Anatomical symptoms of graft incompatibility in Pinus monticola and P. ponderosa

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Summary

Internal symptoms of graft incompatibility were examined in unions of 6-month to 8-year-old ponderosa and western white pine grafts. Internal symptoms in both species could be detected in the 2d and 3d growing seasons following grafting. Common incompatibility symptoms were phloem and cortex necrosis, suberization, internal periderm formation, and invaginated xylem areas. Large masses of atypical axillary parenchyma were found only in the xylem of incompatible ponderosa pine unions. Similar atypical parenchyma was not detected in western white pine grafts. An unusual wavy or undulate pattern of annual growth rings was noted in western white pine seed orchard grafts, but not in any trees grafted in the greenhouse. Practical rogueing programs based solely on anatomical symptoms are not recommended at this time because of the uncertainty of the correlation between long-term graft survival and the internal symptoms described in this report.

Key words: western white pine, ponderosa pine, graft rejection, vegetative propagation, seed orchards.

Zusammenfassung

Titel: Anatomische Symptome der Pfropfunverträglichkeit bei *Pinus monticola* und *Pinus ponderosa*.

Es wurden anatomische Gewebemerkmale der Pfropfunverträglichkeit bei *Pinus ponderosa* und *Pinus monticola* untersucht. Die Pfropflinge waren sechs Monate bis acht Jahre alt. Bei beiden Arten traten Störungen in der zweiten und dritten Wachstumsperiode nach der Pfropfung auf. Allgemeine Merkmale für Pfropfunverträglichkeit waren das Absterben von Phloem- und Cortexzellen, Korkeinlagerung, innere Peridermbildung und Einkerbung des Xylems. Bei *Pinus ponderosa* wurde viel atypisches Markparenchym im Xylem gebildet, was bei *Pinus monticola* nicht der Fall war. Bei *Pinus monticola* waren die Jahrringe an Freiland-Pfropfungen wellenförmig ausgebildet. Bei Pfropfungen im Gewächshaus trat diese ungewöhnliche Erscheinung nicht auf. Praktische Arbeitsprogramme, wie, nur aufgrund anatomischer Symtome, ungeeignete Propfreiser ausgesondert werden könnten, sind zunächst noch nicht möglich, weil die Beziehung zwischen langfristigem Überleben der Pfropfreiser und den in diesem Bericht beschriebenen Symptomen nicht geklärt ist.

Introduction

Graft incompatibility problems have been encountered in most seed orchards. An unknown amount of mortality must be risked when grafting species not previously tested since there is no accurate way to estimate losses under such conditions without actually grafting and observing survival and vigor. Study of losses in other species of the same genus is also of little help because the age of grafted trees varies, the grafting techniques are different, and people are inconsistent on what constitutes an incompatible graft. But such reports do give a general idea of what might occur. For example, incompatibility within *Pinus* that varied from 50% in *P. radiata* (Sweet and Thulin, 1973), 36-46% in *P. resinosa* (Holst, 1962), 38% in *P. patula* (Dyson, 1975), 30% in *P. serotina*, 28% in *P. elliottii*, 22% in *P. virginiana*, 18% in *P. echinata* (Lantz, 1973), 10% in *P. contorta* (Copes, 1975) and 6% in *P. taeda* (Allen, 1967).

Incompatible grafts in western white pine *(P. monticola* Dougl.) seed orchards were reported by Hanover (1962) and by Hoff (1977). Similar graft problems were noted with western white pine and ponderosa pine *(P. ponderosa* Laws.) in a seed orchard near Cottage Grove, Oregon (unpublished report by G. Barnes, 1968, USDA Forest Service Dorena Seed Orchard files). In that report, death of established grafts, scion or stock overgrowth at the union, and severe needle chlorosis were used as criteria to identify incompatible combinations. Four percent of all western white pine and 12% of ponderosa pine grafts, respectively, were said to be incompatible 4 to 5 years after grafting. Since the 1968 survey, additional trees in the same seed orchard have also developed incompatibility symptoms.

