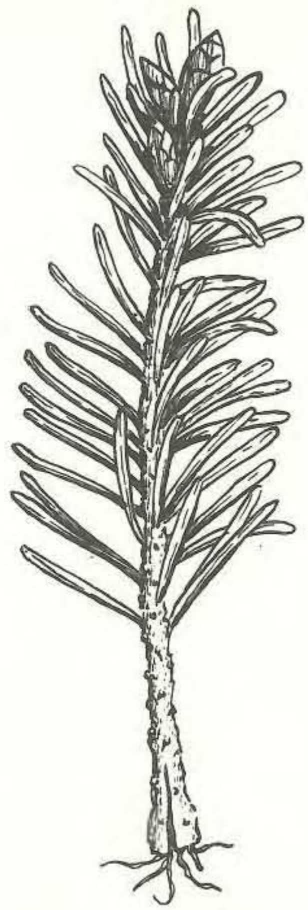

Rooting Studies of Douglas-fir Cuttings

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ABSTRACT

Studies of various aspects of rooting of cuttings from mature Douglas-fir trees are described, i.e., selection of cutting material, time of collection, storage of cuttings, treatment of cuttings (size of cuttings, wounding, root promoting substances, fungicides, paired cuttings), rooting medium, propagation structure and environment during rooting (water, temperature, light). Other studies elucidate photosynthesis during rooting, mortality of cuttings in rooting bed, anatomical and seasonal pattern of rooting, variation in rooting response among trees, and treatment, growth and flowering of rooted cuttings. Recommendations are given for procedures that have produced rooted cuttings from most of the trees tested and have yielded an average of 15% rooted cuttings. These procedures involve collection of cuttings in November, treatment with indolebutyric acid and with Benlate fungicide, placement of cuttings in a rooting medium heated to 20 C but with no air heating, and maintenance of cuttings in a humid and well-lighted atmosphere. Cuttings from some old trees have not rooted with this procedure but have rooted when grafted to cuttings from young trees.

INTRODUCTION

Extensive field selections of Douglas-fir trees, possessing phenotypically superior growth characteristics, have been made in the last two decades in British Columbia and in the States of Washington and Oregon. These trees have been propagated vegetatively by grafting, for use in seed orchards and breeding programs. Although grafting appeared initially to be satisfactory for propagating clonal material, there was a decline in vigor and death in many trees several years after grafting (Duffield and Wheat, 1964). A survey of Douglas-fir seed orchards in Oregon and Washington, in 1966, revealed that about half of the orchard grafts had died by the eighth year (Copes, 1967a).

Death of the grafts was due to a delayed incompatibility, resulting in blockage of phloem translocation between scion and rootstock. The reason for this incompatability is unknown, but Karlsson (1970) found a strong clonal variation. A similar problem has occurred with grafts of other forest trees (Barnes, 1969; Sweet and Thulin, 1973) and with many fruit trees (Mosse, 1962). Copes (1970) studied anatomical aspects and the external symptoms of delayed incompatibility (Copes, 1969) of Douglas-fir. He showed that incompatible grafts can be detected during the second year after grafting from a characteristic wound area in the xylem (Copes, 1967b). By grafting two of the scions on the same rootstock and using one for anatomical examination in the second year, he could select compatible grafts (Copes, 1968). Although this method offers a solution to the incompatibility problem, and has been used extensively in the United States, it is time-consuming and requires a 2-year waiting period.

Vegetative propagation by rooting of cuttings would solve this problem, but previous studies have shown that cuttings from mature Douglas-fir trees do not root well using conventional methods. Cuttings from young trees root readily (Black, 1973; Blankinsop and Callaham, 1960; Bodman _et al._, 1952; Griffith, 1940; Heinrich, 1968; Lanner, 1962); however, superior trees selected up to the present in B.C. are all mature trees. Success with a few mature Douglas-fir trees has been reported (Hough, 1953; McCulloch, 1943) prior to initiation of our studies in 1965.

Rooting of woody plant cuttings has been discussed in books on propagation (Hartmann and Kester, 1968; Komissarov, 1969; Mahlstede and Haber, 1966; Wells, 1955) and in special review articles (Doran, 1957; Nienstaedt _et al._, 1958; Girouard, 1971; Ticknor, 1969). These reviews clearly show that the anatomical and physiological conditions of the cuttings at the time of collection, before they are set out for rooting and during the rooting period, will affect their rooting behavior. Based on our studies, from 1965 to 1972, the effect of some of these conditions and treatments on rooting of Douglas-fir cuttings is summarized in this report, together with other recent advances. The various phases involved from selection of cuttings to treatments of the rooted cuttings are discussed.

MATERIAL

For most of our studies we used cuttings from old trees (70 to 250 years) growing in the vicinity of Victoria, B.C.; about 20 trees were used repeatedly in different years. Some of these trees were good rooters; others were poor. In addition, cuttings were obtained from the B.C. Forest Service's grafted clone banks at Mesachie Lake, Vancouver Island; they comprised many of the plus trees selected in the coastal Douglas-fir region. The B.C. Forest Service also supplied cuttings from parent ortet plus trees. Juvenile cuttings were obtained locally from seedlings growing in the forest or in nurseries.

The study plan specified a sample size of 50 cuttings per tree to be divided into two or more replications and the use of five trees for each treatment. This plan was followed for studies of effects of auxin, collection date and temperature regime. For various reasons, the sample size was reduced in other studies, as described in later sections, but rarely did the material consist of less than 25 cuttings from each of two or more trees, and studies were often repeated in different years. A total of 56,000 cuttings were used.

SELECTION OF CUTTING MATERIAL

Studies of several conifers showed that cuttings from the lower part of the crown root better than those from the top (Farrar and Grace, 1942; Girouard, 1970; Grace, 1939; Hyun and Hong, 1968). Black (1973) found no effect of crown position (cyclophysis) on rootability for Douglas-fir trees up to 24 years old. He demonstrated that branch order position (topophysis) was important. The large first-order lateral branches and the second-order terminals rooted best. Crown exposure may be important. For 82-year-old Douglas-fir, Hough (1953) found that cuttings from the shaded part of the crown rooted better than those from the exposed part, but the opposite result was obtained for 16-year-old trees.

In addition to the position and exposure of the crown, the vigor of the tree may be of importance. Using 3-year-old seedlings of three conifers, Enright (1959) demonstrated that cuttings rooted better if the stock plants were fertilized. Hyun (1967) stated that a high C/N ratio, particularly as a result of low N level, is a feature of good rooting trees. However, Libby and Conkle (1966) found no apparent relationship between rooting and vigor of radiata pine.

Age and length of the shoot should also be considered. Hough (1953) and McCulloch (1943) obtained better rooting of 1-year-old than of 2- and 3-year-old Douglas-fir shoots, and short cutting (5-10 cm) of Norway spruce rooted better than long (10-20 cm) ones (Grace and Farrar, 1940).

Tree age is the single most important factor in rooting, but good rooting ability is usually lost at an early age. Bodman et al. (1952) obtained good rooting for Douglas-fir trees up to the age of 17 years. In the next age class used (42 years), no rooting resulted. Black (1973) found that cuttings from juvenile Douglas-fir trees, less than 9 years old, had a rooting potential of 100%; the potential declined to 5% between 14 and 24 years. Libby et al. (1972) were able to arrest the normal decline in rooting ability of young radiata pine by hedging (heavy shearing). There is evidence that heavy shearing and successive vegetative propagation of old Douglas-fir trees restore their juvenile rooting characteristics (Black, 1973).

Based on studies with other conifers, and for practical reasons, our general procedure has been to collect cuttings from the lower quarter of the crown. In an early experiment (1966), cuttings were taken on the shaded and exposed sides of the crown and from the inner and outer part of the crown of two old trees. Cuttings were collected in the beginning of April before bud flushing, treated with 50 ppm IBA for 24 hours, and set for rooting in a heated (20 C) greenhouse. Rooting percentages were low for all collections from the different crown locations (2 to 6%) and not significantly different. To obtain a good sized cutting from mature trees, the side of the crown free from heavy competition from neighboring trees is preferred. Branches need not be very vigorous; cuttings 3 mm at the base are ideal; 2 mm are acceptable and they root as well as thick cuttings. There is a tendency for more severe decay of very vigorous cuttings.

