EXTERNAL DETECTION OF INCOMPATIBLE DOUGLAS-FIR GRAFTS

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Early detection of incompatible stock-scion combinations has been a problem for many years. External symptoms of incompatibility may not become evident until 10 to 40 years after grafting (7). Delayed incompatibility losses impose a serious handicap to nurserymen and orchardists. Internal incompatibility symptoms which are diagnostic of the relative compatibility of the graft have proven accurate for peach, pear, and plums (*4*), apricots (*5*), pear (*6*), and for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (*2, 3*). Unfortunately, internal detection requires destruction of the graft, a microscope, and some knowledge of microtechnique procedures. Obviously, nurserymen and orchardists would be better served by a simple visual test based exclusively on external appearance of the grafted plant.

Past attempts to find such a test have generally failed. Symptoms such as chlorosis, leaf drop, initiation of cambial activity, initiation of first-year bud elongation, amount of scion and stock growth, abnormally early flower-bud formation, mechanical breakage, scion dieback, and excessive scion growth have been checked for their correlation with incompatibility (*1, 4*). Unfortunately, most symptoms do not appear in delayed incompatible grafts until a number of years after grafting. Even then, they are not specific to incompatibility but may, instead, result from disease, insect attack, or other environmental stresses. One growth characteristic that has been positively correlated with degree of incompatibility in pear, plum, and peach combinations is delay in initiation of root growth each spring (*1*). Root growth was delayed up to 6 weeks in the most incompatible combination. Unfortunately, the usefulness of this correlation is limited because of the difficulty in observing root systems under field conditions.

In 1967 and 1968, observations on vegetative bud burst of Douglas-fir grafts starting their second year of growth showed bud burst to be delayed in a number of the normal-appearing grafts. Anatomical examination revealed that these were incompatible (unpublished data). From this, it appeared that a correlation might exist between delayed bud burst and graft incompatibility. This report presents results of a study on 116 Douglas-fir clones to determine if such a correlation actually exists.

METHODS

In April 1968, 947 Douglas-fir grafts, consisting of 116 different clones, were cleft-grafted for compatibility testing. Grafts were made on 2-0 stocks of a Willamette Valley seed source and grown throughout the study in a lathhouse at Corvallis, Oregon.

Observations on stage of vegetative bud development were made at the start of the second year. Bud development was checked at 7-day intervals, starting March 25 and ending May 20. Stage of development was based solely on the terminal bud of the scion. All 947 grafts were examined at each date. Stage of bud development of each graft was compared with all other grafts of the same clone. Grafts of different clones were not compared because of the inherent differences among clones in time of vegetative bud burst. Each graft which appeared to be retarded was recorded; also, an average bud development stage of each clone was noted for the nondelayed grafts.

The developmental process of bud burst was partitioned into the eight stages as listed in Table 1. The resulting classification represents a workable system for quickly scoring Douglas-fir buds. Characteristics used to determine a development stage were: (1) extent of bud elongation in length and diameter, (2) bud scale color, and (3) shape of the bud tip (Table 1, Figure 1).

All 947 grafts were sacrificed and anatomically examined in June and July 1969; the graft unions were cut into cross sections 30 microns thick with a sliding microtome and an abbreviated safranin-fast green staining schedule (*2*) was used. The stained cross sections were examined under a microscope

Table 1. Stages of vegetative bud development in Douglas-fir.

Stage of Development	Bud Characteristics
1. None	No expansion in length or diameter. Bud color dull, like that of an overwintering bud. Bud tip acute.
2. Slight	Slight increase in length, but little or no increase in diameter. Bud color yellow-red. Bud tip acute.
3. Slight to medium	Acropetal one-fourth to one-half increased in length, but little increase in diameter. Color similar to stage 2. Bud tip acute.
4. Medium	Acropetal one-half to three-fourths increased in length, but only slight increase in diameter. Color similar to stages 2 and 3. Bud tip acute.
5. Medium to maximum	Bud increased in length over the entire bud surface and exhibited a marked increase in diameter. Color similar to stages 2, 3, and 4 Bud tip blunt (obtuse).
6. Maximum	Buds much increased in length and diameter over stage 5 buds. Color much lighter than in the previous stages due to extreme expansion of bud. Bud tip rounded.
7. Burst	Bud scales separated; needle tips exposed. The new stem not visible.
8. Expanded 1-7 inches	New stem visible, and stem elongated 1 to 7 inches.

Fig. 1. Bud development stages used to detect incompatible grafts in Douglas-fir at the start of the second year. Bud stages are, from left to right: (1) no expansion, (2) slight, (3) slight to medium, (4) medium, (5) medium to maximum, (6) maximum, (7) burst.

for regraft areas (wound-xylem), which indicate incompatible grafts (*2*). The anatomical data were then compared with bud-bursting data.

RESULTS AND DISCUSSION

The anatomical test revealed that 35 percent (328/947) of the grafts were incompatible. Average compatibility among clones varied from 100 percent to 0 percent. These extremes undoubtedly represent sampling variation because previous tests have shown no Douglas fir clones to be either 100 percent compatible or 100 percent incompatible when grafted onto a random group of stocks.

Bud observations before April 29, 1969, were too early for meaningful differences in development to be detected. On April 22, 1969, only 65 of the 116 clones had progressed to development stage 1 or 2. On April 29, 81 percent of the clones were at stage 4 or higher, and on May 6, 93 percent of the clones were at stage 4 or higher (Table 2); these were the periods of most accurate bud determination. An evaluation before or after these dates in 1969, except for the seven latest clones, resulted in much lower accuracy in the prediction of compatibility from external bud characteristics.