In the following study, thin cross sections of 6-month to 3-year-old ponderosa pine and western white pine graft unions (referred to as study grafts throughout this paper) were made by microtome and then examined by microscope in order to discover possible causes of graft failure. These grafts were compared with cross sections made by microtome of 4- to 8-year-old incompatible grafts grown in the seed orchard (referred to as seed orchard grafts). Anatomical symptoms for both species are described and may be of diagnostic help in determining union compatibility.

Materials and Methods

The scions of 35- to 100-year-old western white pine were collected on 3-25-71 from grafted ramets of three compatible and three incompatible plus-tree clones at the USDA Forest Service seed orchard near Cottage Grove, Oregon. The compatible or incompatible classification was based on performance of the clones in the orchard. Vegetative buds were beginning to elongate at the time of collection. Within 1 week of collection, 35 cleft-grafts of each clone and 35 autoplastic grafts (a rootstock grafted back upon itself) were made in a greenhouse on 245 potted 2-1 seedlings. Successful grafts were field-planted near Monmouth, Oregon, in November 1971.

Ponderosa pine was field-grafted on 5-1-74 at the same location where the western white pine grafts were planted. Five-year-old (2-1-2) seedlings of a Klamath Falls seed source were used as rootstocks. Scions from the 35- to 100-year-old trees were collected from grafted ramets of three compatible and three incompatible plus-tree clones at the Cottage Grove seed orchard. At the time of collection, the vegetative buds of the scions were in the candle stage of growth. Grafting was delayed until the rootstocks had developed to the growth stage where the newly emerging needles resembled pinfeathers (Parks, 1974). Thirty-five grafts were made of each clone, plus 35 autoplastic grafts. Side-grafts were made on the 1973 leader and protective plastic and kraft bags were placed over the grafts to provide a humid environment around the scion. The protective bags were removed 2 months after grafting.

Western white pine grafts were collected for anatomical study 6, 13, 17 and 31 months after grafting. Ponderosa pine unions were collected 14, 16 and 31 months after grafting. The unions were cut from the trees and preserved in vials containing 50% ethanol. The 6-month-old western white pine unions were dehydrated in tertiary-butyl alcohol, embedded in paraffin, microtomed into 25-μm-thick cross sections and stained with safranin-0 and fast green (Johansen, 1940). Unions of the older grafts were not dehydrated or paraffin embedded but were sliced with a sliding microtome and stained with safranin-0 and fast green, mounted on microscope slides, and examined under a light microscope at 10-100X (Gnose and Copes, 1975).

In 1971, 25 graft-incompatible unions were collected from 4- to 6-year-old ponderosa pine and western white pine ramets growing in the Cottage Grove seed orchard. These unions were cut from orchard-grown trees that exhibited obvious signs of graft incompatibility, such as stock or scion overgrowth, bark invaginations at the union, severe needle chlorosis, abnormally short needles, premature defoliation, or distress cone crops. The unions were collected and prepared for microscopic study as described above for the 13- to 42-month-old grafts; however, the seed orchard unions had to be split in 2-4 pieces before they would fit in the specimen clamp of the sliding microtome.

Results

The most unusual anatomical irregularity seen in the 4- to 8-year-old seed-orchard-grown grafts of western white pine was a wavy or undulate pattern of annual growth rings. Such areas were observed in both union and adjacent non-union areas. Undulations were first visible in the 2d annual growth ring following grafting *(Fig. 1)*. No visible cause of this condition was evident in the adjacent cambial area. Common internal symptoms of graft incompatibility were necrotic tissues in the outer phloem and cortex *(Fig. 2)*, internal periderm development which walled off the necrotic tissues *(Figs. 3 and 6)*, and invaginated or sunken areas where stock and scion tissues prematurely ceased to grow *(Fig. 4)*. Not all symptoms were necessarily found in each western white pine union.