Generally, cuttings 6 to 12 cm long are made from the terminal portion of current shoots. The basal portion of current shoots will also root, but not as well. Contrary to the previous reports with Douglas-fir (Hough, 1953; McCulloch, 1943), we found that 2- and 3-year-old shoots (with the younger shoot intact) root as well as current shoots. If current shoots will not give a 6 cm cutting, older shoots should be included. Our studies have not separated branches of the different orders but the majority have been of third order.

Also tested was the possibility that cuttings from grafted trees (ramets) root better than cuttings from the old trees (ortets), which originally provided the scion material. Cuttings from five ortets and their five ramets, grafted 7 years previously, were used:

Table 1. Rooting of cuttings from ortets and their grafted ramets.

Tree no.	Per cent rooted ortet	ramet
34	34	36
64	12	20
50	12	12
101	2	4
Average	15	18

Cuttings were collected in early November, treated with a basal soak for 24 hrs in 100 ppm IBA and set for rooting in a heated rooting medium (20 C) but with no air heating. The ortets grew in different localities, but the ramets were in the same clone bank. Use of grafted material did not improve rooting and it appeared that rooting was strongly influenced by genotype rather than by the environment in which the ortets were growing. Black (1973) found that Douglas-fir cuttings from grafted ramets rooted slightly better and those from established cutting ramets rooted considerably better than cuttings from the ortets.

TIME OF COLLECTION

In a study with ten coniferous species, Jesinger and Hopp (1967) found that the time the cuttings were collected was more important than hormone concentration and temperature of rooting medium for rooting of cuttings. In Griffith's (1940) experiment, in a heated greenhouse with cuttings from young Douglas-fir trees, the December-January and February-March collections gave better rooting than earlier or later ones. Roberts (1969) recommended collection of Douglas-fir cuttings in January and February.

Our collections have been made at various stages of shoot development ranging from succulent, limber shoots elongated to about

Table 2. Effect of collection time and air-soil temperature regime on rooting of Douglas-fir cuttings.

Air-soil temp regime	Per cent of 50 cuttings																	
	CAWS						WAWS						CACS					
Month-tree No.	1	2	3	4	5	avg	1	2	3	4	5	avg	1	2	3	4	5	avg
Sept.	0	0	-	-	-	0	0	0	-	-	-	0	0	0	-	-	-	0
Oct.	4	0	-	24	-	9	0	0	-	10	-	3	0	8	-	-	24	11
Nov.	14	0	50	52	14	26	0	2	2	14	6	5	-	-	0	0	-	0
Dec.	10	0	18	10	12	10	2	0	26	8	0	7	0	-	18	4	-	7
Jan.	0	0	10	16	24	10	0	0	14	4	0	4	0	-	0	0	-	0
Feb.	0	0	0	18	12	6	0	2	26	20	6	11	0	-	2	0	-	1
March	0	0	0	4	2	1	0	0	4	2	10	3	0	-	0	2	-	1
Average	4	0	16	20	13	9	0	1	14	10	4	6	0	4	4	2	24	3

CAWS: no air heating - soil heated to 20 C

WAWS: air 20 C - soil 20 C

CACS: no air or soil heating

three-quarters of their final length in spring, to fully elongated and rigid shoots during the summer, and at various times during the dormant season until bud break the following spring. No rooting was obtained during early shoot development, but some occurred in mid-September collections when shoots appear, externally, to be fully developed. Although no externally visible change takes place until bud break the following spring, there are important physiological changes, resulting in considerable changes in rooting ability.

The best time for collecting cuttings depends on the temperature conditions used after cuttings have been set for rooting. Cuttings were taken from 5 trees, 70 to 100 years old, and were treated with a 24-hour basal soak in 100 ppm IBA. Best rooting was obtained with collections in November, or early December in some years, provided the rooting medium was heated (20 C) but with no additional air heat (Table 2). For this collection time, rooting was poor if the air was also heated to 20 C or if no heat was supplied to air and rooting medium. With both air and rooting medium heated, the February collection was best, as also shown by Roberts (1969). In an experiment, using cuttings from the selected plus trees collected over a large geographic area in the fall of 1968, there was no effect of collection date, from September 19 to mid-November, in rooting. All cuttings received warm rooting medium and no additional air heat. Generally the rooting response for September and October collected cuttings has been variable from year to year and may depend on climatic conditions. For early fall collections, a chemical index for rooting potential is needed, because there are no visible external changes of the cuttings that can be used as a guide.

STORAGE OF CUTTINGS

Storage may be needed to provide a convenient work schedule, and it has also been reported to increase rooting. Libby and Conkle (1966) got more roots on cuttings from young radiata pine stored from 20 to 50 days at 3 C than from cuttings stored for shorter or longer periods, and an increased speed of rooting was obtained when cuttings were cold-stored from 20 to 90 days. Roberts (1969) reported that rooting of Douglas-fir cuttings, collected November 1, increased with duration of cold storage (0 C) up to 60-90 days, and Black (1973) showed that only dormant cuttings benefitted from cold storage.

In one experiment, cuttings from mature Douglas-fir trees were collected on September 19, October 21 and November 5, and stored moist in

Table 3. Effect of storage of Douglas-fir cuttings from mature trees on their rooting percentage for cuttings collected at different times and set for rooting in different temperature conditions, i.e., no air heating but rooting medium heated to 20 C (CAWS), and both air and rooting medium heated to 20 C (WAWS).

Tree no.	Collection date	Storage							
		no storage		6 weeks-4.4 C		8 weeks-0 C		12 weeks-0 C	
		CAWS	WAWS	CAWS	WAWS	CAWS	WAWS	CAWS	WAWS
1	Sept. 19	4	0	0	0				
2	Sept. 19	0	0	6	0				
	Avg.	2	0	3	0				
1	Oct. 21	4	0	10	0				
2	Oct. 21	0	0	12	0				
4	Oct. 21	24	10	40	4				
	Avg.	9	3	20	2				
3	Nov. 5	56	2			-*	2		
4	Nov. 5	54	14			-*	20		
5	Nov. 5	14	6	18	6			20	10
	Avg.	41	7						

* not tested

plastic bags with wet paper towels for 0, 6, 8, 12 weeks at 0 C or at 4.4 C. All cuttings were treated with a 24-hour basal soak in 100 ppm IBA. Half the cuttings (50 per treatment) were set in an unheated greenhouse (CAWS), the other half in a greenhouse heated to 20 C (WAWS). The rooting medium was heated to 20 C in both cases. Six weeks at 4.4 C increased rooting from 9 to 20% for the October collection when cuttings were subsequently set under cold air and heated rooting medium (CAWS) but not when the air was heated (WAWS). Otherwise, storage for 6 weeks at 4.4 C and for 8 and 12 weeks at 0 C had no effect on rooting (Table 3).

Cold storage did not eliminate the need for subsequent cold air conditions during the rooting period (see section on 'temperature').

Cuttings from seedlings, collected November 1971, were not significantly affected by storage, and rooted 64, 60 and 70% with storage (0 C) of 0, 2 and 10 weeks' duration, respectively. However, seedling cuttings collected November 1968 were affected by storage. Cuttings without storage rooted 80%, whereas those stored for 8 weeks at 0 C rooted only 32%.

Chilling of cuttings at subzero temperatures (-9 and -18 C) for 2 weeks was tested for cuttings collected from two trees at the end of February. No rooting was obtained with or without chilling. Low light (100 ft-c) for 12 hours daily during cold storage (8 weeks at 0 C) had no effect on rooting, whether cuttings were with or without buds.

TREATMENT OF CUTTINGS

(a) Size of cuttings and importance of leaves and buds.

