

## Graft Union Development of the 'Golden Delicious' Apple when Combined with Varied Dwarfing Rootstocks

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### Abstract

Morphological and anatomical characteristics of the graft unions were significant during formative stages of development in that abnormal vascular tissues occurred on the scion side of the union. Dwarfing rootstocks had larger rays than the less dwarfing ones, and the vessels tended to be smaller. Bark thickness increased as the degree of dwarfing increased. Regenerative growth of callus within the graft union was often necrotic or accompanied by growth that was horizontal to the main axis of the plant. Persistent swirling of vascular tissues in the immediate graft union area was associated with the reduction of scion growth.

Current orchard management practices have increased the use of apple dwarfing rootstocks. The graft union compatibility affects not only the vascular system, but determines the ultimate tree performance for the production of high-quality fruit, (2, 4). Morphological and anatomical characteristics of apple rootstocks having varied degrees of dwarfing have previously been reported (13, 14).

Grafting successfully produces compound genetic systems uniting stock, scion and in some cases, stem pieces which are utilized as in interstem trees (15). The formation of a normal graft union is the interaction by both components to form meristematic tissue (1). Callus is produced exclusively from tissues lying outside the xylem cylinder. Those tissues outside the periderm may proliferate with little contribution from the cambium (11).

The anatomical structure of dwarfing rootstocks is different from that of those exhibiting growth of a standard

size tree. Rootstocks producing dwarfing effects have the following characteristics: a higher bark-wood ratio, thicker bark (3, 4, 5), less fibers (3, 6, 7); smaller (and lower proportion of) vessels (2, 6, 7, 11) than that of less dwarfing rootstocks. The shape of the ray in a transverse section of a dwarfing rootstock is nearly square, and is rectangular in shape in less dwarfing rootstocks (3). Stomatal density is lower in dwarfing than in non-dwarfing rootstocks (4). It has been found that smaller vessel walls produced greater flowering and earlier precocity (10).

This study was conducted concerning the morphological characteristics of developing apple graft union of selected dwarfing rootstocks.

### Materials and Methods

The five stock/scion combinations of one-year-old chip-budded nursery trees utilized in the study consisted of 'Golden Delicious'/apple seedling, MM.106, M.26, M.7A and M.9. 'Golden Delicious'/apple seedling was used as a basis for comparison of growth and anatomical development of the graft union.

The trees were planted in a well-drained sandy loam soil in a greenhouse experiment. No fertilizer was applied; however, water was added when the surface became dry. Flower buds were removed to maintain vegetative growth. For statistical analysis, a randomized block design was utilized

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containing eight blocks. Each block had one tree of each stock/scion combination. Longitudinal samples were obtained from each stock/scion combination after one season's growth (Fig. 1-6). A freezing microtome (International Model CTD, Microtome Cryostat) was utilized in sectioning with procedures as previously described (12). Cutting temperatures for this study were between  $-5$  to  $-8^{\circ}\text{C}$  and sectioning was at  $20\mu\text{m}$ . The fresh material was placed in water 10-15 hours before sectioning for softening purposes. Ethanol preserved materials were immersed in running water to remove the alcohol from the specimen. The sections were stained with safranin-0 and fast green as described by Johansen (9).

Twelve representative samples were collected from sequential longitudinal sections of each sub-specimen and then were made into permanent slides. These were observed and photographed with a Zeiss photomicroscope. The anatomical terminology of a normal vascular system was according to that of Esau (8).