Microscopic examination of slides of 4- to 8-year-old ponderosa pine unions from the seed orchard revealed many symptoms which were similar to those just reported for western white pine. Common symptoms were phloem and cortex necrosis and narrow, invaginated union areas. Isola-

Fig. 2. — Necrosis, suberization, and atypical cell organization of phloem and inner cortex cells are shown in the area containing unusually darkstained cells. The dark areas are suberized tissues. Arrows indicate the location of several dark-stained areas.

Fig. 3. — Necrotic area in the cortex was isolated from living cells by development of internal periderm. Arrow shows a walled-off area of dead cells.

Figures 1—7. — International graft incompatibility symptoms in western white pine.

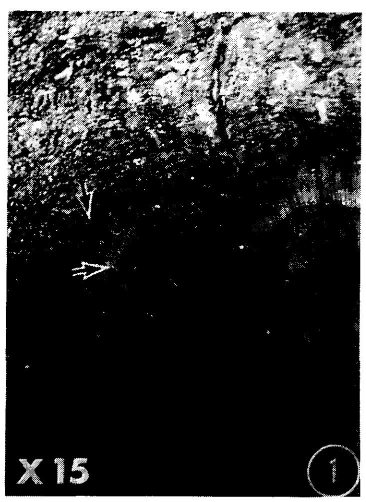

Fig. 1. — The unusual wavy or undulate pattern of annual growth rings was found in some incompatible unions of seed orchard grafts. Areas with wavy annual growth rings are indicated by the arrows

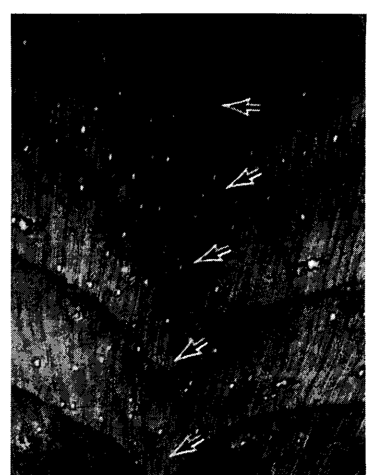

Fig. 4. — Sunken or invaginated areas were common in many unions. The arrows point the location of the invaginated annual rings of 4 consecutive years.

tion of dead tissues through the formation of an internal periderm was not as common in ponderosa pine as it was in western white pine. The most distinctive incompatibility symptoms were the large masses of atypical axial parenchyma *(Fig. 8)*. Parenchyma cells within these areas resembled cells found in xylem rays, but the cells lacked ordered arrangement. The parenchyma areas were also much wider than the widest rays and were filled with dark-stained contents. Formation of atypical parenchyma was often correlated with invaginated annual growth rings at the

Table 1. — Western white pine graft unions determined to be incompatible by external and internal symptoms.

	Seed orchard grafts		Study grafts[1]		
Clone number	Grafts observed	External incompatibility estimate	Grafts	Grafts with short, yellow needles	Internal incompatibility estimate
	(Number)	(%)	(Number)	(%)	(%)
(Incompatible clones)[2]					
18-03-20	4	75	27	41	26
15-01-76	13	54	29	38	64
18-03-0	10	50	31	19	56
Means		60		33	51
(Compatible clones)[1]					
15-01-16	27	4	34	3	48
15-01-37	14	7	28	0	22
15-01-02	28	4	25	0	25
Means		5		1	35
Autoplastic	0	0	22	0	0

1) Data from all 13- to 42-month-old grafts are included.
2) Clones were grouped in compatible and incompatible groups according to seed orchard observations based on external incompatibility symptoms, such as union overgrowth and bark discontinuity.

Table 2. — Ponderosa pine grafts determined to be incompatible by external and internal incompatibility symptoms.

