GRAFTING INCOMPATIBILITY IN DOUGLAS FIR

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Grafting of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) started commercially on the West Coast in the late 1950's with large-scale grafting in clonal seed orchards. Scions collected from superior tree selections were grafted upon wildlings or nursery-run seedling rootstocks. No attempt was made to identify parentage of the stocks or to graft scions upon clonal stocks. Graft mortality from incompatibility became evident as early as the following spring. Each year since that time, mortality has continued with delayed symptoms of incompatibility. Continuing incompatibility losses in the first grafted clones and in later grafts have caused seed orchardists to become increasingly aware of the severity of the problem.

To point up the incompatibility problem, let me repeat graft survival data from three seed orchardists of Oregon and Washington. Mortality from purely technique failures—that occurring during the first 2 or 3 months after grafting—was excluded from data. In one orchard, 1,622 grafts were made; 6 years later only 46 percent were still alive. In a second orchard, only 57 percent of 766 grafts survived 8 years after grafting. A third orchard was reported to have 78 percent of 671 grafts alive after 4 years. The values for these three orchards do not represent all the losses that will occur; many surviving grafts have visible external symptoms of incompatibility. For example, a 100-graft sample from the second orchard revealed that 35 percent of all living grafts had scion overgrowth symptoms. All overgrown grafts will probably die soon. The graft incompatibility problem has become so severe that the practicability of establishing future clonal orchards is in question.

Although pomological literature has numerous papers describing many types of incompatible conditions, little information was available in 1964 about cause and development of symptoms of incompatibility in forest trees and about internal anatomy of Douglas fir grafts. Nor was it possible to correlate Douglas fir condition to that reported for other species in pomological literature. This study was started in 1965 to determine what anatomical symptoms were correlated with scion failure. Primary purposes of this paper are to describe symptom development in grafts from 2 days to 4 years old and to determine what possible cause or causes are responsible for symptom initiation and development.

Materials and Methods

A greenhouse study was designed in which periodic graft sacrifices were made at graft ages of 2 days to 2 years. At

date of collection, sample size ranged from 4 to 44 grafts. Graft union collections were made the first year at 2, 4, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 49, 56, 70, 84, 90-105, 170-180, 220-230 days after grafting. Collections in the second year were made at 13, 15, 17, and 19 months after the date of grafting. Sacrificed graft unions were prepared for microscope observations by embedding in paraffin, rotary microtome sectioning, and staining with safranin and fast green. Both transverse and longitudinal sections were examined under a light microscope at 30-800 X.

Scion material came from 20 clones selected from among the most and the least compatible clones known to Oregon and Washington seed orchardists. Grafts were made in March of 1965 and 1966. A standard top-cleft or similar top graft was used both years. All grafting was done by the author. A number of autoplastic grafts (grafts with scion and stock of identical genotype) were made so that graft unions without stock-scion interaction could also be examined.

Grafting was done in a greenhouse on 2-0 Douglas fir seedling stocks. Seed sources of stocks were at 4,000-foot elevation on Mount Adams, Washington, in 1965 and at low and medium elevations in the Willamette Valley, Oregon, in 1966. Except for the interval from October to March, grafts were grown in a greenhouse from time of grafting until final sacrifice. From October to March, the grafts were placed outdoors for winter chilling.

In addition, dead or dying incompatible grafts, ranging in age from 1 to 4 years, were collected from seed orchards. Many of these grafts belonged to the same clones which were grafted for greenhouse study. Orchard graft unions were cut, unembedded, with a sliding microtome. The same safranin and fast green staining schedule was used for both orchard and greenhouse grafts.

Results and Discussion

First-Year Symptom Development. First detection of a graft incompatibility symptom was at 84 days. The symptom developed as deposits of suberin in intercellular spaces of the bark's cortex. Suberin is a fat or waxlike substance normally found in cork cells of Douglas fir's outer bark. The incompatibility symptom occurred only in tissue areas where stock and scion cells merged. This symptom was present in only 1.5 percent of grafts under 106 days old (Table 1).

