PHASIC DEVELOPMENT AND PHYSIOLOGICAL CONDITIONING IN THE ROOTING OF DOUGLAS FIR SHOOTS

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Timing the taking of cuttings of woody species to coincide with their achieving maximum rooting potential is still one of the goals of propagation research. Determining the physiological status of the stock plant and/or its cuttings at these optimum periods for root regeneration and developing means of physiologically conditioning the shoot before and during the rooting process are providing refinements in vegetative propagation techniques.

The rooting potential of Douglas fir stem cuttings has been correlated with distinct phases of development, both in source tree aging (11) and in annual shoot periodicity (9). Even phases of adventitious root initiation and development after cutting excision respond to specific stimuli (12).

THE STOCK PLANT

Aging and flowering. The loss of rooting potential of cuttings with increasing plant age in many species has been attributed to phase changes during the transition from juvenile to adult. Black (3) found that cuttings from juvenile Douglas fir seedlings under 9 years of age had the potential for rooting 100 percent, but that there was a rapid decline in rootability after this age to less than 5 percent between ages 14 and 24 years, the implication being that the loss in rooting potential coincided with vegetative maturity and the onset of flowering. However, he found little or no difference in cutting rooting potential in different crown levels of trees up to 24 years from seed, as would be expected if the transition from juvenile to adult was progressive from the base of the tree upward. Achterberg (1) reported that Douglas fir cuttings from the lower parts of the crown rooted best. Roberts and Moeller (11) found no reduction in rooting potential of Douglas fir shoots from seedlings up to 15 years of age, at which time they were considered to have reached vegetative maturity since 4 out of 14 trees had been cone bearing for 3 years. However, the cuttings were always taken from the lower one-third of the crown which was possibly juvenile in character.

1978 Experiment. Cuttings from the above mentioned trees were again taken in January, 1978, to determine any change in rooting potential with advancing age and/or increased flowering and cone production. Instead of taking all the cuttings from the lower crown level, 40 cuttings were taken from the lower, mid, and upper one-third of the crown of each of the 14 seedlings. The cuttings were given a standard 5 sec. dip in a 10% Dip 'n Grow- 95% ethanol solution (Dip 'n Grow is a commercial preparation containing 1.0% 3-indolebutyric acid, 0.5% naphthaleneacetic acid, 0.0175% boron, 0.1% Phygon (Dichlone), and 20 % methyl sulfoxide (DMSO)). Twenty cuttings from each crown level were rooted under open-bench misting and another 10 under poly-tent fogging.

RESULTS AND DISCUSSION

The average rooting percentage for cuttings taken from the lower crown level of these trees during their 12th-16th year are given in Table 1 along with the approximate number of cones produced by each tree in 1978. Rooting potential of shoots in the lower portion of the trees did not change significantly over the first 16 years of their existence, although all but 3 of the trees are now producing cones and 4 have produced cones for the last 4 years. However, it appears that the rooting potential of cuttings out of the upper two thirds of these trees is significantly less than in the lower one-third (Table 2). We have no record of when this position advantage developed. It is evident, however, that some trees in the population exhibit this relationship more strongly than others, and that it is not dependent on cone production for expression. This would suggest that loss in cutting rooting potential in Douglas fir with increasing source plant age is not dependent on flowering, cone production or loss of juvenility *per se*, but rather other factors related to physiological maturity. It would appear also that this loss in rooting potential is localized somewhat in certain parts of the tree. This would seem to provide evidence that juvenility persists in the lower parts of the tree, except that many of the cones on the heavy bearing seedling 33 were on the lowest branch whorl and near the ground.