Cuttings of current shoots are cut to a length of 6 to 12 cm depending on the initial size of the shoot. Leaves are stripped from the basal 2.5 cm length to facilitate chemical treatment and insertion of cuttings in the rooting medium, and to provide a uniform compaction of the rooting medium around the stem base. To root well, cuttings need the 3.8 cm top, which a 6 cm cutting will provide. If the current shoot length is less than this, some of the previous year's shoot should be included, as mentioned previously. Removing all leaves from cuttings quickly leads to deterioration and death. This was tested for cuttings from young and old trees given bottom heat in cold and warm air.

Buds may provide rooting inhibitors as well as promoters, and act as competitors for important metabolites. Their role is likely to vary with the season of the year. For a pear variety, Fadl and Hartmann (1967) found that buds promoted rooting when they were in a non-dormant stage, but inhibited rooting when they were dormant. Roberts (1969) obtained some enhancement of rooting by bud removal for cuttings from young Douglas-fir trees, collected in February and March, when they were treated with auxin. However, bud removal was detrimental at most collection times without the auxin treatment. This would indicate that buds, in addition to supplying auxin, may be a source of rooting inhibitors or may compete with the rooting process for needed metabolites.

In addition to seasonal effects of bud removal on rooting, the importance of the buds may depend on the temperature regime under which the cuttings are placed. Cuttings were collected from two old trees (150 years plus) and from seedlings at monthly intervals from November to March. All cuttings were treated with 100 ppm IBA (24-hr soak) and placed in a heated (20 C) rooting medium. For one group, the air was not heated (cold air-warm soil: CAWS); for the other, it was kept at 20 C (warm air-warm soil: WAWS). Half the cuttings had all buds intact; the others had them removed. Buds had no effect on rooting of young cuttings. Cuttings from the two old trees behaved differently (Fig. 1); this is just one example of the difficulty in generalizing about the rooting behavior of Douglas-fir. For tree No. 1, rooting was obtained only for the fall collection placed in cold air, and the buds had no effect on rooting. For tree No. 5, bud removal increased rooting in warm air, but not in the cold air regime. It is interesting to note the increase in rooting from the January to the March collections in warm air and the corresponding decrease in cold air for this tree. It appears that when cuttings from old trees are supplied with auxin, the buds do not promote rooting and they may be somewhat inhibitory in warm air.

(b) Wounding

Wounding of the part of the stem inserted in the rooting medium promotes rooting in many species (Hartmann and Kester, 1968). Removing a slice of bark down one side of the basal stem part, as is done with some cuttings, is detrimental with Douglas-fir. Our standard procedure is to make a 1.5-cm-long vertical slit into the wood on two sides of the stem down to the basal end. These cuts will not promote root initiation and growth up the stem along the slit, as is the case with many other species, and their value has not been established conclusively. However, when roots are few, they have been noted to emerge at the stem base, close to the junction with a slit.

(c) Root promoting substances and methods of application

The beneficial effect of auxin treatment on rooting of cuttings

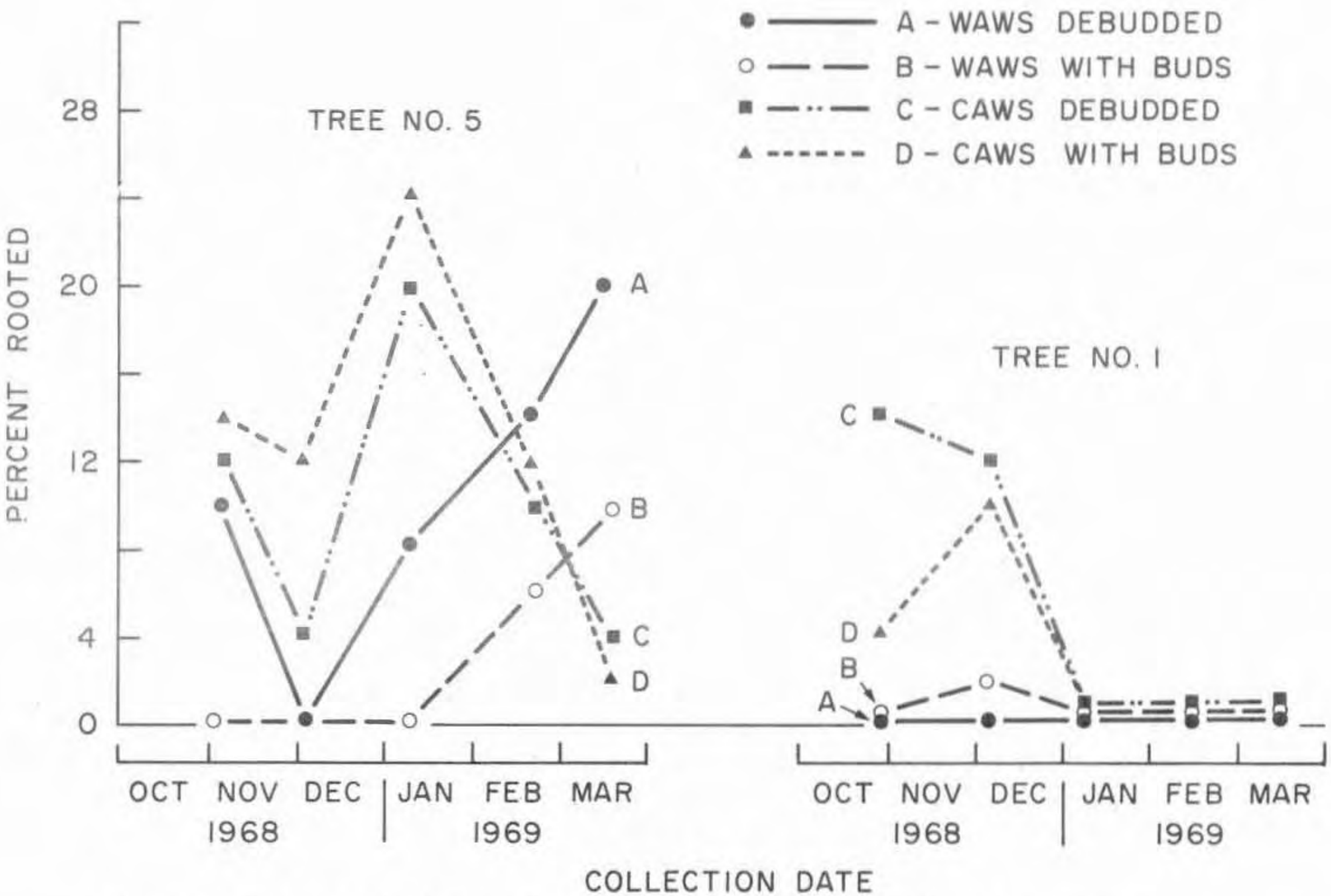

Fig. 1. Effect of bud removal in rooting of cuttings collected from two mature trees in different months and set under two temperature conditions, i.e., warm air-warm soil (WAWS) and cold air-warm soil (CAWS).

from woody plants is well demonstrated (Thimann and Behnke-Rogers, 1950). It is equally clear that auxin is not the only or, in many cases, the major limiting factor in rooting of cuttings. Evidence has accumulated in the last decade for a synergistic action in rooting between auxin and phenolic compounds, termed co-factors (Fadl and Hartmann, 1967; Girouard, 1969; Hess 1962, 1969; Hyun, 1967; Hyun and Hong, 1968). In addition to growth regulators, nutritional factors may be of direct or indirect importance (Hyun and Hong, 1968).

In spite of extensive biochemical investigations in recent years, and successful isolation of some compounds with root promoting activity, only the auxins have been of practical use. Of the several auxins, the synthetic indolebutyric acid (IBA) appears to be the best for most woody plants.

For cuttings from Christmas-tree-sized Douglas-fir, Lanner (1962) and Blankensop and Callaham (1960) did not get improved rooting by use of IBA. For somewhat bigger, although young, Douglas-fir trees, Griffith (1940) made an extensive comparison between the effect of three auxins, i.e., IBA, indoleacetic acid (IAA) and naphthaleneacetic acid (NAA), on rooting of cuttings collected from October to May. IBA was the most effective and NAA the least, and the recommended application was a 24-hour basal soak in 50 ppm IBA.