Examination of grafts 170 to 180 and 220 to 230 days old revealed a higher percent of grafts with suberin zone initiation and a larger suberized tissue area than was found in younger graft unions. Although 1.5 percent of all grafts were suberized at 2 to 105 days, 62 and 33 percent of compatible and incompatible clones, respectively, in 170- to 180-day-old clones and 83 and 86 percent of compatible and incompatible clones, respectively, in 220- to 230-day-old clones were sub-

Table 1. Development of graft incompatibility symptoms in compatible and incompatible clones, by age and date of collection.

Graft age	Scion clone type	Graft with suberin zones initiated	Grafts with xylem wound areas	Suberin penetration into phloem or cambium
			Percent	
2-105 days (Mar.-June)	compatible and incompatible	1.5	—	0
170-180 days (August)	compatible	62	—	0
	incompatible	33	—	7
220-230 days (October)	compatible	83	—	8
	incompatible	86	—	51
13-14 months (Apr.-May)	incompatible[1]	100	—	91
15 months (June)	compatible	33	33	0
	incompatible	100	50	0
17 months (August)	compatible	75	0	0
	incompatible	80	40	0
19 months (October)	compatible	86	50	14
	incompatible	85	31	15
1-4 years	compatible and incompatible	100[2]	100[2]	100[2]

[1]Only incompatible clone grafts had died or were dying at this time.
[2]Signifies grafts grown in seed orchards.

erized. In addition to having a higher percentage of grafts which initiated the symptom, the 170- to 180-day collection had grafts with deeper penetrated bark areas than earlier collections. For example, 7 percent of incompatible clone grafts had suberin penetration to the phloem or cambium, whereas none of the earlier collections had penetration beyond the cortex. The 220- to 230-day-old grafts showed deeper suberin penetration than the 170- to 180-day-old grafts—51 percent of the incompatible clone grafts had been penetrated with suberin from cortex to phloem or cambial regions (Table 1). More than half the grafts from that collection date had suberin areas in cambial tissues. Tissue necrosis was found in both 170- to 180-- and 220- to 230-day collections. Necrotic cells were located near suberized tissues.

Development of suberin zones seemed correlated with periods of cambial activity. Deeper penetration occurred during periods of slow cambial growth. Suberin zones were generally restricted to cortex areas during times of high cambial activity, such as occurred through spring and early summer months (2- to 105-day collections). When cambial activity ceased, or was very slow, suberin zone penetration progressed from the phloem-cortex boundary to phloem and cambial regions.

Little difference existed between percent of compatible and incompatible clone grafts which ultimately initiated suberin zones. Much of the fluctuation between clone types within collection periods could probably be explained by small sample size variation. The chief difference between clone types was depth of suberin penetration during fall and winter months (220-20 days to 13 months). Usually, compatible clone grafts were not as deeply penetrated by suberin as were incompatible clone grafts. This could easily be seen in the 220- to 230-day-old grafts (Table 1). Only 8 percent of compatible clone grafts had suberin penetration of phloem or cambium, yet 51 percent of the incompatible clone grafts had penetrated that deeply. Unequal penetration between clone types is an important fact to remember when the second symptom of incompatibility is described later.

Both compatible and incompatible clones developed some grafts with or without suberin zones, but no suberin zones were ever found in autoplastic grafts. This indicated that the physical act of grafting did not cause suberin zone initiation, but stock and scion tissues of different genotypes were necessary before the zones would appear. The incompatible clone group was found to be antagonistic to more stock genotypes, or more susceptible to the incompatibility factor, than was the compatible clone group. Since all scion clones were found at times to be incompatible, it was concluded that only degree of compatibility varied between and within the two major clone groups.