	Seed orchard grafts		Study grafts[1]	
Clone number	Grafts observed	External incompatibility estimate	Grafts	Internal incompatibility estimate
	(Number)	(%)	(Number)	(%)
(Incompatible clones)[2]				
11-02-36	7	43	6	17
15-02-13	29	32	10	40
11-02-30	5	40	15	13
		38		19
(Compatible clones)[1]				
11-02-7	11	25	12	17
11-02-08	8	36	17	47
11-02-17	7	14	8	38
		25		34
Autoplastic	--	--	25	0

1) Data from all 14- to 31-month-old grafts are included.
2) Clones were grouped into compatible and incompatible groups from orchard observations of external incompatibility symptoms, such as union overgrowth and bark discontinuity.

union. Parenchyma masses were not formed until the 2d year after grafting. After that time, the atypical parenchyma cells formed continuous zones into the xylem of the following year, or atypical parenchyma formation was terminated later in the 2d growing season, only to reappear in xylem the following growing season *(Fig. 9)*.

The younger greenhouse and field-grown study grafts of incompatibility symptoms reported in the older seed orchard grafts. Common symptoms of stock-scion cell antagonism or incompatibility were necrosis, internal periderm development which walled off necrotic areas, atypical axial parenchyma zones, and invaginated cambial and xylem areas. *Tables 1* and *2* indicate the percentages of study grafts found to exhibit internal distress symptoms for western white pine and ponderosa pine. The value for each clone is an average of all the grafts of that clone which were more than 12 months old. The actual percentage of study grafts which would have eventually died from graft incompatibility if they had not been sacrificed for anatomical examination is not known.

Fig. 5. — An easily detected zone of necrotic tissues was present in the bark tissues of the union during the 2d and 3d years following grafting. The arrow indicates the location of a necrotic area in the phloem and inner cortex.

Study of ontogenetic changes in 6- to 42-month-old grafts of both species indicated that between-cell antagonism first appeared in the inner phloem and cambium. The general trend was for sunken xylem and cambial areas to become progressively deeper as graft age increased and for suberized and walled-off areas to become larger and easier to see.

Incompatible western white pine grafts were difficult to identify when only 6 months old. At that age, the only useful symptoms were necrosis and suberization of outer phloem and inner cortex, which could easily be confused with similar symptoms caused by poor grafting technique. Incompatibility detection was much easier during the grafts' 2d- and 3d-year growing seasons since less grafting-technique aberration was present and suberized areas were larger and easier to detect *(Figs. 5* and *6)*. The average incompatibility for the three clones selected as the incompatible group was 51%; the average percentage in the clones selected as the compatible group was 35% *(Table 1)*. Considerable variation in percentage of affected grafts was evident within each group.

The phloem and inner cortex in unions with invaginated xylem and cambia often had distorted and proliferated rays. A growth inhibitor may have caused the invaginated areas by slowing or stopping cell division of cambial initials and phloem and xylem derivatives. No growth retarding influence was noted in the meristematic activity of ray initials or their derivatives; they proliferated and filled the space normally occupied by phloem cells *(Figs. 2* and *7)*.

Many western white pine study grafts had short, chlorotic needles the 2d and 3d years after grafting. Examinations of those grafts indicated a number of the trees that had no internal incompatibility symptoms did have unions that were defective due to poor grafting technique. In most cases the scion and stock were incorrectly aligned when grafted so that a good union of xylem and phloem tissues had not occurred. Many of such grafts had survived with only a union of outer cortex tissues. The needles on such grafts were usually very yellow.

Ontogenetic study of ponderosa pine grafts during the 3-year period following grafting revealed the same familiar pattern of increased severity of incompatibility symptoms in older grafts. Besides atypical axial parenchyma in the

Fig. 6. — Large areas of dead tissue were isolated from living cells by a development of internal periderms. Arrows indicate the location of the internal periderms.