Physiological conditioning. Manipulating the stock plant environment and/or otherwise physiologically conditioning the source plant with chemicals or physical treatment have been used to enhance cutting rootability in a number of species. Light and temperature modifications, as well as feeding organic and inorganic nutrients prior to cutting excision have had significant effects on subsequent rooting. Shoot girdling is another example of attempts used to control movement or accumulation of rooting factors at rooting sites. Whether cuttings are taken when the growth phase and environment interact to produce shoots of high rooting potential, or the stock plant is artificially manipulated to bring about the optimum physiological condition for rooting, it appears that stock plant treatment may be more effective or at least as effective as cutting treatment in bringing about root regeneration in cuttings (4).

Table 1. Changes in rooting potential (rooting percentage) of Douglas fir cuttings with increasing age of source trees and onset of flowering. Cuttings taken from lower crown level.

Seedling	Tree Age in Years				
	12	13	14	15	16
22	—%	100%	85%	65%	85%
30	100	100	55	87	95*
31	—	95	80	68	83
32	—	100*	50	79	100
33	90	75	55	78	85
34	90	80	80	87	85*
35	100	45	35	60	73*
36	25	10	40	49	30*
37	85	55	50	46	80*
38	65	75	60	68	73*
39	—	25	50	81	37*
106	85	45	80	59	53
107	80	85*	95	66	95
109	25	5*	5	32	15
Average	75	64	63	66	71

* First year of cone production.

Table 2. Effect of cutting source (crown level) on rooting potential of Douglas fir stem cuttings from 16-year-old trees, 1978

Seedling Number	Crown Level Upper ⅓	Crown Level Middle ⅓	Crown Level Lower⅓	Number Cones/Tree
22	63%	95%	85%	0
30	83	78	95	17
31	60	48	83	0
32	40	55	100	625
33	65	60	85	540
34	53	75	85	90
35	58	40	73	1
36	18	35	30	150
37	73	47	80	7
38	63	85	73	1
39	45	35	37	300
106	35	65	53	0
107	60	50	95	425
109	3	5	15	360
Average	51%	53%	71%	

Means of 2 replications of 20 cuttings each or total of 560 cuttings per source.

Whitehill, *et al* (13), working with *Pinus sylvestris*, demonstrated that rooting potential of cuttings was determined largely by growth phase of the stock plant. He found stock plants subjected to short days (SD) and cold stored for 60 days at 0°C before excision and then rooted under a 17-hour photoperiod (LD) rooted significantly better (85%) than those under LD (5%) and actively growing at time of excision and cold stored. Rooting *Ilex crenata* 'Hetzii' cuttings was improved by increasing the number of SD given the stock plants during the summer growing period over that of stock plants under LD (6). The opposite results have been obtained with *Salix undulata* (7) and *Populus canadensis* (8), where LD treatment of stock plants has favored the subsequent rooting of cuttings.

1970 Experiment. To determine the effect of stock plant lighting on the rootability of shoots subsequently used for cuttings, trees of several Douglas fir clones were moved into growth chambers for two months under continuous, 16-hour (LD) or 9-hour (SD) illumination. Two trees of each cultivar were given the three light regimes. During the first 5 weeks the air temperature in the chambers was 5°C both day and night and the light intensity during the light periods was 1186 $\mu\omega cm^{-2}\ nm^{-1}$. Ten shoots to be used as cuttings were girdled at the future cutting base to determine if rooting factors would accumulate above the girdle and favor subsequent rooting. The 10 girdled cuttings and 10 not girdled were taken from the trees at the end of 5 weeks, given a standard 5 sec dip treatment in 10% Jiffy Grow- 95% ethanol solution (Jiffy Grow is a commercial preparation containing 0.5% 2-naphthaleneacetic acid, 0.5% 3-indolebutyric acid, and 0.0175% boron), and rooted under open-bed intermittent misting with 21 ± 3°C bottom heat, in a 5:1 (volume) washed sphagnum moss peat mixture. Room temperature was near 10-15°C.