Formation of cambial unions was not necessary for suberin zone initiation. Grafts with both cambial and cortex unions and with only cortex unions were found capable of suberin initiation. Some dead grafts collected in 1966 did not form cambial unions before death, yet they developed suberized zones in all cortex union areas. This indicated that the physical structure of the union had no role in symptom initiation.

Second- to Fourth-Year Symptom Development. The 13-month collection, 4 weeks after the grafts were returned to the greenhouse, revealed more cambial activity in the stock than in the scion. Uneven initial periods of springtime cambial activity are normal for Douglas fir grafts. Stock tissues were juvenile, and scions were usually collected from 40- to 150-year-old trees. The normal timing in seed orchards was for stock branches to burst vegetative buds long before scion branches. The same situation also occurred with greenhouse grafts grown in pots. Even though stock tissues began development earlier than scion tissues, at the start of each growing season, no cambial breakage or tearing was evident. Differences in springtime cambial activity were not correlated with initiation or penetration of suberin zones. It should be noted that the 13-month collection just described was too small for accurate determination of percentage values for suberin

initiation and development or for formation of xylem wound areas.

External symptoms of incompatibility became visible on some grafts during the second year. Scion needle drop and chlorosis at the start of the second year was followed by graft failure within the following 2 months. Twenty-three percent of all grafts living after 220 to 230 days died between the 13th and 14th month. Anatomical examination revealed that suberin zones had penetrated from cortex to cambium in all dead grafts. Death of connecting bark tissues within union zones occurred between ages of 220-230 days and 13-14 months. Suberized areas presented a relatively impermeable barrier to water and nutrient movement between stock and scion cells. Scion needle drop and chlorosis resulted after scion and stock tissues became separated by suberin zones. Even xylem ray cells became necrotic and suberized. This probably eliminated all lateral water movement between xylem tracheids and living bark tissues.

Deepest suberin penetration within a graft occurred in union areas that contained the most vertically disoriented tissues. Lower rates of cambial activity were thought to result in deeper and more extensive suberin development. Number of cambial unions within a graft did not influence initiation of suberin zones but did affect chance of future graft survival. Mortality at the 13- to 14-month collection date was greatest in grafts with fewest cambial unions. A greater number of union areas increased probability that at least one area would not develop suberin zones into the cambium. Grafts with some unpenetrated cambial area would survive for at least another year. Initiation of suberin zones was not affected by physical structure of the union, but collections made in the first and second years showed suberin zone penetration to be altered by union structure.

A pattern of suberin zone initiation and development, similar to that found in grafts of the first year, was recorded at 15, 17, and 19 months. Suberin zones were generally restricted to the cortex during active growth periods (15-month collection), and then suberin zones progressed inwards during period of slow cambial activity (17- and 19-month collections). Percentage of grafts with phloem or cambial penetration in the second year was less than was recorded for the 220- to 230-day collections. The probable cause of lower second-year values was death of most severe incompatible combinations during the 13th and 14th months. Although suberin penetration depth was generally less than at 220 to 230 days, percentage of grafts which had initiated suberin zones was very similar between the 2 years. Eighty-six and 85 percent of compatible and incompatible clone grafts, respectively, initiated suberin zones in October of the second year (19-month collection). Those values closely matched the October collection values of the first year (220- to 230-day collection), when 83

and 86 percent of the compatible and incompatible clones, respectively, initiated suberin zones (Table 1).

A second characteristic internal symptom of Douglas fir graft incompatibility was first noted in the 15-month collection. This symptom will be called a xylem wound area (Fig. 1). Xylem wound areas, as seen in transverse view, developed at the start of the second growing season where stock and scion cells merged. The areas were composed of irregularly shaped tracheids that were disoriented vertically; lignified callus cells that had dark-stained cell contents; and necrotic areas of crushed, suberized, cambial and phloem tissue. Wound callus cells were formed only for a short time after regrafting occurred, and then irregular, disoriented tracheids developed. More normal tracheids were differentiated later in the growing season from derivatives of the xylem wound cells. Vertical xylem disorientation became less, and cross-section tracheid diameters returned to nearly normal size and shape. At the end of the growing season, some grafts developed normal tracheids, but other grafts continued to produce disoriented tracheids.