Our studies include many of the chemicals reported in the literature to have had some effect on rooting. In agreement with Griffith (1940), IBA was the most effective of the auxins IAA, IBA and NAA. Several vitamins (B_1, B_2, B_3, B_5, B_6, C and H) were tested, using a 24-hour basal soak in a 25 ppm solution combined with 100 ppm IBA. None were stimulatory to rooting. Boron has increased rooting of holly (Weiser and Blaney, 1960) but has not affected Douglas-fir in concentrations of 25 and 10 ppm with a 24-hour soak. Because of the renewed interest in ethylene as a growth regulator and early reports of its effect on rooting of tomato (Zimmerman and Wilcoxon, 1935), this gas was tested for cuttings from three old Douglas-fir trees. Ethylene was absorbed to saturation in water, which contains 12.2% ethylene when prepared at 20 C (Crocker et al., 1935), and the cutting end was soaked in this solution for ½, 1, 2, 3 or 24 hours. Some cuttings were completely submerged for 2 or for 18 hours. No IBA was applied. These ethylene treatments did not affect rooting and, surprisingly, no other effect was apparent. Argenine and adenine in 10 and 100 ppm (24-hr soak) and 1 and 3% sucrose (24-hr soak) had no effect on rooting. Several phenolic rooting co-factors were tested in combination with IBA. They included catechol, caffeic acid, quinic acid, chlorogenic acid and tannic acid in 10^{-3} and 10^{-4} M concentrations (24-hr soak). They were used singly or all combined for cuttings from three old trees, one of which was known to be difficult and another easy to root. No increase in rooting was obtained, and catechol and tannic acid decreased rooting in the 10^{-3} M concentration.

The IBA has been applied in different ways, i.e., by soaking the basal cutting end in weak solutions (25, 50, 75, 100, 150 and 200 ppm) for various periods of time (12, 24 and 48 hr), by dipping for 5 sec in stronger solutions (1-5000 ppm), and by dipping the end in an IBA-talc powder mix using IBA concentrations from 0.01 to 4%. Various commercial rooting preparations have also been tested. The most consistently good results were obtained with a 24-hour soak in 100 ppm IBA and with a 0.8%

IBA powder application. For other rooting studies, we have standardized on the former method. Cuttings from young plants have also benefitted from IBA treatment, but only half the above-stated concentration should be applied. The amount of ethyl alcohol used to dissolve the IBA should be kept at a minimum to avoid damage. We use 110 ml of 90% alcohol to dissolve 1 g IBA; when this solution is diluted to 100 ppm IBA, the alcohol concentration is 1.1%. The water soluble potassium salt of IBA has not given better rooting or reduced mortality in the rooting bed. Cuttings should not be treated with IBA before storage.

Retreatment of cuttings with IBA has been tested on a limited scale. In one experiment, cuttings were treated with 100 ppm in a 24-hour soak before being set for rooting in mid-November in cold air-warm soil. Those not rooted in early May were divided into two groups; one had the basal end recut and dipped in 1% IBA powder and the other was left untreated. Of the retreated cuttings, 54 rooted, compared to 32 for the control.

(d) Fungicides

Pathogens have usually not been a problem during cold storage but mold did develop on one collection of cuttings. This was remedied by a 2-minute dip of the cuttings in a 5% solution of a commercial bleach containing 5.25% available chlorine, followed by a rinse in water.

As discussed later, mortality in the rooting bed is at times considerable. For some trees, in some years, the physiological conditions of the cuttings make them intolerant to the treatment and the environment needed for good rooting, and causes physiological disorders and death.

Serious mortality has also been caused by pathogens, but insects have not been a problem.

Various fungicides were tested to prevent infection in the rooting bed. In the early studies, the part of the stem to be inserted in the rooting medium was dipped in a talc powder with 4% Arasan. Although this prevented some fungal attack, it was not completely effective. Hough (1953) found this fungicide treatment essential for rooting of Douglas-fir cuttings. Captan (1 tablespoon of 50% W Captan per 2 gal water) was not helpful as a soil drench or in a 5% talc powder mix applied to the stem base. It has provided good control of botrytis when sprinkled periodically on the cutting top. Botrytis can be especially destructive during flushing of new shoots. Soil treatment with Terrachlor, Morsodren, and Panodrench has not given sufficient protection.

The best treatment is an application of the systemic fungicide benomyl (trademark Benlate, Dupont). Benlate (50% active benomyl) was tested both as a basal powder dip in concentrations from 2.5 to 10% in talc and as a basal 24-hour soak of the cuttings in 75, 100, 150, 200 and 300 ppm, with or without a supplementary soil drench of 300 or 600 ppm. The recommended treatment is a 24-hour soak of 150 ppm Benlate for cuttings from mature trees, but only 75 ppm for seedling material, or a 10% Benlate powder. Benlate is not water soluble, but is applied in the 150 ppm concentration, together with the alcohol-dissolved IBA solution. Carville (1971) found that a 5% (a.i.) benomyl powder treatment gave the highest percentage rooting of rhododendron cuttings, and this fungicide is becoming widely used in propagation of cuttings.

(e) Paired cuttings

Since cuttings from young Douglas-fir trees root readily, we investigated whether they would stimulate rooting of cuttings from mature trees when grafted together in pairs (Fig. 2). Early results were

Fig. 2. Cuttings from a young and an old tree grafted together before insertion in rooting bed.

promising (Brix, 1967; Brix and Barker, 1971). Stimulation of rooting of the old cutting with this technique appears to originate from the roots initiated on the seedling cutting, although presence of the seedling top did increase the rate of rooting of the old cutting.

Further tests have shown this to be a valuable method. Cuttings from six mature trees rooted 22% when paired with young cuttings, compared to 11% for single cuttings from the same trees. Perhaps most significant was the finding that cuttings from two of the old trees did not root at all when single, but rooted 8 and 14% when paired with seedling cuttings. Similar encouraging results were obtained in another study using cuttings collected at different times from two old trees that had been difficult to root by other means (Table 4).

Table 4. Rooting of single cuttings from young and old trees and of cuttings from old trees when paired with young cuttings

Tree	Collection date	Per cent rooted and date inspected		
		Single	Paired	
		Oct 1972	Oct 1972	Oct 1973
G6 (old)	Nov 1971	8	48	48
G6 (old)	Dec 1971	5	27	30
Average old tree		7	38	39
Average young trees		62	-	-
GS2 (old)	Dec 1971	0	10	25
GS2 (old)	Jan 1972	0	50	50
Average old tree		0	30	38
Average young trees		54	-	-

ROOTING MEDIUM

In addition to giving physical support to the cuttings, the rooting medium must supply adequate moisture and, at the same time, provide good aeration to the stem base. The medium can therefore become too dry or too wet. The best medium is likely to vary with the watering system used. For instance, straight perlite or coarse sand may be a good medium with an automatic watering system supplying an abundance of water, but a medium with much peat moss will become too wet for some cuttings. On the other hand, with less water supply some peat moss may be desirable. In early studies, we used intermittent misting controlled by evaporation of water from a steel mesh (Geiger, Mist-A-Matic), and peat moss in excess of one-third total volume was detrimental. The medium now used is equal volumes of fine peat moss, washed coarse sand and coarse grade perlite. This mixture provides sufficient water and aeration with the intermittent misting system, and is suitable for cuttings also in high air-humidity under a plastic cover with just twice a week watering. A vermiculite-sand-peat moss mixture is not as good, and perlite alone becomes too dry under the last-mentioned watering regime.

PROPAGATION STRUCTURE

In early experiments, cuttings were set in greenhouse benches. After it was shown that cold air combined with bottom heat was beneficial for fall-collected cuttings, it was thought that a more favorable temperature differential between air and rooting medium might be obtained during the winter in an outdoor frame. This would also have the advantage of simplifying the facility requirement.