During the second 4-week period, the air temperature was raised to 15°C and held constant where lighting was continuous. The air temperature for the SD and LD treatments was 10°C during the dark period and 15°C during the light period. Ten cuttings from girdled and non-girdled shoots were again taken for rooting tests. Rooting percentage was determined after 120 days in the rooting bench.

RESULTS AND DISCUSSION

The data presented in Table 3 suggests that stock plant lighting can significantly affect the rooting potential of cuttings subsequently taken from these plants. However, further study is needed to determine whether this rooting response is a photoperiodic one or related to photosynthesis and net assimilation or other physiological processes. The results are similar to those obtained by Whitehill (13) with *Pinus sylvestris* and Kelly (6) with *Ilex crenata*. It is interesting in these experiments, as well as our own, to note that the stock plant SD treatment enhanced the rooting of cuttings taken from these plants, while it has been established that LD or additional light favors rooting of cuttings after excision and during the rooting process in Douglas fir (2). We have never been able to establish a consistent rooting response from girdling in this species.

Table 3. Effect of stock plant lighting and shoot girdling on rooting of Douglas fir stem cuttings excised from these plants after one month's treatment, 1970.

Period I. Air temp. 5°C day and night, Jan. 6-Feb. 14. Light intensity 1187$\mu\omega cm^{-2}\ nm^{-1}$.

Clone 40	Lighting Regime					
	Continuous		16-hours		9-hours	
Replication	Girdled	Not-girdled	Girdled	Not-girdled	Girdled	Not-girdled
1	0%	8%	40%	60%	80%	100%
2	0	0	60	20	100	80
Av.	0	4	50	40	99	90

Period II. Air temp. 15°C with continuous lighting and 10°C during dark period and 15°C during light period for long and short day treatment, FEb. 14-Mar. 14. Light intensity was 2723$\mu\omega cm^{-2}\ nm^{-1}$.

Clone 40	*Lighting Regime*					
	Continuous		16-hours		9-hours	
Replication	Girdled	Not-girdled	Girdled	Not-girdled	Girdled	Not-girdled
1	0	0	0	60	40	100
2	0	0	20	40	40	60
Av.	0	0	10	50	40	80

THE CUTTING

Shoot's seasonal periodicity and rooting. The shoot apex of Douglas fir is characterized by a remarkable growth periodicity. The close relationship of this periodicity to the rooting potential of stem cuttings has been established (9). No treatment has been effective in rooting Douglas fir cuttings during maximum bud dormancy (rest) which coincides with the cessation of initiatory activity of the apex (10). Cutting rooting potential increases progressively in the shoot from this peak of rest (based on speed of bud break) to a maximum in January and February when the chilling requirement of the buds has been fully met. At mid-rest (November) the shoot has reached the physiological-morphological state at which exogenous auxin treatment will promote rooting of excised cuttings. At the end of rest (January) cuttings will root in fair percentage without added auxin.

Rooting environment. Within limits rooting can also be enhanced by environmental manipulations during the rooting process. Responses to rooting temperature, photoperiod, light energy, misting, aeration have been reported for a number of species of woody plants and the requirements are quite specific for a given species. A number of these treatments have been used with Douglas fir to change the physiological status of the cutting during rooting and thus further enhance rooting percentage and quality of roots. Bhella and Roberts (2) found that an 18-hour photoperiod (LD) significantly increased cambial activity, rooting, bud respiration, and also hastened bud break of stem cuttings as compared with similar cuttings propagated under a 9-hour photoperiod (SD). Rooting response was modified by stage of rest at which the cutting was taken and by the temperature of the rooting medium. Daylength response was greatest in December. Optimum rooting temperature was 26°C during early rest (September-November) but shifted to 18°C toward the end of rest (December-January).

1974 Experiment. It appeared in Bhella and Roberts' experiments that the LD enhancement of rooting was related to rest, because cuttings taken from September 15 to December 15 initiated significantly more roots under LD than SD, but those taken at the end of rest (January 15) showed no response to photoperiod. Since this was considered an important point in elucidating the nature of the LD effect on rooting, we essentially repeated Bhella and Roberts' experiment in 1974 taking cuttings in October, December, and January for rooting. We are reporting here only the results for cuttings taken in January.