### Results and Discussion

*Golden Delicious/apple seedling* (Fig. 1) The orientation of vascular tissues at the graft union was in a normal longitudinal direction from the axis of the stem in a two-year-old tree (Fig. 1-B). However, callus development (CA) was apparent, indicating the inherent vigor of the seedling rootstock (Fig. 1-A). Small areas within the develop-

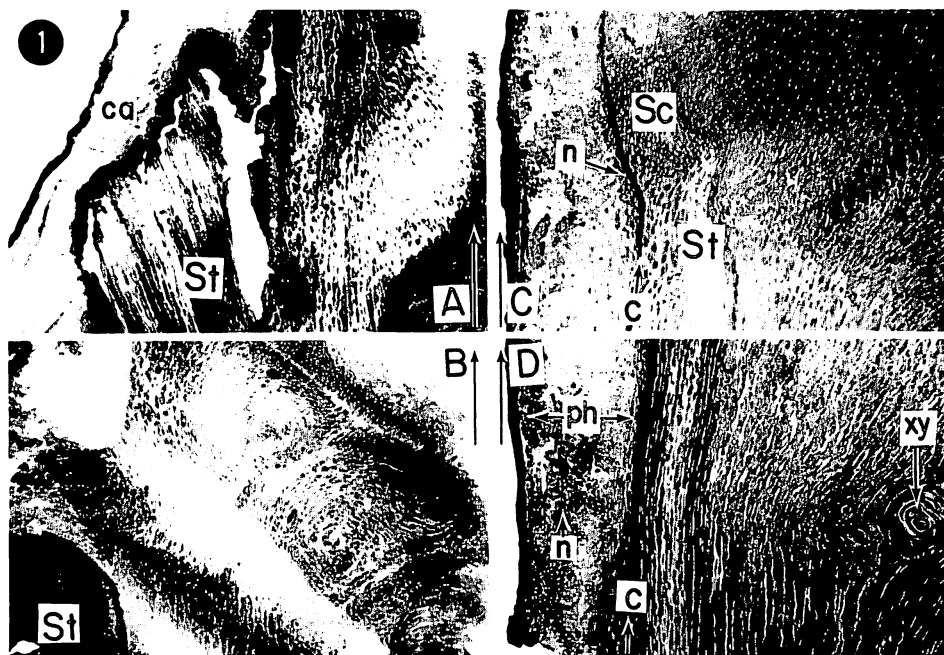
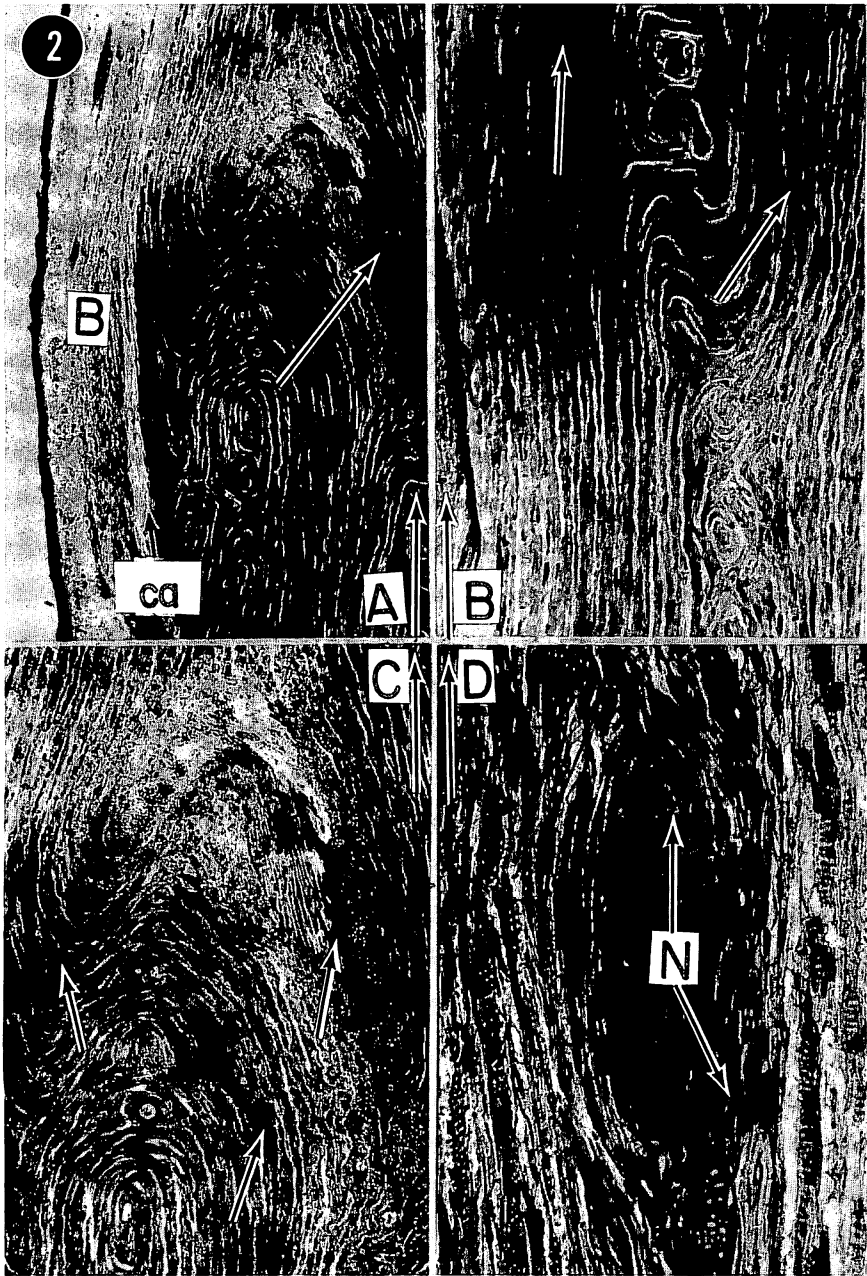