The outside frame measured 70 x 244 cm inside and had 25 cm high sides (Figs. 3 and 4). The frame was placed on gravel for better drainage, and sand 5-8 cm deep covered the bottom. A 18 m (115 V, 3.5 amp) leaded heating cable was embedded in the sand. Eight flats 33 x 61 x 10 cm could be placed closely together, the spaces between them carefully filled with sand to minimize heat escape. The sensing bulb for the thermoregulator was embedded in sand between two flats at the same depth as the basal end of the cuttings. The temperature could be maintained at 20$\pm$1.5 C. The frame was covered with a tight fitting, clear plastic sheet to maintain high humidity around the cutting tops. Watering was needed only once or twice a week. To avoid buildup of heat under the plastic cover during sunny periods, the frames were oriented east-west, lengthwise, and a shade was fitted on the south side and at the ends to a height just sufficient to exclude direct sunlight throughout the summer. A clear, plastic sheet, fitted to the top of the shade, covered the open front and prevented rain and snow from falling on the inside plastic cover. The inside of the frame and shade was painted white for better light reflection and the shallow width prevented excessive shade at any location.

For comparison, cuttings were collected during four periods from mid-December to mid-March from two old trees; half were set in the greenhouse and the remainder in the outside frame. Bottom heat only was supplied at both places. No difference in rooting between these groups of cuttings was found. Similar comparisons performed in other years has shown the outside frame to be as good or better than the greenhouse.

ENVIRONMENT DURING ROOTING

(a) Watering and humidity control

To avoid excessive desiccation of cuttings, both the rooting medium and the air must be kept at high humidity. In the beginning, this was accomplished by intermittent misting, using a control system (Geiger

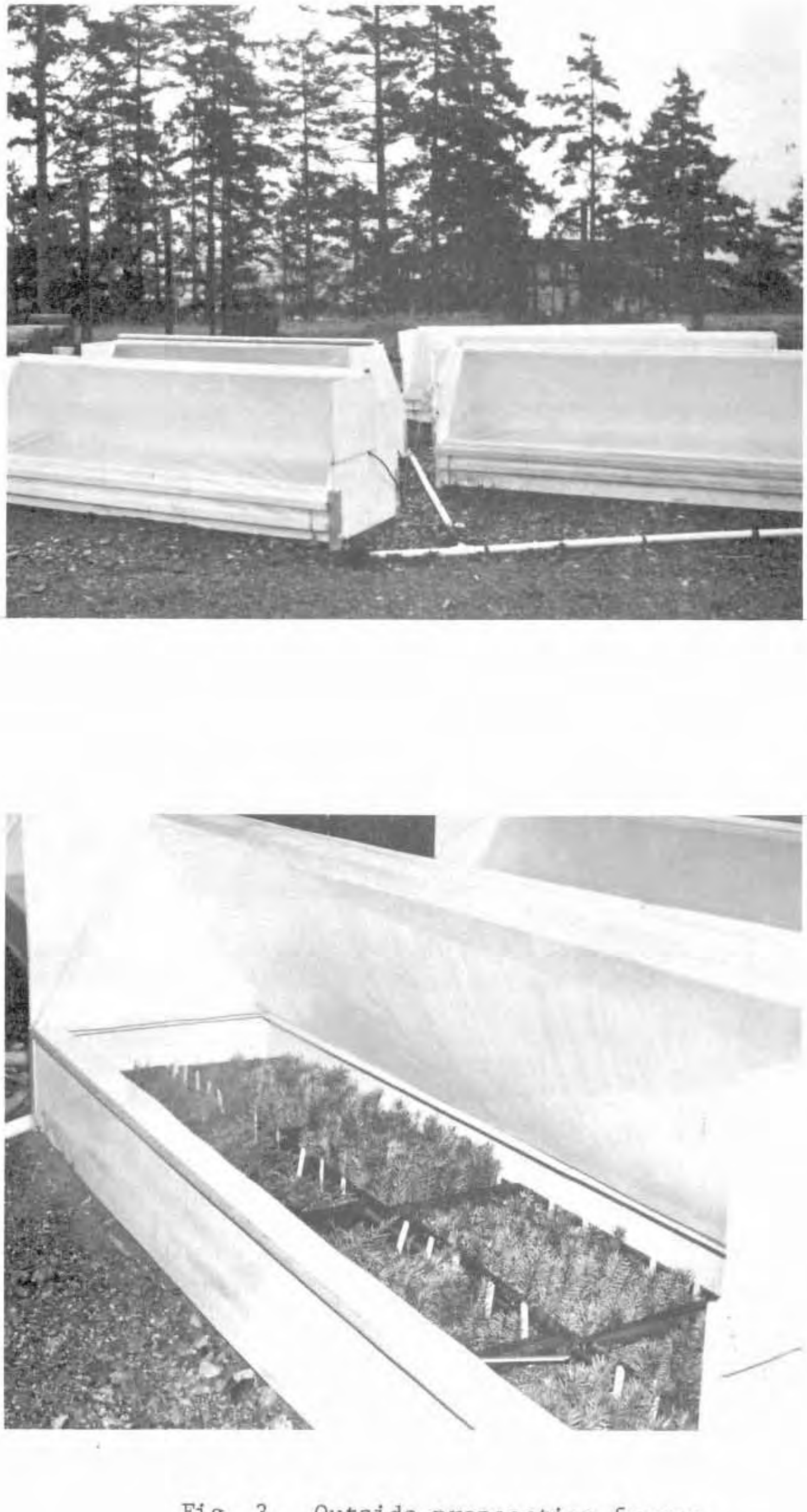

Fig. 3. Outside propagation frames.

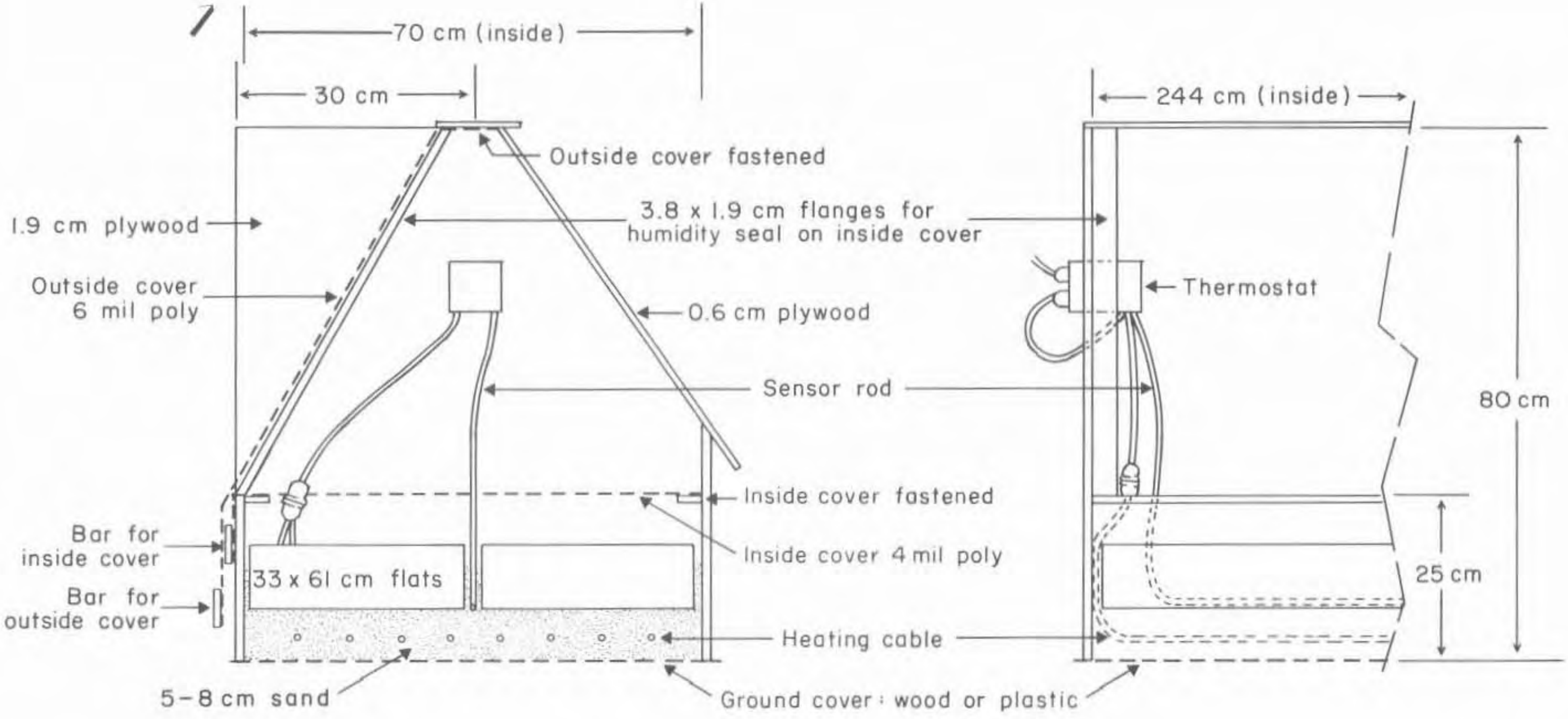

Fig. 4. Diagram of outside propagation frames.

