "seam" and "patch" terminology will be used for the remainder of the paper.

Data on graft survival are presented in Table 1, based on the numbers of plants living in the autumn of 1983. A few plants died of unknown causes between that time and the summer of 1986. It can be seen, even without analyses, that the percentage of living graft combinations was significantly higher in grafts where both stock and donor had the same cambial isoperoxidase bands. Inversion of the donor bark ring apparently had little effect. Graft failures observed during this stage were likely caused by the inability of the stock to regenerate "bridging" tissue from ray parenchyma. No attempt was made to determine whether such failures could be ascribed to "incompatibility" or technique problems, since it was expected that most stock plants would develop a continuous vascular connection along the "seam". However, if the rate of technique—caused failures were the same in all types of grafts, there could still be a significant portion of the failures caused by "incompability".

Dye movement from injections made in the stocks below the "seam" areas of the grafts showed complete continuity of the xylem vascular system. Isoperoxidase determinations on the "seam" cambial zone indicated that the "seam" tissue had been regenerated from the stock.

The gross morphology of the graft unions, showing the path of dye movement from injections into the stocks below the "patch" areas of the grafts are illustrated in Figs. 2, 3, and 4. Cross-sections through the patch mid-point of certain grafts are shown in Figs. 5 and 6. In all the cases where stock and donor had the same isoenzymes, and the ring had been placed in normal orientation, the dye movement indicated a complete restoration of xylem vascular continuity across the "patch" (Figs. 2-1, 2-3, 5-1, 5-2). In those grafts that had rings of similar isoperoxidase constitution placed in an inverted position on the stocks, the xylem did not reorient, but the more rapid growth of tissue from the "seam" tended to overgrow (undergrow) the "patch" (Figs. 2-2, 5-3). (Although the bark of the patch donor tended to remain in place, and was not "overgrown", the tissues derived from patch meristematic cells were "overgrown" by seam tissues "undergrowing" the patch bark).

In all grafts in which stock and donor had different isoenzyme constitutions, there was *no* restoration of xylem vascular continuity across the patch. Although the bark in the patch area appeared intact in the living plants, bark peeling often revealed necrotic areas under the patch ranging from nearly complete (Figs. 3-3, 3-2, 6-1) to various small zones at the upper graft line (in decreasing order; Figs. 3-1, 4-1, 4-3, 4-5, 5-1). It must be remembered that it is at this upper graft boundary that the effects of phloem discontinuity were most profound. The growth and spread of normal tissue from the "seam" area therefore was more extensive in the lower portion of the patch. In those instances where the patch zone tended to remain more or less intact (Figs. 3-1, 6-2, 3-4, 4-2, 4-4), there was often little necrosis, but still there was no xylem vascular development across the patch. Dye movement occurred only through stock tissues and tissues regenerated from the stock "seam".

The isoperoxidase pattern of meristematic cells (not necessarily cambium, in the true sense) under the patch was that of the donor plant. In cases where the patch tissue had been largely overgrown by seam tissue (Figs. 4-1, 4-3, 4-5), it was difficult to find any patch meristematic cells for analysis.

Reversal of the normal orientation of rings of dissimilar isoenzyme constitution (Figs. 3-4, 4-4, 6-3) tended to retard the envelopment of the stock by seam-derived tissue (compare with Figs. 2-2, 5-3). Apparently, the lack of reorientation of cambial initials was coupled with differences in isoperoxidase constitution in producing this result. Cultivars of Chinese chestnut were typed as follows: 'Orrin' (AB),

**Table 1. Survival of bark ring-grafts of Chinese chestnut having similar or different cambial peroxidase (1982–1983).**

| | Normal Position | | | Inverted Position | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. made | No. Living | % Living | No. made | No. Living | % Living | No. made | No. Living | % Living |
| Self | 15 | 13 | 86.7 | 14 | 10 | 71.4 | 29 | 23 | 79.3 |
| Same | 8 | 6 | 75.0 | 8 | 6 | 75.0 | 16 | 12 | 75.0 |
| Different | 44 | 21 | 47.7 | 27 | 14 | 51.8 | 71 | 35 | 49.3 |
| Total | 67 | 40 | 59.7 | 49 | 30 | 61.2 | 116 | 70 | 60.3 |

