

# GRAFT INCOMPATIBILITY IN INTERIOR DOUGLAS-FIR

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## ABSTRACT

Scions from 21 mature trees were grafted and used as ortets in a test of anatomical and electrophoretic methods of detecting delayed graft incompatibility in interior Douglas-fir. Both methods were found to be effective. Incompatibility, as determined by anatomical examination in these interior Douglas-fir clones, averaged 34%. Electrophoretic analysis of isozymes 19-20 months after grafting averaged 83% accurate in detecting incompatible grafts. Of the incompatible grafts, 60% also had externally visible bark fissures along the contact zone where the scion and rootstock joined. One clone with greater than 90% compatibility was found and several other potentially good clones were identified. The ortets of the most compatible clones have been used for breeding rootstocks of high graft compatibility.

**Keywords:** Interior Douglas-fir, graft incompatibility, seed orchards

## INTRODUCTION

In recent years many organizations have expanded their planting programs for interior Douglas-fir and increasing interest has been shown in the use of genetically improved seed. The Inland Empire Tree Improvement Cooperative initiated a program in the late 1970s to improve the growth and form of this species. Cooperators in the program selected phenotypically superior trees from wild populations and established progeny tests throughout eastern Washington, northern Idaho, and western Montana. The next step was the establishment of either seedling or clonal seed orchards of superior genetic quality. Seedling orchards are planted with progeny from selected trees, whereas clonal orchards can be composed of rooted cuttings or grafts of scion material taken from selected trees.

There are a number of advantages to grafted orchards compared to seedling orchards. Grafted orchards:

- Preserve and use good genotypes;
- Maximize genetic gain and profit;
- Flower earlier than seedling orchards for some species;
- Have fewer problems with related matings within the orchard;
- Can benefit from frost-hardy or disease-resistant rootstocks;
- Can achieve accelerated early growth when grafted to vigorous, established rootstocks.

The two major disadvantages of grafting are plagiotropism (branch-like growth habit) and graft incompatibility. Plagiotropism is a fairly common, but usually temporary problem when grafting to potted rootstock, and it can be minimized

by grafting onto vigorous, established rootstock in the field. Graft incompatibility is a more serious and longer-term problem because it is symptomatic of inherent physiological antagonism between the scion and rootstock, which eventually results in the death of the graft.

Graft incompatibility in clonal seed orchards of coastal Douglas-fir was a major problem in western Oregon and Washington during the 1960s and early 1970s. Most troublesome were grafts that appeared healthy for the first 10-12 years and then died suddenly from delayed incompatibility. These grafts occupied valuable orchard space and did not produce the seed needed to justify the cost of establishment. Regrafting was costly and often unsuccessful because of new incompatibility symptoms and suppression of the new grafts by older, adjacent trees.

Methods for identification of incompatible coastal grafts, which relied on external symptoms such as scion overgrowth, foliage-chlorosis and altered phenology, were not reliable for young grafts. This led to the development of two laboratory techniques by Copes (1970, 1978) which relied on the early development of *internal* incompatibility symptoms in the graft unions.

The first technique involved anatomical examination of two-year-old graft union cross-sections to detect wounds in the xylem and/or suberin (wax-like deposits) in the phloem. This technique became widely accepted because it was nearly 100% accurate in identifying incompatible grafts. However, it required destructive sampling of the graft union and therefore the rootstock had to be double-grafted so that a back-up graft remained in the orchard for seed production.

The second technique developed by Copes (1978) involved the detection of elevated levels of certain enzymes in the bark tissue of incompatible graft unions. While not as accurate as the anatomical examination, this second technique was non-destructive and therefore required only a single graft for the orchard. These two techniques were also used to identify and select highly graft compatible parent trees that could be bred to produce compatible coastal Douglas-fir rootstock (Copes 1981).

Little direct knowledge is available on graft incompatibility in interior Douglas-fir, other than a report by Copes (1969) in which he noted that approximately the same percentage of incompatibility occurred with interior grafts and coastal grafts. However, all trees in that study were grown in coastal Oregon. Since changes in environment can influence symptom development and expression (Copes, pers. comm.), it was not clear that the symptoms or the methods developed to detect incompatibility in coastal Douglas-fir in coastal environments would also apply to interior Douglas-fir grafts growing in interior environments.

This study was conducted to:

- Assess the level of incompatibility in interior Douglas-fir grafts grown in the Inland Empire by using the anatomical examination;
- Identify highly graft-compatible interior trees that could be bred to produce compatible rootstocks;
- Test the accuracy of the enzyme analysis relative to the anatomical examination.

## MATERIALS AND METHODS

Twenty-one mature interior Douglas-fir trees were grafted as scions in this study. The trees had been phenotypically selected in north central Idaho for superior growth rate and form. The trees ranged in age from 42-82 years and came from elevations ranging from 2,500-5,000 feet.

The first scions were collected in late February 1981 and top cleft-grafted in mid-March onto 413 two-year-old potted interior Douglas-fir seedlings of nursery-run origin. In early March 1982, scions were collected from only 7 of the original 21 trees (in order to increase the number of observations per clone). A total of 369 grafts were made in mid-March 1982 on potted nursery-run seedlings. All grafting took place in the Potlatch Corporation greenhouse in Lewiston, Idaho.

Bark samples from 206 of the 1982 graft unions were collected at 19-20 months after grafting. A template was placed on the bark at the contact zone between the scion and rootstock so that samples consisted of similar sized pieces of bark which included both scion and rootstock tissue. Enzymes in these samples were analyzed using the horizontal starch gel electrophoresis methods described by Copes (1978) and Conkle *et al.* (1981). Bark samples that showed the darkest staining peroxidase and esterase enzyme bands were scored as incompatible grafts. Accuracy of the enzyme analysis in detecting incompatible unions was evaluated as percent agreement with the later results from anatomical testing of the same grafts.