Figure 1. Longitudinal section between 'Golden Delicious' and seedling rootstock after one year's growth. A. Callus (CA) overgrowth where the rootstock was removed; B. Swirling xylem, cambium and phloem at the apex of the seedling rootstock; C. Orientation of phloem and xylem, with a small amount of necrotic (arrow) (N) tissue in the union area; D. Necrotic non-functioning phloem and cortex with a small area of swirling xylem (XY) at the union; arrows on Fig. margin indicate longitudinal direction. All X28. St = Stock; Sc = Scion; C = Cambium.



**Figure 2.** Longitudinal section of a 'Golden Delicious'/MM.106 graft union showing necrotic callus and swirling vascular tissue. A. Small, separated necrotic callus (arrow) and swirling vascular tissues contiguous to the bark (B) with a well-defined cambium (CA); B. Partially necrotic vascular tissue (arrows) and swirling xylem; C. Necrotic callus (arrows) interspersed with the union area; D. Swirling and necrotic xylem (N) (arrows). Arrows on margin indicate direction of longitudinal section. A, X28; B, C, X45; D, X178.

ing regenerative tissue between the stock and scion were necrotic as a result of the graft union growth (Fig. 1-A, B). Swirling of the xylem and phloem appeared between the stock/scion tissues (Fig. 1-D). However, where the union had been established and callused over, swirling of the vascular tissues subsided. Phloem (non-conducting) near the graft wound was necrotic (N, arrows, Fig. 1-D) but did not extend into adjacent areas. Continuity between the stock and scion was evident (arrows, Fig. 1-C). Early growth malformations occurring within the graft union with seedling rootstock did not prevent subsequent vigorous growth.

'Golden Delicious'/MM.106 Regenerative tissues between the stock and

scion (Fig. 2) showed normal unification of the newly formed xylem and phloem, with the development of axial orientation between the two components.

Necrosis (N, Fig. 2-D) in the cortex (outer bark) during nursery growth might be related to mechanical damage rather than to incompatibility. This was not apparent in its subsequent growth.

