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GRAFT UNION FORMATION IN DOUGLAS-FIR[1]

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ABSTRACT

Greenhouse-grown Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) graft unions were examined between 2 and 84 days after grafting. Room temperature was maintained at 60–70 F throughout the growing season. In most respects grafts of Douglas-fir followed development patterns previously reported for spruce and pine grafts, but specific differences were noted in contributing cell types, time of formation, and mode of healing. The time interval from first occurrence to occurrence in 80% of the grafts is as follows: contact layers, 2 days; callus bridges, 10–14 days; periderm, 10–17 days; cambia, 17–23 days. Callus bridges were generally of secondary phloem or cortex origin. Callus lignification began along cut edges of the union at 14 days and was completed across the entire length of the union by 17 days. Lignified tracheids were continuous across union zones at 35 days. When proper grafting techniques were used, all tissue systems necessary for a successful union were present 35 days after grafting. Poor grafting techniques at times retarded cambium formation for 3 months or more.

GRAFTING of food-producing plants has been done by man for thousands of years, but grafting of forest tree species is relatively new. Widespread interest in forestry grafting did not occur until the 1930's, and in the United States grafting of Douglas-fir did not begin on a large scale until the mid-1950's. Since that time, loss of many grafts with incompatibility symptoms has promoted great interest in the union healing process.

No paper has been published which describes normal union healing in Douglas-fir grafts, and few papers have been written which describe in detail anatomical union formation in other conifers (Mergen, 1954; Dormling, 1963; Nenjuhin, 1966). Microscopic structure and symptom development of incompatible Douglas-fir grafts have been described (Copes, 1967a), but union development during the first 90 days was not mentioned.

A general model of graft union development for most conifers follows this sequence: contact or isolation layer formation, cell enlargement, callus formation, phellogen formation, and vascular cambium formation. This sequence is in the approximate order of occurrence which follows grafting in many plants. Definitions and descriptions of graft-union terminology are found in Esau (1965). The following paper describes normal anatomy of Douglas-fir graft unions collected 2 to 84 days after grafting.

METHODS—Cleft grafts were made in March of 1965 and 1966 on potted, 2-year-old Douglas-fir seedlings. Twenty scion clones were grafted the 1st year, and 16 of the same clones were grafted the 2nd year. Scion clones averaged more than 50 years old; thus physiologically adult scions were grafted on 2-year-old stocks. Air temperature in the greenhouse was maintained at 70 F from 8 AM to 6 PM and at 60 F the remainder of the time.

Collections of 9 to 21 graft unions were made 2, 4, 7, 10, 14, 17, 21, 24, 28, 35, 38, 42, 49, 56, 70, 84 days following grafting. The unions were fixed in FAA or a chromic acid fixative, dehydrated with tertiary-butyl-alcohol and embedded in 62 C paraffin (Johansen, 1940). Before sectioning with a rotary microtome, paraffin-embedded unions were soaked in an acid-alcohol softening solution (Gifford, 1950) for 3 days, and in a soap-glycerin solution (Alcorn and Ark, 1953) for 2 days. Sectioned unions were stained with safranin and fast green (Johansen, 1940).

OBSERVATIONS AND DISCUSSION—Graft-union development varied between and within clones and grafts of each collection. Within individual unions, areas near the middle of the union usually contained the most advanced development; acropetal and basipetal areas were slower to develop. To reduce the position effect and technique variation, results are based on the most advanced stage of development found in each graft sampled.

Contact layer formation—Contact layers were seen in phloem and cortex union zones of 2-day-old grafts. As in guayule (Artschwager, 1951), the zone in Douglas-fir consisted of dead cells, cell fragments, and dried cytoplasm. Suberization of contact layers was slight through the first

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7 days, but by 10 days the layers had become well suberized.

Little, if any, contact layer was evident over the cut xylem surfaces. Thus Douglas-fir reacted like *Picea abies* but unlike *Pinus sylvestris* (Dormling, 1963). The proportion of living cell types in xylem areas was inadequate to establish an identifiable contact layer. Resin may have formed a protective layer over the cut xylem tissues, but xylene in the microtechnique solutions would have removed any resin present.

Cell enlargement—Cell enlargement was not visible in 2-day-old Douglas-fir grafts, but at 4 days enlarged epithelial cells plugged some cut resin canals (Fig. 1). Plugging of resin canals occurred only in the stock close to a cut surface. After 7 days, some enlargement was seen in phloem-ray cells and phloem parenchyma within 10–15 cells of the cut surface.