Mist-A-Matic) acting on a solenoid valve inserted in the water line. Mist falling on a balanced stainless steel screen, and evaporation from it, tripped a mercury microswitch connected to the solenoid valve. Various types of nozzles were used from time to time. This system was fairly reliable, although occasional power and mechanical failures were experienced. For this reason, and to simplify equipment needs, we tested a plastic-covered frame, both in the greenhouse and outside, for maintenance of high humidity. Sprinkling was needed only once or twice a week, and the only possible failure would be caused by lack of a completely tight plastic cover. This system has worked well and has been used exclusively for the last 3 years.

(b) Temperature

For rooting to occur, various chemical substances must be produced or mobilized in the top of the cuttings and translocated to the basal stem portion, where they are utilized in root initiation and development. The processes involved in the top and at the base are therefore quite different and may require different environmental conditions, such as temperature. Also, the optimum temperature regime during-rooting may depend on the physiological conditions of the cuttings and therefore on the season of collection.

Heating the stem base of cuttings while keeping the top at a lower temperature has favored rooting of cuttings of some plants (Hartmann, 1969; Sandved, 1963). Three temperature regimes were tested for Douglas-fir cuttings: (1) air and rooting medium not heated except to keep frost-free (cold air-cold soil, CACS); (2) air not heated but rooting medium heated to 20 C (cold air-warm soil, CAWS), and (3) both air and rooting medium heated to 20 C (warm air-warm soil, WAWS). In one study, cuttings were collected in mid-November (1967) from four old trees and their rooting percentages were 32, 22, 20 and 18 with the cold air-warm soil treatment, whereas none rooted with the other temperature regimes (Brix and Barker, 1969). Interestingly enough, western hemlock collected in the fall rooted best with no heating of air or soil. In a subsequent study, Douglas-fir cuttings were collected monthly from September (1968) to the end of March (1969) from five trees, 70 to 100 years old. Trees with large crowns almost to the ground were selected, since an abundance of easily accessible cutting material was needed. Plastic-covered frames in heated and unheated greenhouse compartments were used for rooting. Heating of the rooting medium will inevitably affect the air temperature around the top of the cuttings. On the average, during the winter months, taking early morning and afternoon temperatures, the unheated greenhouse temperature was 6.7 C and the air under the plastic cover at the top of the cuttings was 12.2 C when the soil at the cutting base was heated to 20 C. All cuttings were treated with 4% Arasan and a 24-hour soak with 100 ppm IBA.

Results confirm the earlier observation that best rooting is obtained when cuttings are collected in November and placed in CAWS (Table 2). The cold air becomes less beneficial the later the collection, and no beneficial effect was obtained by February. Tree No. 1 rooted in significant numbers only for the November and December collections under CAWS. Tree No. 2 gave a poor return, but rooted 32% in the previous year's trial for the November collection under CAWS, so year-to-year variation in rooting can be extensive. For cuttings from seedlings, good rooting was obtained with November and December collections in WAWS after cold storage (2 months at 0 C) and also without storage. Cold storage of cuttings from old trees did not substitute for the cold air treatment in the cutting bed.

Without cold storage or cold-air treatment, bud flushing in the spring is delayed or absent for fall-collected cuttings from both old and young trees. Very short shoots are often produced and sometimes only with aborted leaves. However, this shoot is able to produce a bud that can form a normal shoot the following year, or a bud may form in a leaf axis of the old shoot. Even chilled cuttings produce a short shoot with reduced leaf size in the rooting bed.

(c) Light

Indications are that a heavy shading is detrimental to rooting. Since cuttings remain in the rooting bed for several months, it is likely that carbohydrate supply will become limiting unless light is sufficient to sustain some photosynthesis. In the section on mortality, it is shown that shading affects cutting mortality. We therefore admit as much diffuse light as possible in our outdoor frames, but exclude direct sunlight, since this results in excessive heating under the plastic cover. Only natural daylight has been used, except in one growth-room study. The effect of photoperiod should be tested, although Baker and Link (1963) found that long photoperiod did not improve rooting of cuttings of the majority of the 26 woody ornamental species they studied, and any improvement was masked by the use of IBA.

PHOTOSYNTHESIS DURING ROOTING

As discussed in previous sections, maintenance of a favorable environment is of great importance, since cuttings remain in the rooting bed for several months. One of the first studies undertaken (1965-66), using intermittent misting, was to ascertain whether this system would prevent a serious desiccation of the cuttings and enable them to continue photosynthesizing. At the time of the study, cuttings were kept at a temperature of 20 C in the air and rooting medium, which consisted of equal volumes of peat moss and sand.

Rates of CO_2 uptake were measured with an infrared CO_2 analyzer (Beckman model 15 A) for shoots intact on seedlings, at a light intensity of 1000 ft-c and at 20 C. Subsequent rates measured with the same light and temperature were expressed as a percentage of this rate. The shoots were then excised and the basal end recut under water. They were inserted in the rooting medium, contained in a plastic pot, through holes in a plastic cover (3 mm thick). The holes were sealed during gas exchange measurements to provide a gas-tight barrier between the top of the cuttings and the rooting medium. Between measurements, the pot was placed in the rooting bed under intermittent misting. During CO_2 measurements in the laboratory, the cutting tops were enclosed in a chamber through which air was circulated in a closed system with the CO_2 analyzer. Cuttings were sprayed with a mist of water at 5-minute intervals through a hole in the chamber to simulate conditions in the rooting bed. For the first 3 days in the bed, photosynthesis was close to the rate for the shoots on the intact plant, but declined rapidly the next 2 days to 58% (Fig. 5). One month later, and for 3 weeks thereafter, the rate was 28%. After about