Xylem wound areas were formed in grafts of both compatible and incompatible clones. They occurred only in grafts of 15-, 17-, and 19-month collections that also contained suberin zones, but not all suberized grafts developed xylem wound areas (Table 1). Size of xylem areas was positively correlated with size of suberin zones located in bark opposite them. Both incompatibility symptoms varied in size within union areas of individual grafts. It is concluded that xylem wound areas were formed as a result of prior suberin zone penetration of the cambial zone. Autoplastic grafts did not develop suberin zones; thus, no xylem wound areas were found.

Comparison of cell types located in xylem wound areas with cell types and tissue organization developed after first grafting indicated that regrafting of stock and scion tissues had occurred at the start of the second season. As growth started the following spring, suberin zones and cell necrosis had either caused cambial death or severely disrupted cambial continuity. When spring growth began, a continuous cambial zone no longer existed between stock and scion. Mitotic activity of stock and scion cells near suberized and necrotic cambial areas resulted in formation of extensive callus areas. If grafts were to survive, a continuous bridge of living cells had to fill the gap between stock and scion. Grafts that succeeded in breaking through suberin zones later differentiated new cambiums across bridge areas. Newly organized cambium formed irregular and vertically disoriented tracheids. Cell types in regraft areas were different from those formed immediately before or after regrafting and provided excellent visual evidence of severe scion-stock incompatibility in Douglas fir. Thus, regrafting resulted in for-

mation of xylem wound areas. This symptom will be of diagnostic value for estimating incompatibility of new clonal selections for future orchards.

Examinations of 2- to 4-year-old seed orchard grafts, which were dead or dying of incompatibility when collected, revealed that xylem wound areas developed in all xylem union areas at the start of the second year's growth (Fig. 1). No graft formed xylem wound areas one year and then reverted to normal the following years. Also, no graft union that was normal the first few years developed xylem wound areas in later years. If a stock-scion combination was incompatible enough to cause xylem wounding, it began the process at the start of the second year. Xylem wound area was found in all union zones where cambium-to-cambium contact existed between stock and scion. The grafts had previously survived by regrafting each year until the time came when regrafting failed and the graft finally died. After regrafting failed, incompatibility symptoms in older seed orchard grafts were seen externally as scion overgrowths, and still later, as chlorosis and needle drop. Grafts only 2 to 3 years old normally died without developing scion overgrowths.

A summary of the graft mortality and union anatomy data revealed that 26 and 63 percent of compatible and incompatible and scion clones, respectively, showed symptoms of incompatibility severe enough to cause death or regrafting at the start of the second year. Regrafting will ultimately fail and then graft death will occur. It should be remembered that only worst incompatible and most compatible clones were examined in this study. A collection of grafts from nonselected clones might have an incompatibility value between 26 and 63 percent.

Symptoms of Douglas fir graft incompatibility resemble but are slightly different from symptoms reported by pomologists. Repeated regrafting is an old story to apple workers, so this symptom is not specific for Douglas fir. Major characteristic separating Douglas fir incompatibility from other plants was area of suberin initiation. Pomological literature reported that suberin zones begin in phloem or cambium. This study has shown that suberin zones in Douglas fir are always initiated at some point in the cortex. The zone is never formed first in phloem or cambial areas but reaches those tissues only after inward penetration from its cortical point of origin.

Cause(s) of incompatibility in Douglas fir are uncertain. However, it is evident that a simple growth rate difference between stock and scion is not the cause, that different periods of growth initiation had no effect on stock-scion compatibility, and that physical act of union formation did not cause incompatibility. The results of this study suggest that inverse correlation exists between rate of suberin penetration and amount of cambial activity. A biochemical antagonism, or possibly an antigen-antibody reaction, might exist between

stock and scion tissues. Also, the possibility of viral infections cannot be overlooked.