Callus formation—Callus was first visible between the 5th and 7th days. In 7-day-old grafts some callus cells had already given rise to several derivatives. Initiation of cell division in Douglas-fir between the 5th and 7th days was slower than the 1st day in *Citrus* (Mendel, 1936), 2nd day in peach (Fletcher, 1964), or 3rd and 4th days in *Picea abies* and *Pinus sylvestris* (Dormling, 1963).

All types of parenchyma cells, from terminal-xylem parenchyma cells to cortex parenchyma cells, were capable of division. Chief contributors to callus growth were ray and phloem parenchyma

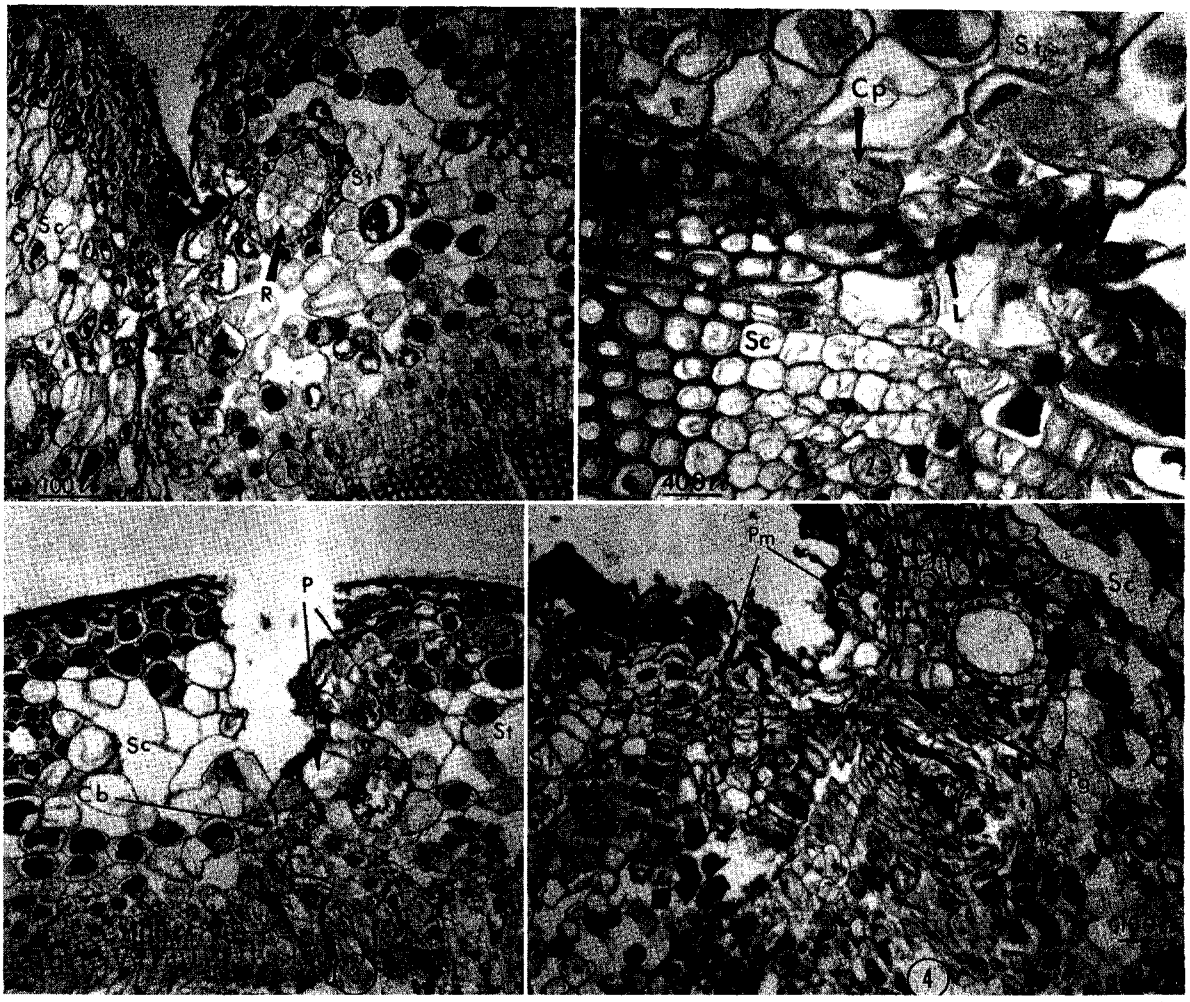

Fig. 1–4. Transverse sections of graft unions.—Fig. 1. Enlarged epithelial cells plugged a resin canal in a 4-day-old graft. The canal was located close to the cut surface in the stock tissues.—Fig. 2. Mitosis occurred in stock tissues of a 7-day-old graft. The cell plate was oriented parallel to the cut surface.—Fig. 3. Periderm formation began in stock tissues of a 10-day-old graft. No development was visible in scion tissues. A callus bridge formed where the contact layer was broken.—Fig. 4. Phellem tissues developed in the 17-day-old graft. The periderm was completed on both sides of the union.—Key to labeling: Cb, callus bridge; Cp, cell plate; L, contact layer; Lf, contact layer fragment; P, union periderm; Pg, phellogen; Pm, phellem; R, resin canal; Sc, scion; St, stock.

cells located in the zone of the crushed, nonfunctional sieve cells. Cambial and xylem cells contributed little to early callus formation. Unexpectedly, in the hundreds of slides examined only one mitotic figure was found from a mature xylem parenchyma cell.