A pattern horizontal to the main stem axis developed in the vascular system, accompanied by swirling in small areas, which with growth, finally assumed a normal longitudinal orientation. This development from the first growing season produced an abundance of callus tissue (CT), with proliferation between the stock and scion

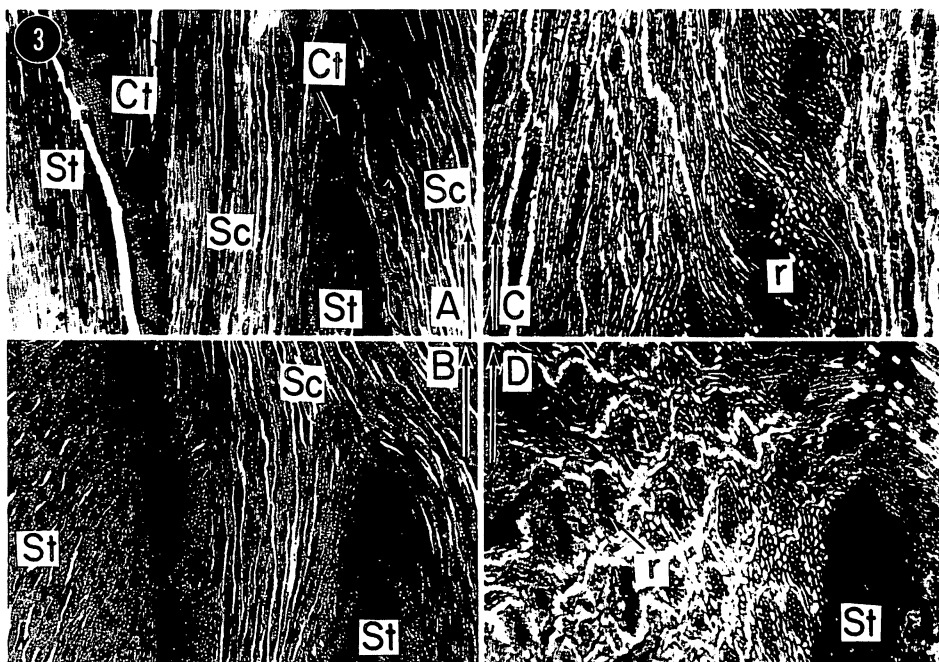


Figure 3. Longitudinal section of a 'Golden Delicious'/MM.106 graft union. A. Callus tissue (CT) partially filled the space between xylem of the rootstock (ST) and the scion (SC) without any vascular connection at this point; B. Callus (partially differentiated into vascular tissues) was the only vascular connection between the stock and scion; C. A strong union, characterized by vascular tissue development with immature xylem rays (r); D. Regenerative development of vascular tissue and rays (r). Arrows on margin indicate direction of longitudinal section. A, B, X71; C, D, X178.

area (Fig. 2-A, D). MM.106 rootstock produced more necrosis and swirling of the vascular elements than seedling rootstock.

The graft union of 'Golden Delicious'/MM.106 showed regenerative tissue in the subsequent longitudinal development between the stock and scion during the second year's growth (Fig. 3-A, D), including longitudinal orientation with the main axis of the trunk (Fig. 3-A).

There was good cambium development between the vascular elements when the stock and scion were united. However, in Fig. 3-D, the area of functioning phloem was restricted at the point of union. This was contiguous to developing xylem vessels where some necrosis was apparent and

was at the area where developing cells of the vascular system were forming. These appeared to be adjusting into a normal longitudinal pattern of growth.

*'Golden Delicious'/M.26* Thickness of the functioning and non-functioning phloem was similar in both stock and scion but a necrotic line developed along the outer functioning phloem of the M.26 bark. Xylem elements were oriented horizontally to the main axis of the stem within the graft union (Fig. 4-A, D). Xylem oriented horizontally to the stem axis occurred in a swirling pattern with some necrotic callus tissue. Breakage occurred in this area in some of the unions (Fig. 4-A, D). Xylem rays and parenchyma tissue in the scion were necrotic at the graft