Grafts were 19, 20, or 31 months old when destructively sampled for the anatomical test. The unions had two or three annual increments of wood following grafting. Laboratory methods followed those of Gnose and Copes (1975) with slight modification.

Three factors were considered in estimating the graft incompatibility status of each clone. First, all graft failures that were determined to be the result of poor grafting technique were not considered. Since graft incompatibility is an inherent physiological problem, we wanted to analyze only well-matched unions. Thus, the estimate of incompatibility included grafts that died from early incompatibility plus those that were determined to be incompatible based on the anatomical examination.

## RESULTS AND DISCUSSION

Average survival after five months for the 1981 and 1982 grafts was 85% and 91%, respectively. Microscopic examination of cross-sections for the anatomical test showed that well-matched, compatible graft unions were free of cell necrosis in the phloem, cambium, and xylem at the interface where scion

and rootstock tissues had grown together. The earliest symptom of graft incompatibility was seen as dark-staining suberin or wax-like deposits in the phloem and bark cortex where scion and rootstock tissues were in contact. A later-developing and more easily recognizable symptom referred to as "wound-xylem" by Copes (1970) could be seen as pockets of dark-staining cell necrosis at the beginning of the second annual increment of wood, again at the interface where scion and rootstock tissues had grown together.

Average incompatibility of the interior grafts in this study was 34%, which is similar to Copes' estimate of 35% for coastal Douglas-fir. Of this 34%, 7% died from early incompatibility and 27% had a more delayed type of incompatibility that required the anatomical test for detection.

Although clonal incompatibility estimates for the 1981 and 1982 grafts showed some differences, clonal rankings were the same for both years for six of the seven clones (Figure 1). Only clone 224 fell out of rank in 1982. This discrepancy could have been influenced by the relatively small number of samples tested in 1981, fluctuations in climate between years (which can cause symptoms to be expressed at different rates), and the different rootstock genotypes that were grafted each year.

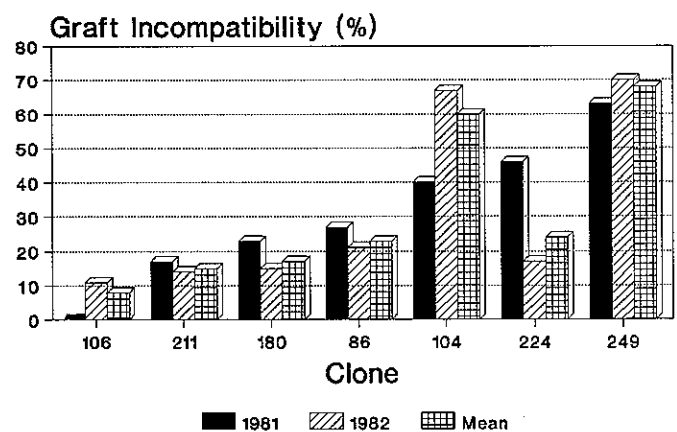


Figure 1. — Graft incompatibility (%) in 7 interior Douglas-fir clones.

Two clones, 106 and 211, had relatively low levels of incompatibility in both years. Copes (1981) reported an average of one out of ten coastal trees that were screened, to be more than 90% graft compatible. Although our sample of clones was small, our findings with interior Douglas-fir are generally consistent with that figure.

Also observed in this study was an external symptom of incompatibility that may be useful as a simple visual screening

procedure to reduce the number of grafts requiring the more elaborate anatomical test. Approximately 60% of the well-matched incompatible graft unions developed some degree of external fissuring on the bark at the contact zone between the scion and rootstock. Similar bark symptoms were reported for coastal Douglas-fir grafts by Karlsson (1970) who called it "breaks" in the bark. By comparison, well-matched compatible grafts had relatively smooth unions. This symptom should only be used with well-matched graft unions, however, because mismatched surfaces of compatible unions show similar symptoms.

By using the darkest staining enzyme bands as indicators of incompatibility, the enzyme analysis proved 83% effective (on the average) for identifying incompatible interior Douglas-fir grafts. The accuracy of this technique among the seven clones tested with large sample sizes ranged from 69%-93%.

Copes (1974) has estimated the heritability of graft compatibility for coastal Douglas-fir at 0.81. If interior Douglas-fir has a similar high heritability for this trait as coastal Douglas-fir, then the graft compatibility of full-sib rootstock families resulting from controlled crosses is expected to be considerably higher than the 66% average compatibility found for the 21 trees in this study. In an effort to produce highly graft compatible rootstock, Potlatch Corporation performed controlled crosses among the four trees in this study with the lowest levels of incompatibility in both years. Approximately 4,000 filled seeds have been harvested from these crosses.

## SUMMARY

Graft incompatibility in interior Douglas-fir grown in the Inland Empire averages 34%, which is similar to the 35% average for coastal Douglas-fir, and individual highly graft-compatible clones can be identified in approximately the same frequency as in the coastal variety. The enzyme analysis was 83% accurate in the early identification of incompatible grafts, but the accuracy varied widely from clone to clone.

Although graft incompatibility is as large a problem with the interior variety as it is with the coastal variety of Douglas-fir, it can be circumvented by the use of these early detection methods. Furthermore, if the interior variety parallels the coastal variety in the inheritance patterns from graft compatibility, it should be possible to produce compatible rootstock through selection and breeding.

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## ADDITIONAL INFORMATION

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