Extensive areas of callus formed in spaces between graft components by 10 days. Rate of cell division was high through the 2nd and 3rd weeks. At 21 days, most open areas within the union zones had filled with callus. Callus found between xylem and pith tissues of the stock and scion originated from cells located outside the xylem cylinder.

Cell expansion by callus and other parenchyma cells located adjacent to the contact layers presumably resulted in the buildup of considerable pressures against the layers. Contact layer breakage was first observed at 10 days and was observed in 80% of the grafts 14 days or older. Early unions of callus tissue, called callus bridges, developed where contact layers were no longer continuous. Bridges developed most often in cortex and nonfunctional phloem areas. Very few bridge areas formed directly from cambial-derived callus.

Callus contribution by the scion exceeded that of the stock through the first 7 days, but by 10 days callus production by the stock exceeded that of the scion. Initial slower callus production by the stock was surprising since stock tissues at the time of grafting were actively growing and scion tissues were relatively dormant. This reversed order of stock-scion callus contribution has also been noted for *Picea abies* and *Pinus sylvestris* (Dormling, 1963). Two possible causes of the reversal in Douglas-fir are water and hormone availability. First, a water deficit in scion tissues after 7 days may have slowed mitotic activity in Douglas-fir. A similar water stress was noted in grafts of three *Pinus* and one *Picea* species. Those grafts lost weight for 10 days before they began to gain weight (Nenjuhin, 1965). Second, polar-moving hormones may have been insufficient in stock tissues of the union until the contact layer was bridged. The supply of endogenously produced hormones or the acropetal movement of hormones in Douglas-fir stocks is too limited to stimulate rapid callus formation (Copes, 1967b). But once the contact layer was bridged, the hormones could move basipetally from the scion to the stock and thus promote increased mitotic activity.

Through the first 2 weeks, division planes during mitosis in all cells located near the cut surface were parallel to the cut edge (Fig. 2). Enlarged cortex cells located some distance from the cut edge underwent mitosis without apparent orientation to the wound surface. A different situation had been reported for *Picea abies* and *Pinus sylvestris* grafts; first-cell divisions were not oriented in any specific direction, but cell plates formed in older grafts were oriented parallel to the wound surface (Dormling, 1963). It is impossible to determine from the results of the present study if the control of cell plate direction in Douglas-fir is a response to hormone gradient, to direction of least pressure, or to the combined effect of pressure and hormones.

Phellogen formation—Periderm formation across union zones is necessary before the grafts are effectively protected from insects and diseases. No such periderms were seen in unions younger than 10 days. Before the 10th day, contact layers functioned as the only protective barriers between living cells and the outer environment. By 10 days the cells in callus bridges located farthest from the cambium had divided parallel to the outer surface and had formed a zone of cells which resembled a periderm. The newly formed periderm segment was continuous from the cut edge of the old periderm to a point in the union halfway between the stock and scion. No identifiable periderm was seen on the scion side of the union (Fig. 3). In Douglas-fir, as in *Pinus sylvestris* and *Picea abies* (Dormling, 1963), old phellogen tissues did not contribute to new phellogen formation.

Although phellogen began development by 10 days, the tissues were not completed in 80% of the grafts with well-matched unions until the 17th day and much later across poorly matched unions. Greater periderm development was evident on the stock side of the union than on the scion side; four or five cells in radial tiers developed on the stock side, but only two cells in a tier developed on the scion side. Phellem formation was not seen on the scion side of the union until 17–24 days (Fig. 4). At that time suberized cell walls were present on all union surfaces exposed to the external environment. Time of phellogen formation was similar to the 14 days reported for grafts of apple (Mosse and Labern, 1960) and the 15–20 days for *Picea abies* and *Pinus sylvestris* (Dormling, 1963), but it differed widely from the 6 weeks reported for *Pinus elliottii* (Mergen, 1954).