Summary

Two internal symptoms of incompatibility were found during the first 2 years—(1) initiation and penetration of suberin zones in bark areas of the unions and (2) initiation and development of xylem wound areas in xylem areas of unions. The suberin symptom was first seen in the cortex of an 84-day-old graft. Xylem wound areas became visible when the grafts were 15 months old. The suberin symptom became increasingly evident as age increased during the first year. Incompatible clones differed from compatible clones anatomically only in depth of suberin penetration. No similar symptoms were ever found in any autoplastic graft.

During the first year, necrotic phloem and cambial cells developed near suberized tissues. Incompatible grafts, which did not die before or soon after start of the second growing season, regrafted. Xylem wound areas developed only where deep suberin zone penetration occurred. Potential incompatibility losses were 26 percent for compatible clones and 63 percent for incompatible clones.

Cause(s) of graft incompatibility in Douglas fir are still not known. Growth rate, grafting technique, and spring

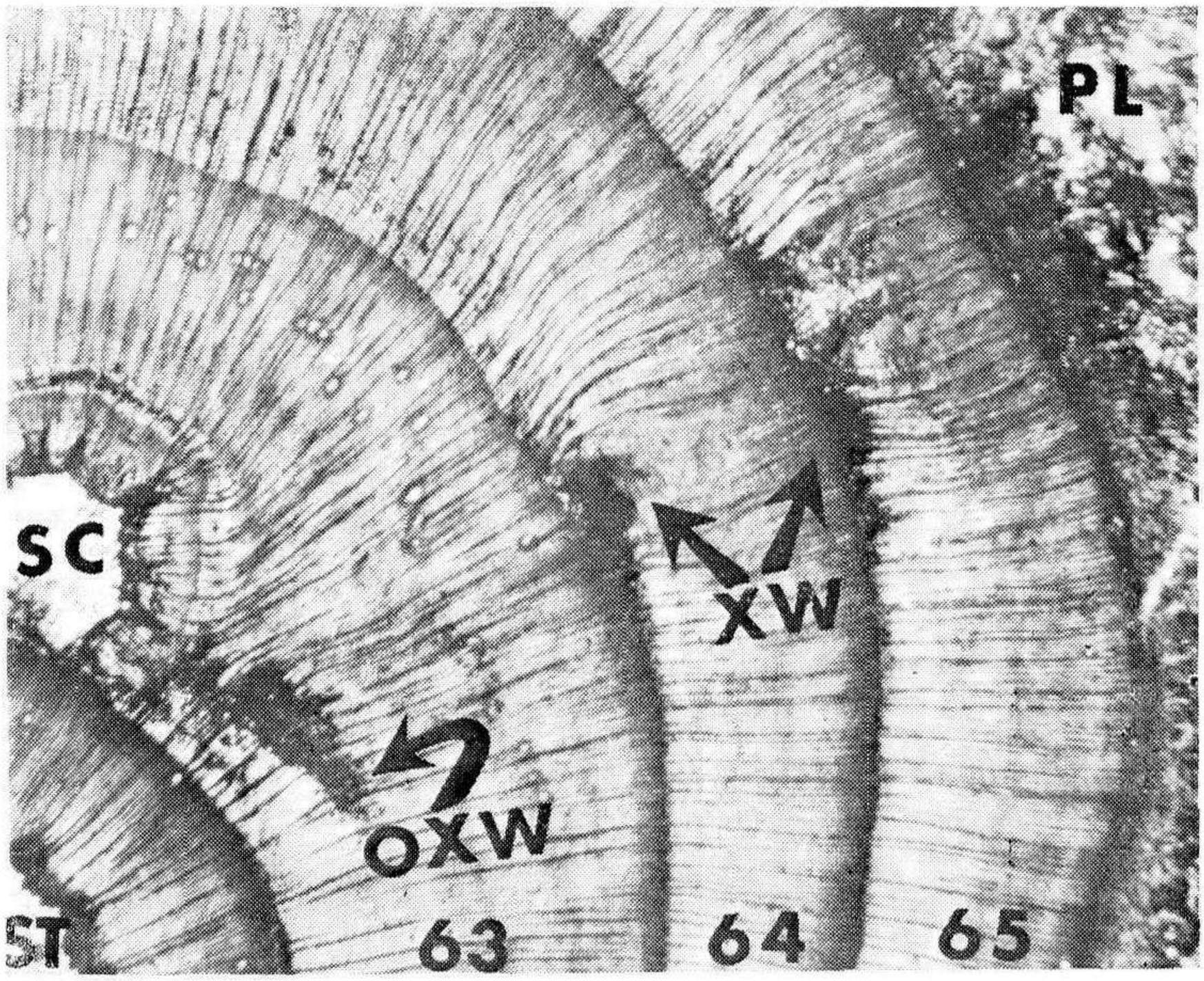

Figure 1. Three-year-old graft union containing two xylem wound areas formed at the start of the second and third year's annual growth. Transverse view. 40X. (Xylem wound areas, XW; original graft xylem wound cells, OXW; scion, SC; stock, ST; phloem, PL)

phenology have been ruled out as causes. Viral infections or biochemical antagonisms caused through stock-scion interaction might be causes of symptom initiation.

MODERATOR DOUGLASS: The next speaker is Ralph Jack, who is the owner of the Silver Falls Nursery and Christmas Tree Farm, Silverton, Oregon, which is in the Cascade Foothills, east of Salem. Mr. Jack has specialized in growing some 200 varieties of trees and shrubs for the wholesale market; these include Christmas trees, Christmas tree planting stocks, specimen trees, ornamentals and bonzais. Ralph Jack:

FIELD PRODUCTION OF CONIFERS

RALPH A. JACK
Silver Falls Nursery and Christmas Tree Farm
Silverton, Oregon

Our nursery and tree farm has as objectives: (1) raising seedlings and transplants for our use in growing Christmas trees, which we wholesale and (2) producing container stock for wholesale to nurseries, as well as for our own mail-order retail business.

We are located at Silverton Hills near Silverton, Oregon, in the Cascade foothills at an elevation of 1500 feet. We are 15 airline miles east of Salem, Oregon. Our soil is Olympic clay loam and is of a medium texture. Locally it is called "shot" soil.

We gather some conifer seeds for our own use, such as noble fir, western and mountain hemlock, *Abies magnifica* and *Abies concolor*. Noble fir is collected in the Cascades at 3500-4000 feet elevation. *Abies concolor* and *Abies magnifica* are collected in the Sierra Nevada mountains of California at about 7000 and 8000 feet elevation, respectively. We buy most of our seeds.

Seed is stratified in one of two ways; (1) with damp peat in plastic bags—50% seeds and 50% peat with moisture squeezed out—or (2) soaked overnight, drained and placed in plastic bags. For both methods we keep the seed in cold storage at 34° to 41° F. from five to eight weeks. We try to plant them just as soon as sprouts appear.

Seed bed preparation includes plowing with a rotary plow which breaks the soil into particles about ⅛ inch size. Vapam has been used in the past for soil sterilization. Beds are 34 inches wide, and are cultivated and raked. Seeds are broadcast by hand, then covered with ¼ to ⅜ inches of fine soil. This is either done by hand or by a trailer following a tractor. Sifted soil is shoveled onto a 4′ x 4′ plywood piece with a long handle. One man rides the trailer and shakes the board to drop the soil off evenly onto the seeds.

Seed beds are enclosed by wooden frames 3′ x 12′, made of 1 x 4 lumber. Hardware cloth (½″ x ½″ mesh) is nailed