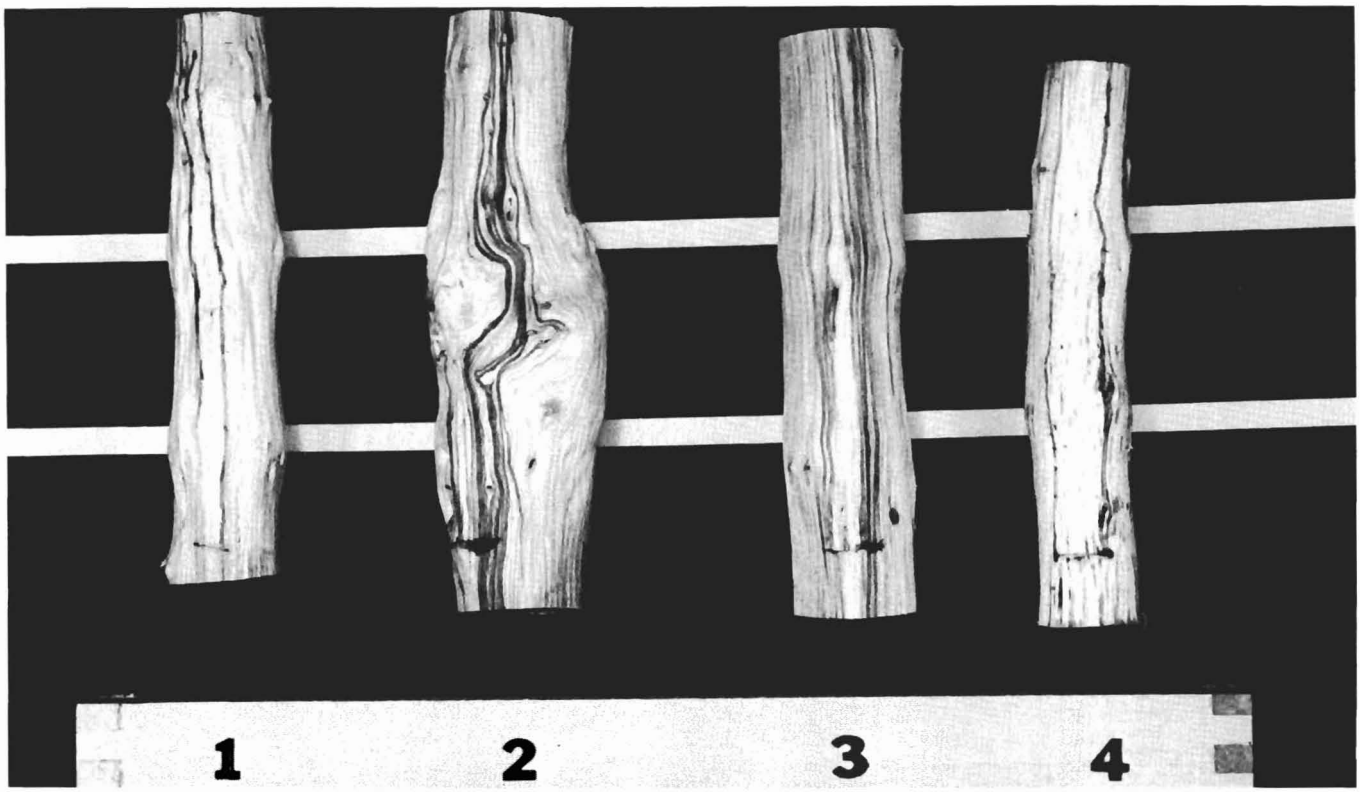



**Fig. 2.** Dye movement, shown by dark vertical lines, in xylem from below "patch" area of bark-ring grafts in Chinese chestnuts of similar cambial isoperoxidase constitution. (1) AB ring on AB stock, (2) AB (inverted) on AB, (3) B on B, (4) B self. Dark horizontal lines behind stem sections indicate approximate position of graft. Horizontal wound near base of stem section is site of dye injection.