Periderm-like zones encircled some contact layer fragments in grafts 21 days or older (Fig. 5). Presence of dead tissue within the cortex stimulated oriented division of adjacent parenchyma cells. As a result the fragment was surrounded by a continuous sheath of cells. Phellem-like cells in the sheath were seen adjacent to the fragment.

Vascular cambium formation—Identifiable cambial zones were first observed at 17 days and were present in 80% of the grafts by 28 days. Early cambia developed only where close contact existed between cambial and phloem regions of the stock and scion. In 17- to 28-day-old grafts, the usual condition was to have cambia completed across only one or two of four potential union

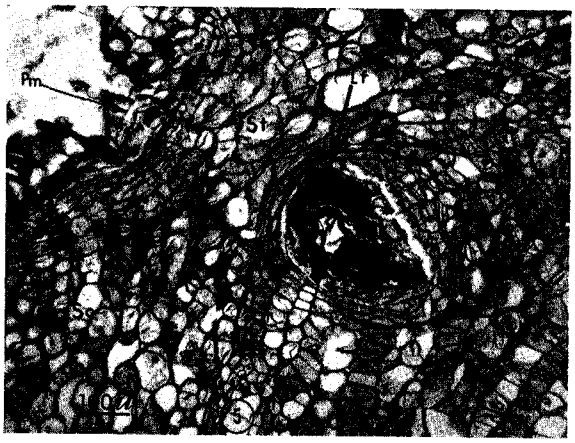

Fig. 5. Transverse section of a graft union. A contact layer fragment surrounded by a periderm-like ring of tissue in a 35-day-old graft. Phellem-like cells differentiated next to the fragment.—See key to labeling, Fig. 1–4.

areas (as seen in transverse view) of well-matched cleft grafts. The appearance at 17–28 days was similar to the 21 days reported for *Pinus sylvestris* and *Picea abies* (Dormling, 1963), but this was longer than the 14 days reported for peach (Fletcher, 1964) and 15 days for *Citrus* (Mendel, 1936). Most 35- to 38-day-old grafts contained cambia in at least two union areas. As time progressed the previously unbridged union areas also developed cambia. When stock and scion with unequal diameters were grafted, or when stock and scion cambial zones were not placed opposite each other, cambial unions were often delayed for 3 months or more.

Cambia formed in union zones through differentiation of callus cells in bridge areas. Less than 20% of the cambial unions originated from callus produced solely by cambia of stock and scion. Most cambia were initiated in bridge areas of secondary phloem or inner cortex origin.

Newly formed cambial initials in young union zones had larger diameters and more irregular shapes than initials found in ungrafted tissues or in older union zones. Cambial cell diameter and size variability were greatest at 17–28 days and then decreased until, at 40–49 days, they closely matched diameters and shapes found in ungrafted cambia. The same conditions also applied to the tracheids which differentiated from the cambial initials.

Vertical tissue was extremely disoriented in the first-formed vascular tissues. Extent and persistence of disorientation in the cambium was often directly associated with grafting technique. Well-made grafts had little tissue disorientation, but poorly made grafts continued to form extensive areas of disoriented xylem and phloem many months after grafting.

Phloem cells which differentiated 17–35 days after grafting consisted mainly of parenchyma-like cells and ray cells. The parenchyma-like cells retained their nuclei rather than becoming enucleated as did normal sieve cells in adjacent ungrafted areas. Cell diameters were two to three times that of normal sieve cells. By 38 days the cambial cells of the union were producing phloem cells containing sieve areas, but cell diameters were still slightly larger than normal. Normal-sized sieve cells appeared in most unions after 42–49 days.

Lignification at 14 days was restricted to callus along cut edges of the stock and scion. Cells located near the middle of the callus bridges were not lignified, but by 17 days some lignified callus cells had formed across the entire unions. By 35 days cambial mitosis, differentiation, and lignification had progressed and as many as eight lignified tracheids in radial tiers had formed in the unions. Continued cambial activity after the 35th day resulted in many 84-day-old unions having more than 50 lignified tracheids in radial tiers.

Although cambia were recognizable in some grafts at 17–21 days, vegetative bud burst did not occur until about 28 days after grafting. At that time tracheids were present in most union zones. Poorly made grafts were slow to form cambial unions and were also delayed in bud burst. Initiation of shoot growth by the scion was a good indication that a cambium was present and that tracheids had differentiated in the union.

Development after 35 days—Normal union development was complete after 35 days. Development beyond 35 days was simply an enlargement of tissue systems that were already functioning. Changes which occurred were as follows: more normal-appearing phloem and xylem differentiated, thicker periderms formed, vascular unions developed in previously unbridged union zones, and abnormalities induced by poor grafting techniques were reduced or eliminated. The appearance of internal incompatibility symptoms was not observed until after union formation had occurred.

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