During the collection of bud-bursting data, differences among ramets as small as two stages were recorded. For example, when nondelayed grafts of a clone were at stage 6, other grafts of the same clone had to be stage 4 or less to be recorded as delayed. But when bud-burst data were compared with the anatomical test results, it was apparent that differ-

Table 2. Average bud development of 111 to 116 Douglas-fir clones on four observation dates, 1969.

Stage of bud development	April 29	May 6	May 13	May 20
		Number of clones		
1. None	3	0	0	0
2. Slight	12	1	0	0
3. Slight to medium	6	6	0	0
4. Medium	38	13	2	0
5. Medium to maximum	15	20	0	0
6. Maximum	37	52	9	2
7. Burst	0	16	21	4
8. Expanded 1-7 inches	0	5	84	110
Totals	111	113	116	116

ences of three stages or larger were required for the differences to be significantly related to incompatibility. Also, to reduce human errors in bud ranking and data recording, a graft was not predicted to be incompatible unless it had been recorded as delayed in at least two different observation periods. If these two criteria were not met, the probability of incorrectly predicting a compatible graft to be incompatible was much increased.

Delay in second-year bud development in Douglas-fir grafts was directly associated with incompatibility. Of the 191 grafts predicted to be incompatible by delayed bud development, 184 proved to be actually incompatible by anatomical test. Thus, only 1.9 percent (7/619) of the compatible grafts had been incorrectly identified as incompatible.

Of the 947 grafts making up the study, 328 were shown to be incompatible by anatomical test. External bud development observations were effective in identifying only 184 of the 328 (56 percent). An examination of bud development and anatomical test data indicated that all degrees of detection accuracy exist among clones; in some clones, all of the incompatible ramets could be detected externally, yet in other clones with the same percentage of incompatibility, none of the incompatible grafts could be externally detected. To investigate the cause of this variation, externally detected and undetected incompatible grafts were anatomically rechecked for possible tissue differences. Microscopic examinations suggested that the ability to predict incompatibility from bud-burst data was directly related to the number of xylem union areas connecting the stock and scion and the size of the regraft areas (wound-xylem) as seen in cross section. Delayed bursting grafts generally had fewer union zones and larger regraft areas. Apparently vascular discontinuities resulting from necrotic phloem and cambial tissues at the time of bud expansion in the spring caused the delay in bud development until the stock and scion regrafted and re-established vascular connections in the phloem and cambial regions. Grafts with many union zones of contact and small regraft areas were not noticeably slowed in stage of bud development; thus, they escaped external detection.

Although 100 percent detection of incompatible grafts has not been attained, the 56-percent success is high enough to recommend bud development screening to Douglas-fir seed orchardists. If, for example, the present group of 116 clones had been externally checked, and if the delayed grafts had been rogued, an 81-precent compatible orchard would have resulted rather than the unrogued 65-percent likely to result without bud screening.

The correlation between bud development at the start of the second year of growth and graft incompatibility in Douglas-fir suggests that horticulturists might benefit by rechecking the relationship of these phenomena in all graft combinations that form regraft areas similar to those found in Douglas-fir grafts.

SUMMARY

Delay in vegetative bud development was correlated with the presence of internal incompatibility symptoms for 116 Douglas fir clones. Of 191 grafts classified as delayed in developing, 184 were found to be incompatible when anatomically checked. Of 619 compatible grafts, seven were incorrectly identified by the delayed bud development as being incompatible. Delay in bud development was found to be related to the number of union areas shared between the stock and scion and by the size of the regraft areas (wound-xylem). Regraft areas occurred in all delayed incompatible grafts. Incompatible grafts that had numerous union zones between stock and scion, plus small regraft areas, were not detected by bud characteristics. Forty-four percent of the incompatible grafts were not identified.

External screening is a usable tool for Douglas-fir seed orchardists. This study showed that an 81 percent compatible seed orchard could have been produced if the grafts showing delayed bud burst had been rogued, as opposed to a 65 percent compatible orchard that would have resulted if no roguing were done.

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MODERATOR LAGERSTEDT: Thank you, Don. Next, Barrie Coate will discuss some aspects of sanitation in the nursery. Barrie:

THE IMPORTANCE OF CLEANLINESS IN THE PROPAGATION HOUSE

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Many important propagation procedures become so routine that we often relax our attention to them. I'm speaking of the day-to-day details of cleanliness in the propagation program. Disease organisms can and do travel all the way from the cutting bench, through the greenhouse into 1 and 5 gallon stock, as the diseased crop is transferred to larger containers. It is very difficult to convince some nurserymen that meticulous disease control in the propagation department is worth the man-hours it requires. However, consider the value of 25 5-gallon saleable plants lost to disease in a month as compared with one man-hour per day spent on cleaning propagating tables, floors, and equipment per month.

25 5-gallon plants @ $3.00 ea.	— $75.00
25 hrs @ $2.50 per hr.	— $62.50

Even if only 25 5's per month are lost to disease, and this can be prevented, money would be saved. Another point in favor of good sanitation practices is the fact that chemical control over disease, once the disease is present, is poor at best.

Once *Rhizoctonia solani* or *Phytophthora sp.* are established enough for detection of the symptoms, at best we can only hope to prevent its spread to the remainder of the crop or to other crops. It is virtually impossible to eliminate these diseases once they begin to affect a crop.

Many nurserymen are "living with" infected stock maintained under "low stress" conditions. When this diseased stock is shipped to higher stress conditions (retail nurseries, high temperature areas, poor water areas) it often declines or dies, leaving the purchaser with a poor memory of the supplier and no repeat orders.

One question we should ask ourselves periodically is, "when did I last empty and disinfect my greenhouse?" The answer should be — "not more than 6 months ago."

Probably the easiest, least costly, and most rewarding single sanitation effort one can make is as follows: a 2% formaldahyde drench applied through a large sprayer. This will