Cuttings from clones 45, 48, 111, and 150 were taken on January 24 from the field and given the standard cutting treatments reported earlier in this paper. After treatment, 10 cuttings of each cultivar were placed in growth chamber rooting trays (2) maintained at 10°, 16°, 21°, and 27°C rooting temperatures. On series of trays were placed in a growth chamber under LD conditions (18-hour photoperiod with light intensity at 8 klx or 750 ft-c) and another in a chamber under SD conditions (9-hour photoperiod at 16 klx or 1500 ft-c). Half of the cuttings were harvested at the end of 120 days to determine rooting percentage. At that time the air temperature in the chambers was raised from 10°C to 16°C for the next 80 days, after which the remaining cuttings were harvested for evaluation of rooting. These results are presented in Table 4.

RESULTS AND DISCUSSION

Contrary to Bhella and Roberts' earlier results, there was increased rooting in this experiment with LD or increased lighting even after rest (January) and this has been verified more recently by Haugh (5), who has also demonstrated that it is not a photoperiodic response but one related to light energy levels and carbohydrate status in the cutting. As observed by Bhella,

Table 4. Effect of daylength and rooting temperature on rooting percentage of Douglas fir stem cuttings after 120 and 200 days in rooting trays. 1974.

Series 1. 1/24 - 5/24, 10°C air temp., 120 days in rooting tray.

	Cutting base temp.							
	27°C		21°C		16°C		10°C	
Clone	SD	LD	SD	LD	SD	LD	SD	LD
45	0%	0%	30%	20%	20%	20%	0%	0%
48	0	0	0	50	0	10	0	0
111	0	20	10	30	0	20	0	0
150	0	10	0	30	0	20	0	0
Av.	0	7.5	10	33	5	18	0	0

Series 2. 1/24 - 8/14, 10°C air temp from 1/24 - 5/24 and 16°C from 5/24 - 8/14; 200 days in rooting tray

	27°C		21°C		16°C		10°C	
Clone	SD	LD	SD	LD	SD	LD	SD	LD
45	0%	0%	0%	10%	30%	90%	0%	40%
48	0	0	40	10	10	60	0	0
111	0	0	10	10	10	90	0	10
150	0	0	20	30	10	40	0	0
Av.	0	0	18	15	15	70	0	13

the rooting response to lighting in this experiment showed an interaction with rooting temperature.

One should not try to explain what physiological changes are taking place with such environmental manipulations without in-depth studies, but they do illustrate what can be done in conditioning cuttings during the rooting process. Hansen, et al. (4) and Haugh (5) have shown that carbohydrate status and carbohydrate-auxin balance may be the mechanisms by which increased light energy increases root initiation and development in the cutting bench.

CONCLUSIONS

Evidence continues to accumulate that the current season's shoot, that we excise for vegetative propagation of woody species, has a predetermined potential for rooting that may far outweigh any after-the-fact chemical stimulant we might give for predisposing it to root. The shifting physiological balances occurring during stock plant aging and in the seasonal periodicity of shoot development seem to have controlling influence over root regeneration potential. Our ability to identify the morphological stages and the physiological status of these shoots at time of maximum rooting potential and to manipulate the stock plant and cutting toward these optimum balances is one of the challenges of propagation research. When we fully understand these developmental stages and how to physiologically condition them, then we will be in a position to produce two or three generations of rooted cuttings a year in growth chambers as an assembly line.

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ROOT REGENERATION OF EVERGREEN PLANTS

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Abstract. The root regeneration potential of 6 species of bare-rooted conifers and broadleaf evergreens was studied in raised sawdust beds under natural conditions at Corvallis, Oregon. All plants were successfully rooted; how-