Fig. 7. — Meristematic activity of axial cells in the cambium was retarded in many incompatible unions, but ray and parenchyma cells proliferated in areas with reduced phloem growth. The arrow indicates an area with prolific ray and parenchyma growth.

Figures 8—12. — Graft incompatibility symptoms in ponderosa pine.

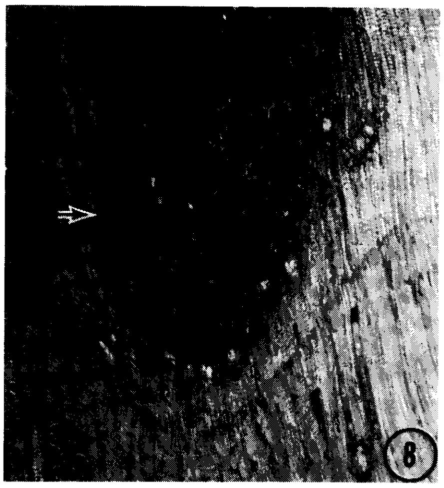

Fig. 8. — Large zones of atypical axial parenchyma formed in unions of many grafts. Such zones of dark-stained cells shown at arrow were absent in compatible grafts.

Fig. 9. — Zones of axial parenchyma cells could be continuous after initation into the following growth increments, or they could disappear later the 2d year and reappear in the 3d and 4th years. Several areas which terminated and started again are shown by arrows.

xylem and necrotic areas in the phloem and cortex, an unusual growth phenomenon was detected in several grafts: broad or wide areas of union and adjacent nonunion tissues were sunken below the level of the surrounding tissues *(Fig. 10)*. The depressed areas probably resulted from retarded cambial activity in those areas. The areas were different from the narrow, invaginated areas already mentioned because of the shape and size of the sunken area. Thickness of the phloem (as seen in cross section) on the areas joining the depressed areas contained progressively more phloem cells as the distance increased from the bottom of the depressed areas *(Fig. 11)*. Small raised areas of xylem and phloem were found along the bottom surface of the depressions. These small raised areas were likely formed by the activity of isolated groups of cells which had retained their meristematic activity after the surrounding cambial and derivatives cells had ceased to divide *(Fig. 12)*. This is indicated by the profusion of phloem cells on top of each small raised area and by the thin layer of phloem at the base of each raised area.

The percentage of ponderosa pine grafts exhibiting internal incompatibility was a surprise since 34% incompatibility was detected in the compatible clone group and only 23% incompatibility in the "so called" incompatible group *(Table 2)*. Apparently external union and foliage symptoms in ponderosa pine do not provide accurate criteria for classifying clones by compatibility.

No evidence of between-cell antagonism was found in autoplastic grafts of rootstocks of either species. Some suberization in union zones was found. but this was always correlated with the original graft wound. Lack of incompatibility symptoms in autoplastic grafts proved that the actual grafting technique procedures were not responsible for the incompatibility problems detected in homoplastic grafts.

Discussion

Scion or stock overgrowth, premature needle drop, abnormally short needles, and distress cone crops in western white pine and ponderosa pine were preceded by formation of internal incompatibility symptoms, such as phloem or cortex necrosis, suberization, walling-off of necrotic areas, and invaginated or sunken areas in unions. The invaginated areas found in western white pine unions closely resemble the invaginated zones reported in *Pinus contorta* unions (Copes, 1975).