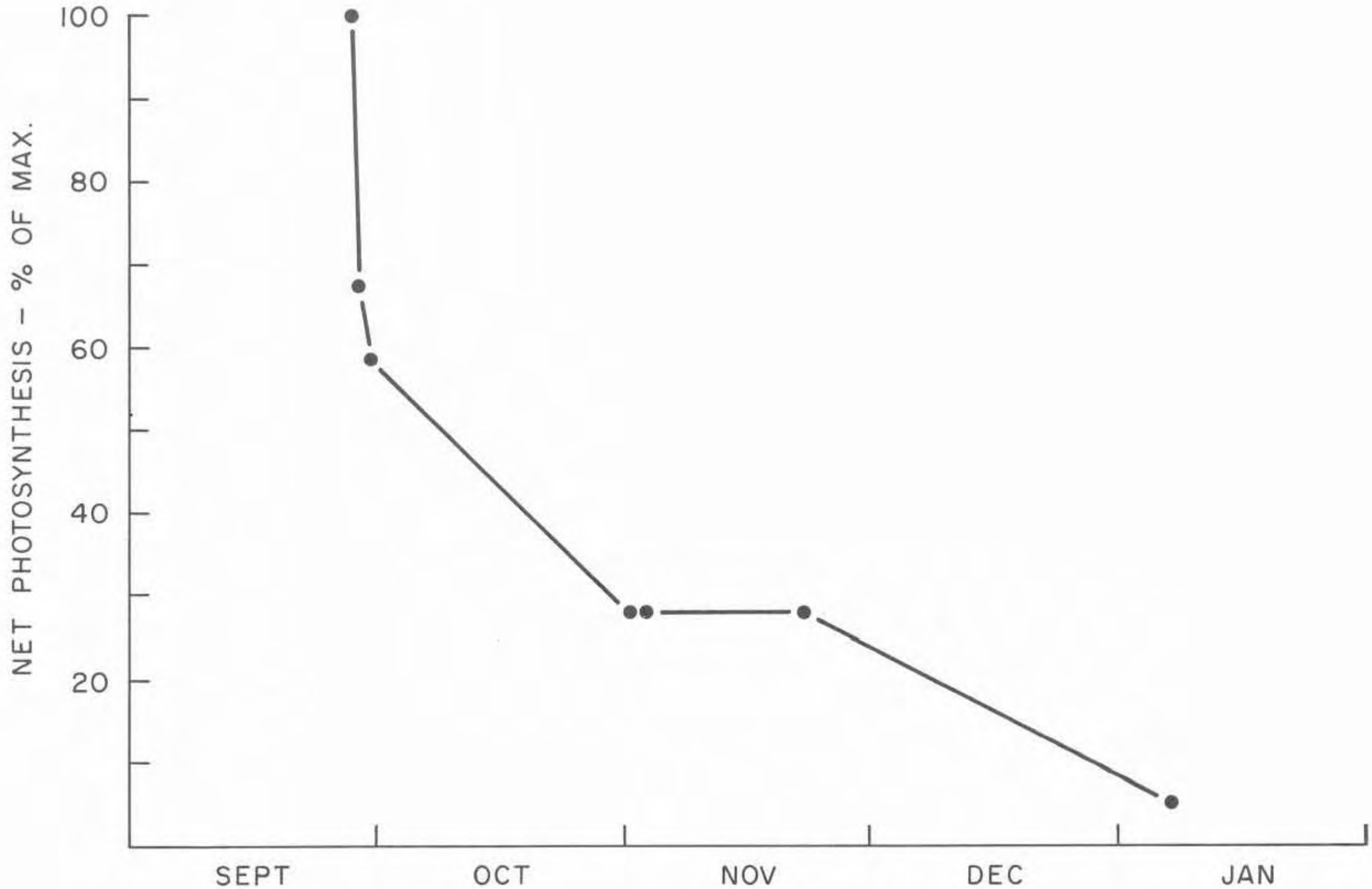

Fig. 5. Time change in rates of photosynthesis of Douglas-fir cuttings as a percentage of the rate before excision on Sept. 27.

3½ months in the rooting bed, the rate had declined to 5%, which was the same rate by which CO_2 was released in dark respiration. No carbohydrate depletion takes place during this length of time under the conditions of the study, and it appears that the photosynthetic capacity of the cuttings can take advantage of a favorable light intensity, though this relationship was not studied.

MORTALITY

Mortality of cuttings in the rooting bed has been a major problem in some years. Decay usually begins at the stem base and is sometimes confined to a part of the stem in the rooting medium. Roots may then form at the base of the living tissue. If the decay progresses above the rooting medium, the cuttings will eventually die. As discussed under the section on fungicides, pathogens cause some of this decay, but another reason may be a physiological deterioration associated with the condition of the cuttings when collected and the treatments they receive. Van Elk (1969) found that some conifer cuttings, placed in a hot environment, may decay at the base when treated with hormones or Captan though, under more favorable temperature conditions, both substances increased rooting, especially if combined. For some crabapple varieties, Howard (1968) showed that it was unlikely that basal rot was caused by fungal pathogens. The rot occurred mostly in cuttings collected in fall and early winter rather than late winter and spring, and increased with an increase in IBA concentration and at high temperature. He believed that high IBA predisposed the cuttings to rotting.

Cuttings collected in the fall have, in some years, suffered more from decay than winter collections and changes in susceptibility can be abrupt. For instance, cuttings were collected from the same trees in two periods, 26 November to 2 December and 7 to 19 December, and set for rooting immediately thereafter. Mortality was 66% for the first collection and only 22% for the latter.

The effect of light intensity during rooting on mortality was studied, using shadings of 0, 50 and 75% of the natural light in a greenhouse. Cuttings were collected in late November and in mid-March from two old trees and a group of juvenile trees. All cuttings were set for rooting in mid-March in warm air and rooting medium after treatment with a 24-hour soak in 100 ppm IBA and 150 ppm Benlate. Mortality at the end of May was not significantly different between collection dates or between juvenile and old cuttings, but cuttings from one of the old trees were significantly different from the rest and reached 100% mortality in the 2 shaded plots. The average mortality was 31, 55 and 79% in the 0, 50 and 75% shade, respectively. This points out the importance of providing good light conditions in the rooting bed. Differences among trees in cutting mortality have also been pronounced in some of our other studies, even for trees growing in close proximity.

The effect of IBA treatment on mortality was studied with and without storage (5 weeks at 0 C) of cuttings from old and juvenile trees collected in late November. The IBA was applied in a 24-hour soak at 100 ppm and as a 0.8% powder dip and also in the form of KIBA. No significant effect of cutting storage, auxins or their mode of application on mortality was found, but IBA may be harmful in higher concentrations and when used with non-dormant cuttings.

ANATOMICAL AND SEASONAL PATTERN OF ROOTING

The morphological and anatomical basis for rooting of cuttings from woody plants was reviewed by Girouard (1967). For Douglas-fir, Heaman and Owens (1972) found that root primordia arise in the basal callus, which is proliferated from the lowest cells of the vascular cambium, in association with differentiating phloem and wound cambium. As mentioned previously, root initiation only occurred from the basal stem cut, in our studies, and not along the wound up the stem, as is the case with cuttings from many other plants.

As found by Griffith (1940) and Blankensop and Callaham (1960), callus formation is generally evident within 2-3 weeks after cuttings are set for rooting and is accompanied by a swelling of the stem base. The extent of swelling is restricted by the presence of buds near the base (Heaman and Owens, 1972). The amount of callus is no indication of how well the cuttings will root and there has been a tendency, in our studies, for production of more callus on cuttings from old than from young trees.

The time required for rooting of cuttings from young Douglas-fir trees was studied by Griffith (1940). Cuttings collected in October-November and in April-May required 120 to 150 days, but those collected in February-March required only 60 to 100 days. Blankinsop and Callaham (1960) showed a periodicity in rooting of cuttings from young Douglas-fir trees. Cuttingsset in December started rooting in mid-April; few roots appeared in May and June, but rooting increased in July.

Cuttings were inspected periodically for rooting while in the rooting bed. The seasonal pattern of rooting for the 1967/68 season for cuttings in cold air-warm soil in the greenhouse is shown in Figure 6 as a percentage of the total rooted by the end of August 1968. This record was made for cuttings collected and set for rooting at different times. For the November-December collection from old trees, a few rooted as early as January and a significant number rooted in February. Half of the cuttings that were going to root had done so by the end of April, except for the February-March collections which did not reach the 50% level until the end of June. Cuttings from seedlings followed a similar rooting pattern. Although variable for different trees and treatments, many cuttings flushed by mid-April in the temperature regime studied. Bud flushing was accompanied by a period of good rooting. However, rooting occurred long before and long after bud flushing for most cuttings and cuttings may flush without producing any roots.