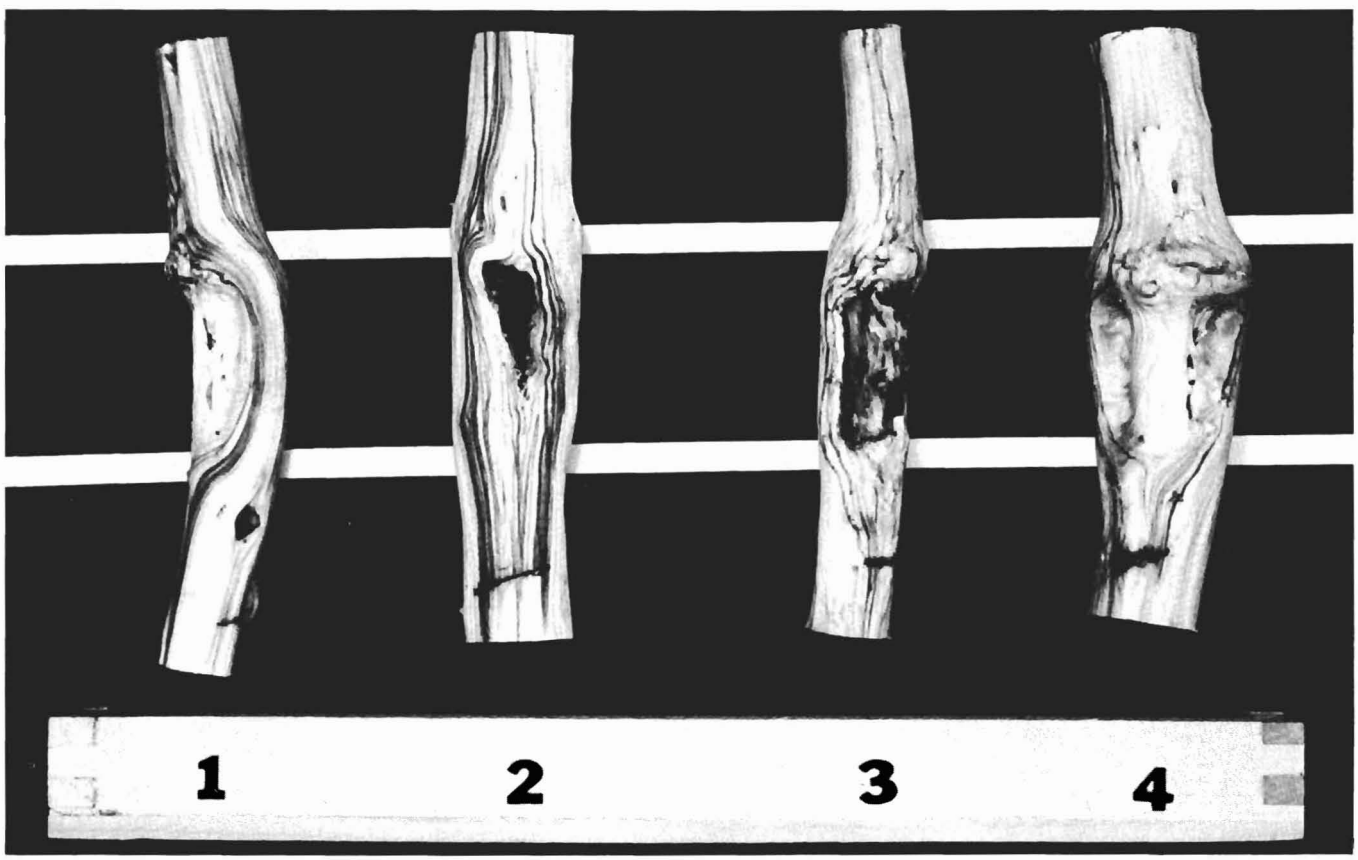



**Fig. 3.** Dye movement, shown by dark vertical lines, in xylem from below "patch" area of bark-ring grafts in Chinese chestnuts of differing cambial isoperoxidase constitution. (1) (2) (3) show AB ring on B stock, (4) is AB (inverted) on B. Dark horizontal lines behind stem sections indicate approximate position of graft. Horizontal wound near base of stem section is site of dye injection.