Unions with evidence of extensive between-cell antagonism were detected in both pine species during the 2d and 3d growing seasons following grafting. First-year detection of incompatible grafts was possible but was difficult because of possible confusion with symptoms resulting from poor grafting technique. Other symptoms whose relationship to incompatibility are presently unclear are the broad, sunken areas seen in a few ponderosa pine unions and the wavy or undulate growth ring pattern observed only in seed orchard grafts of western white pine. Neither condition was preceded by phloem, cambial or cortex necrosis, or any other visible sign of between-cell antagonism. Some relatioship, however, with incompatibility is suggested since nei-

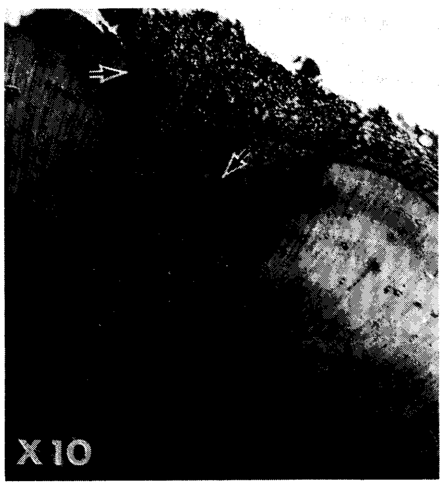

Fig. 10. — A broad area within the union was found where reduced xylem growth caused the affected areas to recess below adjacent normal areas. The arrows indicate the location of the sunken area.

Fig. 11. — The phloem area indicated in *fig. 10* by the small arrows is shown in greater magnification. Notice that the phloem zone became thicker (left arrows) as distance increased from the bottom of the sunken zone (right arrow).

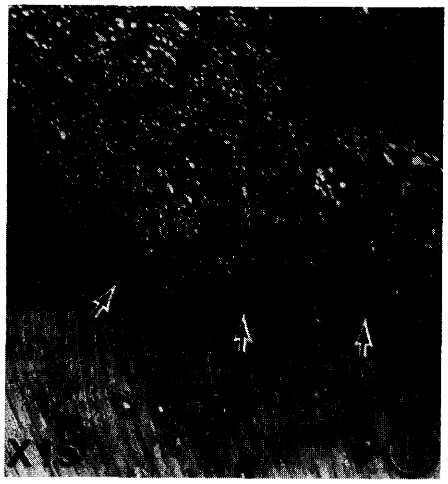

Fig. 12. — Small island-like areas of cells with meristematic activity were found in the bottom of the sunken zones. This activity resulted in the formation of small raised areas of xylem and phloem which are shown by the arrows.

ther symptom appeared until the year after grafting. The symptoms appeared only in grafts with stock and scion of different genotypes and never in autoplastic grafts.

The most reliable internal symptom for detecting incompatible ponderosa pine grafts was presence of the atypical axial parenchyma in the xylem of union zones. The same symptom could not be used in western white pine since it did not occur in that species. A practical incompatibility detection program would be easier to conduct for ponderosa pine than for western white pine because of the ease with which the atypical parenchyma can be detected, but neither species can be evaluated as easily or as accurately as is possible with Douglas-fir grafts. At this time, it would also be difficult to know exactly which grafts to rogue since the long-term effect of the internal symptoms on the pine grafts is not yet certain.

The sizable differences in average incompatibility were noted when seed orchard grafts were compared with the same clones grafted in the greenhouse and later transplanted and grown under different field conditions. These differences in average compatibility may have occurred because the rootstocks in the two areas were from different seed sources or because of different environments under which the grafts developed. Irregularity in average incompatibility values between study and orchard grafts was greater for ponderosa pine, but rather large differences were also evident for one compatible and one incompatible western white pine clone. Variable incompatibility values were previously noted in a western white pine seed orchard in Idaho where replacement grafts of the original clones were made a number of years after the original grafts (personal communication from R. J. Hoff, 1976). In that orchard, the seed source of the rootstocks was different, as was the environment following grafting. An even greater difference between the compatibility of the identical *Pinus radiata* clones at two different seed orchards in New Zealand was reported (Sweet and Thulin, 1973).

I have found that poor grafting techniques can induce western white pine ramets to grow yellow needles. Similar needles form on many incompatible graft unions. This fact negates use of needle chlorosis in identifying incompatible western white pine grafts. Few ponderosa pine grafts were found with chlorotic needles, so its relationship with graft incompatibility is not known.

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