ROOTING PERCENTAGES AND VARIATION AMONG TREES

Good and poor rooting trees have been recognized for many forest tree species (Hyun, 1967; Schreiner, 1967), and Black (1973) found greater variability among old than among young Douglas-fir trees. This tree to tree variability has also been evident in our studies, not only in rooting behavior but also in mortality, as pointed out earlier. Several old trees have been used from year to year. Some trees have been consistently poor and others good 'rooters', whereas others have shown variable response. The rooting percentage in a study with cuttings from 32 selected plus trees, all over 80 years old, varied from zero to 82% and averaged 15% for all trees; 78% of the trees produced some rooted cuttings, although only 25 cuttings were used per tree. Tree-to-tree variation in rooting and mortality of the cuttings is also illustrated in other sections. Some of

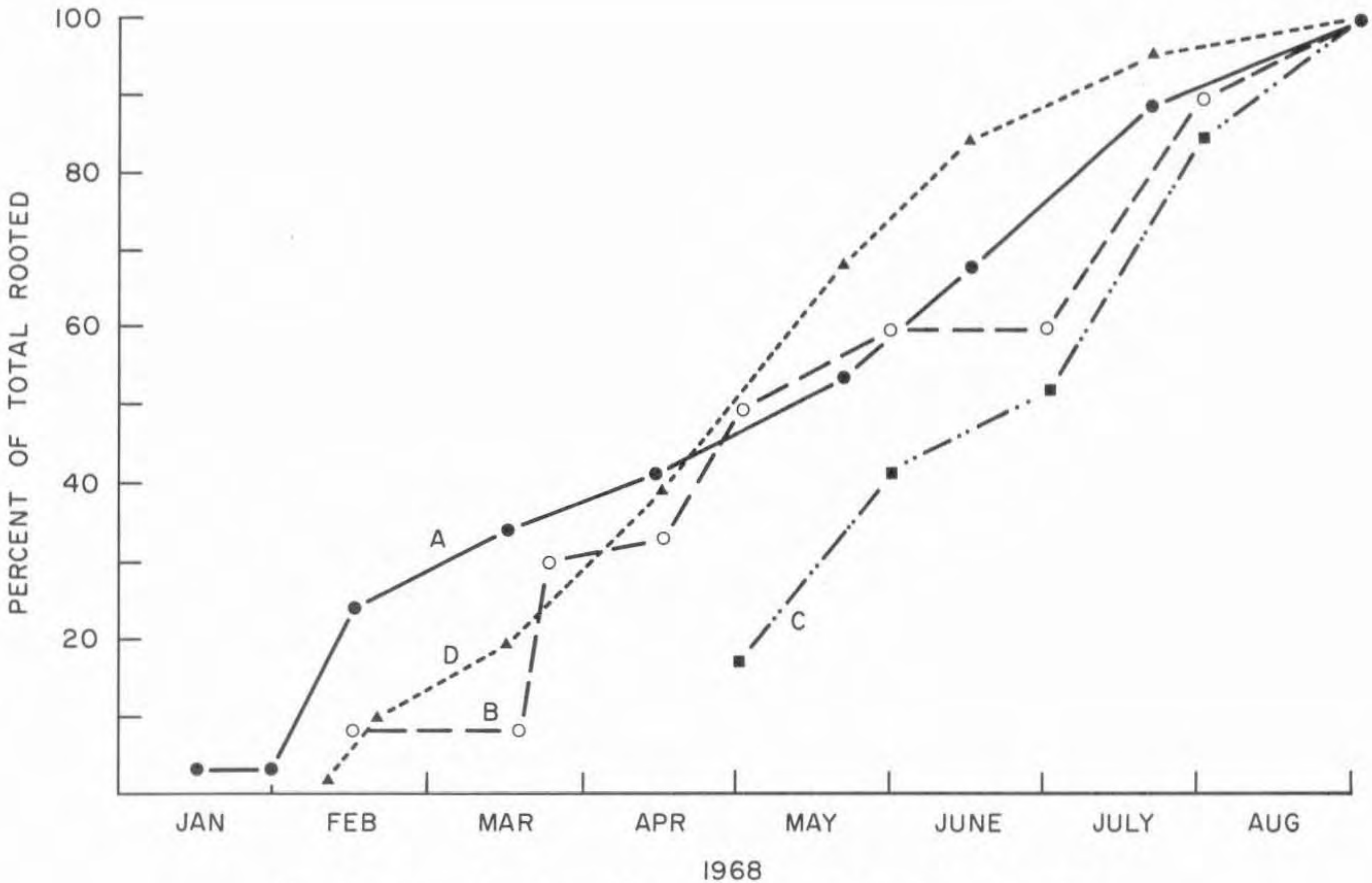

Fig. 6. Seasonal pattern of rooting for cuttings from old and young Douglas-fir trees collected at different times. Data expressed as a percentage of cuttings rooted by the end of August. a, b, and c: cuttings from old Douglas-fir trees collected and set Nov.-early Dec., Dec.-Jan. and Feb.-March, respectively. d: cuttings from young Douglas-fir trees.

this variation is likely caused by the environment in which they grow. However, trees growing in close proximity have also differed in rooting, and the study comparing rooting of ortets and their ramets seems to indicate a strong genetic influence. This type of variation complicated our study tremendously, and makes it difficult to prescribe a treatment that will work for all trees and in every year.

TREATMENT, GROWTH AND FLOWERING OF ROOTED CUTTINGS

When cuttings have 2 cm or longer roots, they are transplanted into 5-cm square plastic pots with a mixture of equal volume of peat moss and sand. For the following month they are given a weekly application of a $CaNO_3$ solution (20 ppm N) and kept in a humid location shaded from direct sunlight. Thereafter they are fertilized with a complete nutrient solution until the fall after rooting using 1 tablespoon of a 28-14-14 formulation per 9 liter solution and about 10 cc/5-cm pot/week. The concentration of the solution is 310, 130 and 160 ppm of N, P and K, respectively. A good root system has developed by then but shoot growth is usually limited to the 2- to 8-cm shoot developed in the rooting bed. Juvenile shoots generally produce the best growth, as also shown by Black (1973). Cuttings are transplanted into 10-cm plastic pots, placed in an outside shadehouse, and given a weekly supply of the 28-14-14 solution (about 20cc/pot) during the following growing season. They are then ready for transplanting into 18-cm pots in which they grow for 1 year, giving them two or three growing seasons from rooting. Most cuttings will then be ready for transplanting in the field; they are sturdy and have a good root system. The total height growth at this time is variable, but may average

Fig. 7. Rooted cuttings from Douglas-fir plus trees outplanted at B.C. Forest Service Mesachie Lake Experiment Station.

Fig. 8. Douglas-fir cuttings with seed cones 2 years after rooting.

25 to 30 cm. A leader growth of 30 cm or more is common during the first growing season in the field. Plants retain the plagiotropic branch-type growth for several years and are therefore staked both in the pots and after outplanting (Fig. 7). Terminal cuttings from the top section of the crown and upright shoots resulting from shearing produce orthotropic plants more rapidly than other shoots (Black, 1973).

Since the objective of rooting cuttings, in most cases, is to establish clonal material for breeding and seed production, the flowering performance is a major concern. Our material is still too young to give a long-term prediction, but in 1971, many of the cuttings produced male and female flowers. Some had been rooted only 2 years so the flowers were produced on the first shoot initiated after rooting (Fig. 8).

CONCLUSIONS

A great many factors related to conditions of cuttings when collected and their subsequent treatment will affect the rooting of Douglas-fir cuttings. The single most important factor is tree age. Genetic differences appear to be of major importance in rooting of cuttings from mature trees. Cuttings from different trees can respond quite differently in mortality and in rooting in response to treatments such as temperature, bud removal and chemical regulators, and to time of cutting collection. Some treatments may therefore be beneficial to cuttings from some trees but not to those from others. This feature has greatly compounded the work involved in prescribing treatments that generally facilitate rooting of cuttings from mature trees. However, with the treatments prescribed in this report, cuttings from most of the trees tested have rooted with a success that should be acceptable for establishment of clonal material to be used in seed orchards and tree breeding. If some trees prove to be difficult to root, using single cuttings, the paired cutting method offers a possibility for rooting. Growth after rooting is slower initially than one would expect with grafted stock, but is satisfactory some years after establishment. Both pollen and seed cones have been produced on several clones a few years after rooting, but no long-term prediction of cone production can be made at this time.

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