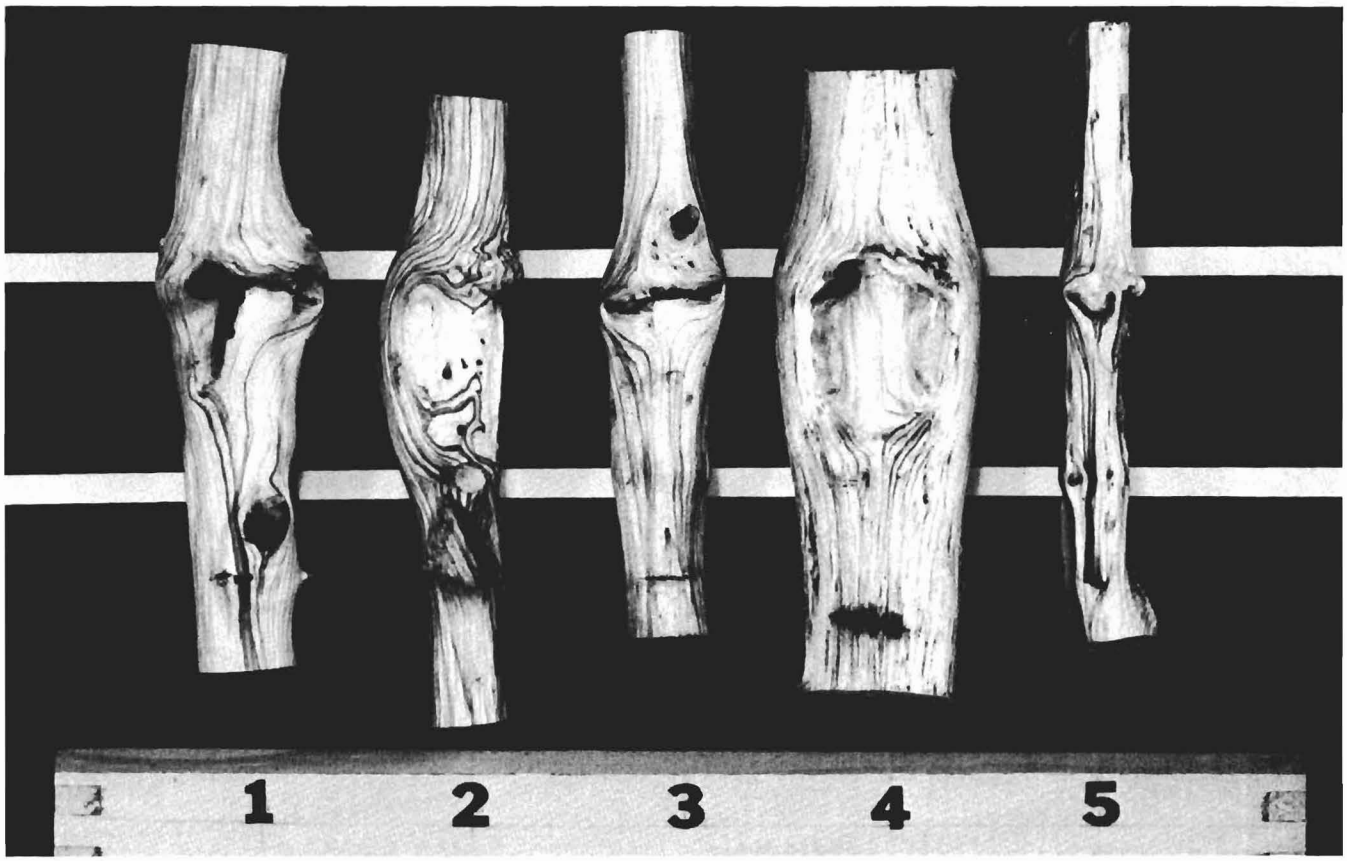


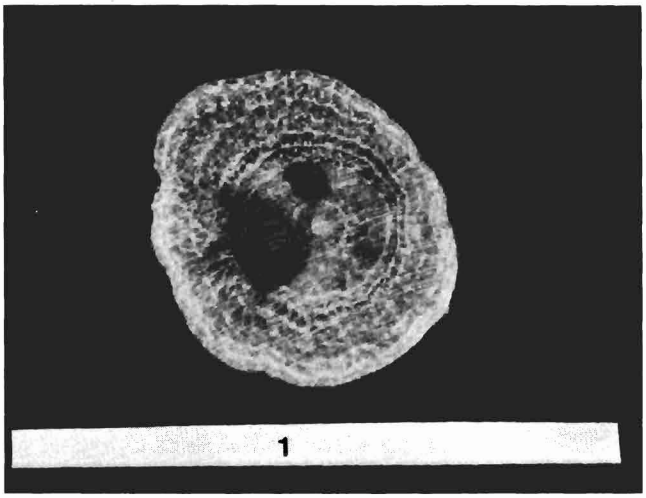

**Fig. 4.** **Dye movement, shown by dark vertical lines, in xylem from below "patch" area of bark-ring grafts in Chinese chestnuts of differing cambial isoperoxidase constitution. (1) (2) (3) show B ring on AB stock, (4) is B (Inverted) on AB, (5) is BC on AB. Dark horizontal lines behind stem sections indicate approximate position of graft. Horizontal wound near base of stem section is site of dye injection.**