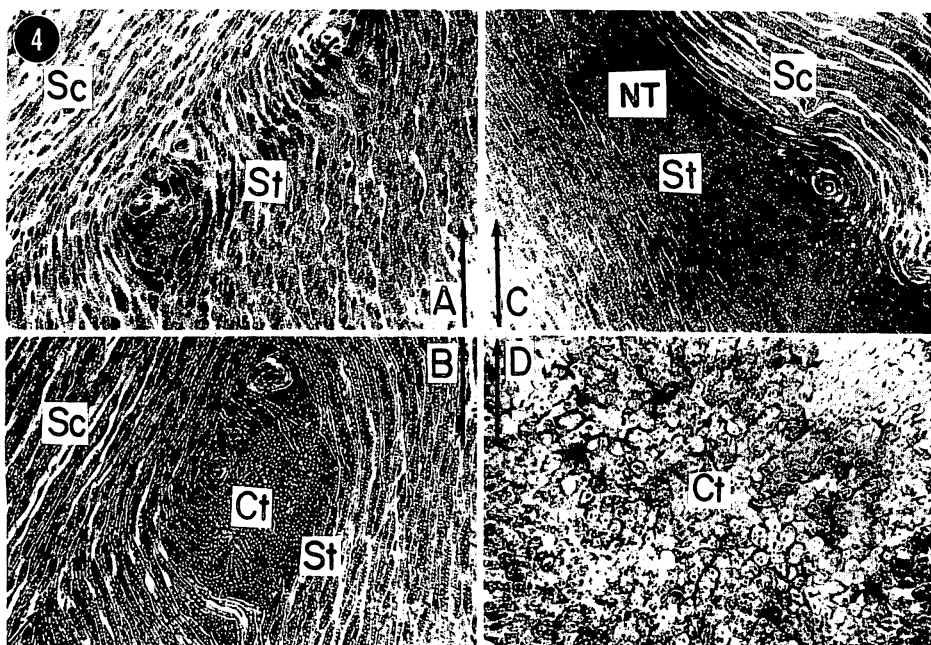
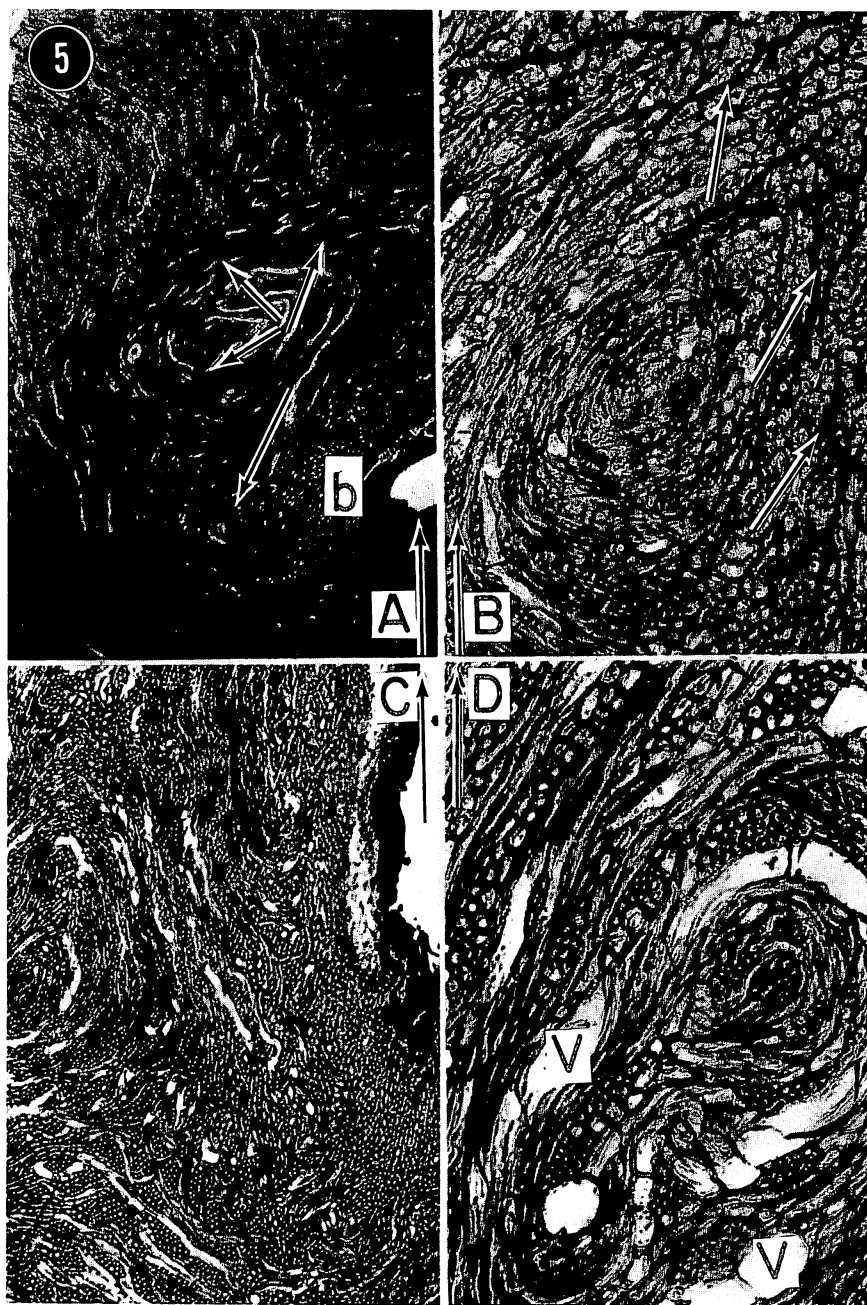