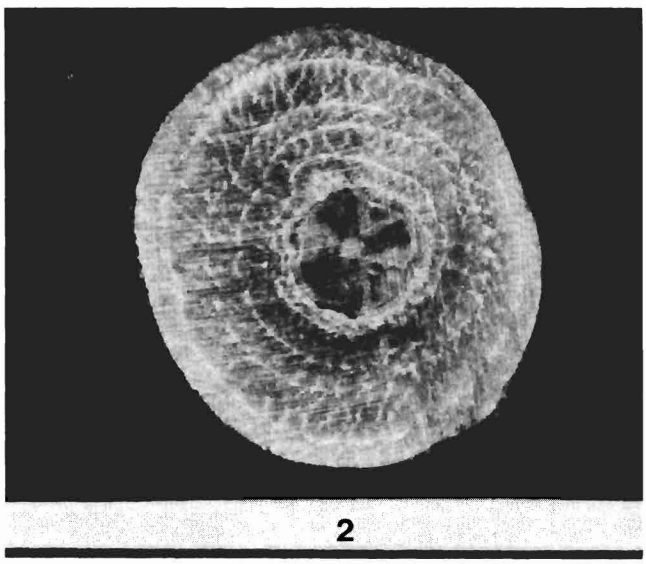
'Nanking' (AB), 'Redwing' (AB), and 'Crane' (BC). Hybrids involving Chinese chestnut were 'Eaton' (B), and 'Sleeping Giant' (B). The cultivar 'Clapper', a supposed hybrid of *C. crenata* Sieb, & Zucc. and *C. dentata* Borkh., had only isozyme band A. Considering the potential variability in seedling understocks it may not be surprising that graft incombability is a problem in Chinese chestnut.

## Significance to the Nursery Industry

Commercial success in grafting propagation of selected clones and cultivars of Chinese chestnut, and probably other species and hybrids of *Castanea*, depends on matching the cambial isoperoxidase patterns in stock and scion. There are several ways in which greater grafting success can be achieved.

The "best" method would be to "type" all of the seedling understocks and only utilize certain stocks for certain cultivars. It is not likely that many nurseries would be able to do this on their own or even consider the expense of contracting for such analyses, because it would be an annual chore. A better alternative would be the development of "seed orchards" to produce seedlings with the enzyme patterns required for the propagation of certain cultivars. It must be emphasized here that we do not know the specifics of isoperoxidase inheritance in Chinese chestnut. However, past experiences would indicate that the enzyme bands are inherited as co-dominants and a cross of an "A" individual with a "B" individual would produce a progeny in which *every* seedling would be AB. (Selfing is not a problem in *Castanea*.) These seedlings could then be used as rootstocks for any cultivar with an AB enzyme phenotype. A similar scheme could be used for mass production of "A" seedlings and "B" seedlings.