Figure 4. Longitudinal section of a transition zone within the graft union of 'Golden Delicious'/M.26. A. Scion had smaller and fewer rays but larger and more vessel volume when compared with the rootstock (ST) xylem; tissue at the union line was slightly necrotic with occasional swirling of xylem or isolated callus tissues; B. Callus tissue (CT) interspersed along the union line; C. In the union, there was a mass of necrotic tissue (NT) accompanied by swirling xylem; D. Callus tissue (CT) at the union containing starch granules. Arrows on margin indicate direction of longitudinal section. A, C, X71; B, X113; D, X283.



**Figure 5.** Longitudinal section of 'Golden Delicious'/M.9 near the upper edge of the graft wound showing swirling and necrotic tissue. A. Necrotic and swirling xylem (arrows); B. Necrotic tissue in swirling xylem (arrows); C. Outer portion of the union; swirling xylem persisted, but not in the necrotic tissues; D. Non-necrotic, swirling xylem. Arrows on margin indicate direction of longitudinal section. b, bark; V, vessel; A, X71; B, X283; C, X113; D, X453.

union (arrows, Fig. 4-A, D). Small callus areas (CT) were present along the union line (Fig. 4-D).

The graft union of 'Golden Delicious'/M.26 of a two-year-old tree is shown in Fig. 4-A, D with swirling phloem at the distal end. Also, swirling xylem extended to the scion with small to large areas, varying with different necrosis within the union. This pattern of growth continued horizontally within the stem axis (Fig. 4-C). Cambium orientation was in a normal vertical direction, except in areas where swirling xylem and phloem were noted. The xylem (occasionally necrotic) and callus islands (CT) were found at the union line after growth in the nursery and throughout the subsequent growing season (Fig. 4-B).

'Golden Delicious'/M.9 Functioning and non-functioning phloem of 'Golden Delicious' was significantly thinner than that of M.9 (Fig. 5-A, C), indicating that the thickness of non-functioning phloem (Fig. 5-A, C), could be a contributing factor for dwarfing in M.9 trees.

Swirling growth of the vascular system in the union area of 1- and 2-year-old trees was accompanied by necrotic xylem parenchyma and ray cells (5-B, D). These unions were weaker and abnormal radial horizontal ray development was observed.

'Golden Delicious'/M.7A Phloem, cambium and xylem developed in a direction vertical to the stem axis (Fig. 6-A, C), in contrast to the horizontal direction at the distal and proximal ends of

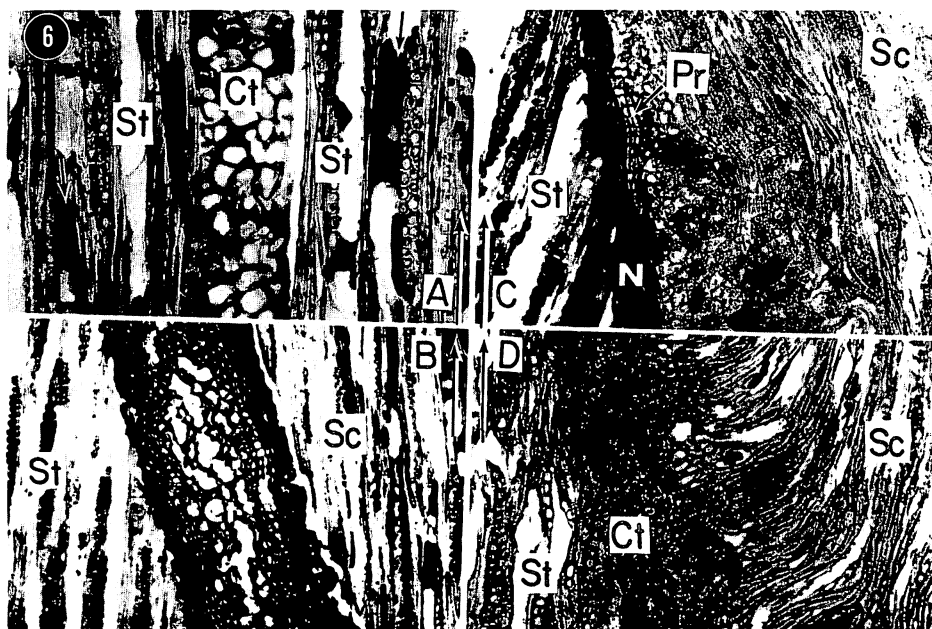


Figure 6. Longitudinal section of 'Golden Delicious'/M.7-A with callus differentiation. A, B. Space between the mature xylem of the rootstock and scion engulfed with callus (CT); B. Crushed necrotic callus (CT); C. Callus tissue that differentiated into vascular tissue was united with meristematic tissue of the scion (SC). Callus united with mature or non-meristematic tissue of the rootstock (ST) was sealed off by a wound periderm (PR); D. Both sides of the callus had undergone differentiation and united with the vascular tissue of the rootstock (ST) and scion (SC). Arrows in margin indicate direction of longitudinal section. A, X453; B, X226; C, D, X283.

the graft union. Callus formed between the rootstock and scion as shown in Fig. 6-A, B, D. Mature xylem between the stock and scion became necrotic (arrow, Fig. 6-A), Callus (CT) was separated from the xylem by a wound periderm that became necrotic (Pr and N, Fig. 6-C). Contiguous to the graft wound, a band of callus was present between the rootstock and scion developing a vascular connection on both sides of the union.

The more vigorous rootstocks had longitudinal orientation of vascular tissues with normal cambial development occurring between the stock and scion. Variations in the rootstocks exhibiting more dwarfing characteristics had swirling phloem, abnormal orientation of the xylem rays and different degrees of necrosis within the graft union. This could be a precursor to the graft union breakage as the trees attain commercial production.

This research extends the basic information for interpretation concerning graft union growth upon trees during the formative years. An understanding of incompatibilities between the rootstock and scion is necessary for adjustments of production practices in order to maintain tree vigor and for optimum production of high-quality fruit.

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