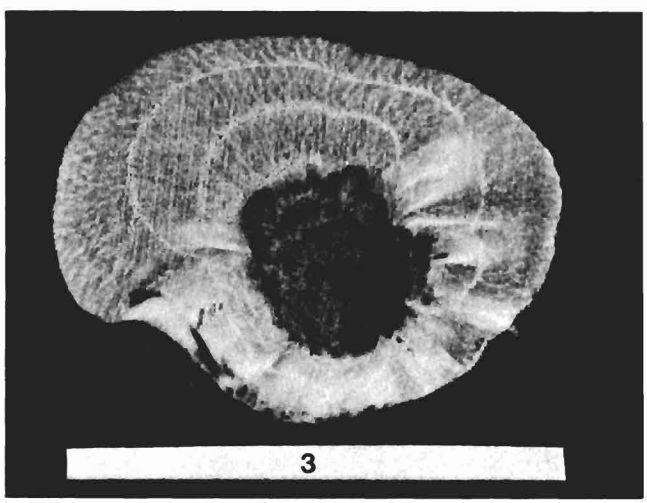
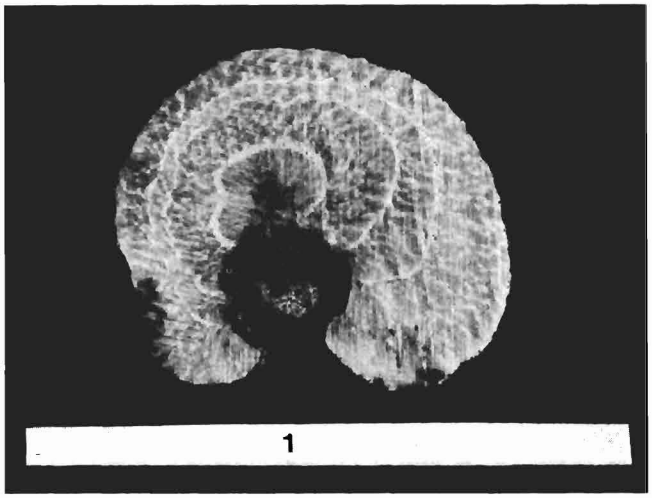
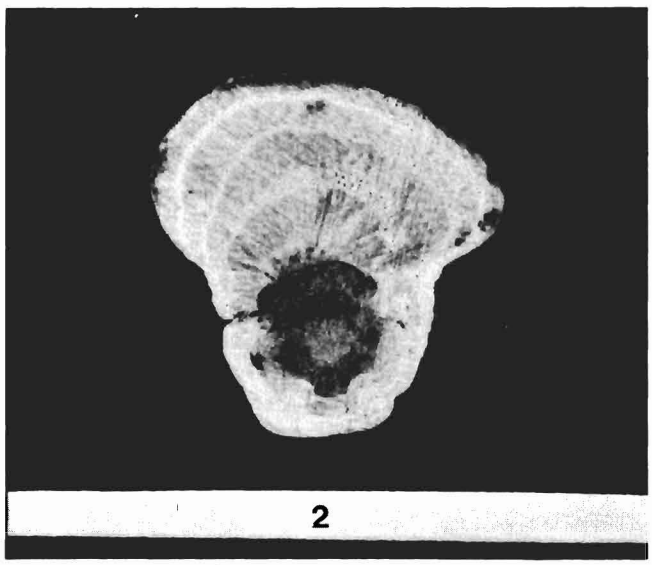




**Fig. 5.** Sawn and sanded cross-sections through mid-point of bark graft of stem sections shown in Fig. 2. (1) AB on AB (Fig. 2-1); (2) B on B (Fig. 2-4); (3) AB inverted on AB (Fig. 2-2). "Seam" area at top of picture. White bar is 2.6cm (1 in) long.



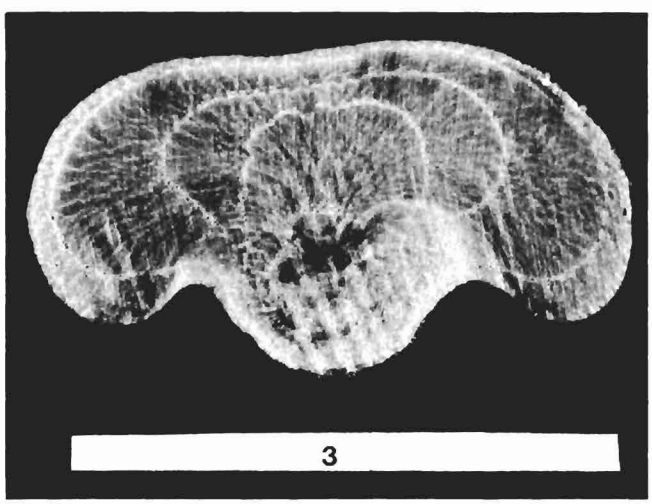




**Fig. 6.** Sawn and sanded cross-sections through mid-point of bark graft of stem sections shown in Figs. 3 and 4: (1) AB on B (Fig. 3-2); AB on B (Fig. 3-1); B inverted on AB (Fig. 4-4). "Seam" area at top of picture. White bar is 2.6cm (1 in) long.

## Literature Cited

1. Dickson, A.G. and E.W. Samuels. 1956. The mechanism of controlled growth of dwarf apple trees. J. Arnold Arb. 37:307–313.

2. Garner, R.J. 1979. The grafter's handbook. Ed. 4, Oxford Univ. Press, 319 p.

3. Mosse, B. 1960. Further observations on the effects of ring-grafting peaches with an incompatible rootstock variety. J. Hort Sci. 35:275–281.

4. Santamour, F.S., Jr. 1980. How to build a better tree. New Horizons from the Horticultural Research Institute—1980, p. 1–3.

5. Santamour, F.S., Jr. 1988. Graft compatibility in woody plants: An expanded perspective. J. Environ. Hort. 6:27–32.

6. Santamour, F.S., Jr., A.J. McArdle, and R.A. Jaynes. 1986. Cambial isoperoxidase patterns in *Castanea*. J. Environ. Hort. 4:14–16.

7. Sax, K. 1954. The control of tree growth by phloem blocks. J. Arnold Arb. 35:251–257.

8. Sax, K. 1957. Dwarf ornamental and fruit trees. Proc. Intern. Plant Prop. Soc. 7:146–153.

9. Sax, K. and A.Q. Dickson. 1956. Phloem polarity in bark regeneration. J. Arnold Arb. 37:173–179.

10. Thair, B.W. and T.A. Steeves. 1976. Response of the vascular cambium to reorientation in patch grafts. Can. J. Bot. 54:361–373.


# Chemical and Biological Stability of Indole-3-Butyric Acid (IBA) After Long-term Storage at Selected Temperatures and Light Regimes[1]


**James A. Robbins,[2] Mark J. Campidonica, and David W. Burger[3]**

*Department of Environmental Horticulture, University of California, Davis, CA 95616*



## Abstract

Concentrated [4.9 mM (1,000 ppm) and 24.6 mM (5,000 ppm)] IBA solutions in 50% isopropyl alcohol were stored in amber and clear glass bottles at 3 temperatures [22–25°, 6°, 0°C (72–77°, 43°, 32°F)]. No significant change in biological activity of the solutions or breakdown of IBA was observed for solutions stored for 4 and 6 months. Solution color changed during storage. Color development was dependant on storage temperature, but not on exposure to light. *Chemical names used:* IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; NAA = 1-naphthaleneacetic acid



**Index words:** propagation, growth regulators, rooting, auxins, cuttings


## Introduction

Auxins are the class of plant growth substances most often associated with the promotion of adventitious root formation. Zimmerman and Wilcoxon (11) demonstrated that two synthetic auxins, IBA and α-NAA, induce greater rooting than the naturally occurring auxin, IAA. Auxins, when used as rooting compounds are often applied as concentrated solutions (2, 7, 8, 10). Although the use of synthetic auxins (IBA, NAA) is of central importance in plant propagation (1), little is known about the shelf-life of concentrated solutions.

Hartmann and Kester (3) indicate that an uncontaminated solution of NAA maintains its activity for as long as a year and is entirely light-stable although no data are presented to support this claim. For IBA, they state that a 20hr exposure to strong sunlight causes only a slight change in concentration. Pinney (8) suggests that a concentrated solution of IBA (in 95% ethyl alcohol) can be stored for up to 3 months in the dark at 4°C (39°F) with neglible change in concentration. Again, no data are presented to substantiate this claim. To date, no quantitative information has been found in the literature on the shelf-life of IBA solutions.


[1]Received for publication June 8, 1987; in revised form January 8, 1988.


[2]Present address of senior author: Department of Horticulture, Kansas State University, Manhattan, KS 66506.

[3]Former post-doctoral student, graduate student, and Assistant Professor, resp.

The purpose of this study was to provide practical information about the effect of storage conditions on the long-term shelf-life of concentrated IBA solutions. The concentration, solvent, and storage environment were chosen to represent conditions that a plant propagator would encounter.

## Materials and Methods

IBA was purchased from two suppliers, United States Biochemical (USB) (Cleveland, OH 44122) and Sigma (St. Lous, MO 63178). A bulk quantity of IBA was prepared for each concentration [24.6 mM (5,000 ppm), 4.9 mM (1,000 ppm), 0 mM] and chemical supplier. Fifty percent isopropyl alcohol, as opposed to ethyl alcohol, was used as the solvent since isopropyl alcohol is more readily available to the public. The two alcohols yield equivalent rooting results (9). The color of freshly prepared solutions depended on the chemical supplier. A 24.6 mM (5,000 ppm) solution of IBA prepared using the USB product was a light-yellow color. The intensity of this color is proportional to concentration. The solution [24.6 mM (5,000 ppm)] prepared from the Sigma product was clear.

The bulk supply for each solution was dispensed (60 ml/bottle) into clear or amber glass bottles. Caps for the bottles were vinyl-lined. Two bottles were prepared for each treatment (temperature, bottle color, concentration, and chemical supplier). During the storage period, bottles were located in one of three locations (laboratory shelf, refrigerator, freezer). One series of bottles was stored on an open shelf