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April 1980

ROOTING WESTERN WHITE PINE, *PINUS MONTICOLA* DOUGL.,
NEEDLE FASCICLES AND BRANCH CUTTINGSDuane S. Andrews¹

ABSTRACT

Three experiments were conducted to investigate effects of IBA, kinetin, GA₃, B-nine, sucrose, and of an acid and a base on the rooting of western white pine needle fascicles and branch cuttings. IBA, B-nine, sucrose, and acid were all effective in enhancing root initiation.

KEYWORDS: Vegetative propagation, rooting, rooting white pine, rooting needle fascicles

Clonal lines are useful for investigations of genetics, physiology, and disease and insect resistance. Asexual propagation by cuttings is preferable to grafting because rootstocks can affect the development of grafted scions. Cuttings of western white pine (*Pinus monticola* Dougl.) are relatively difficult to root. Cuttings from seedlings up to 2 years of age root relatively well without any chemical treatment. With an auxin treatment, a rooting success of 75 to 100 percent is quite common. McDonald and Hoff (1969), and Hoff and McDonald (1968), rooted needle fascicles from western white pine seedlings. Toda and Isikawa (1971) reported that needle fascicles have the same trend in rooting in relation to age of the ortet as stem cuttings. Because of the small size and ease of handling, needle fascicles are ideal for larger studies to be carried out in growth chambers. However, western white pine and pines in general outgrow the capability of forming adventitious roots relatively fast beyond 2 years of age. According to Girouard (1971), there are few reports of rooting cuttings taken from pine trees over 10 years of age. However, because young seedlings have a limited number of branches for taking cuttings, development of clonal lines takes considerable time. Therefore present investigations were undertaken to find a combination of chemical treatments that enhance the rooting of cuttings from older trees.

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Previous research suggests that synthetic auxins promote rooting of pine cuttings. IBA, IAA, IPA and NAA² are several of the auxins that have been used to promote rooting; IBA seems to be the most effective (Girouard 1971). Even though cytokinins are known to be essential for cell division and differentiation; their effect on rooting is unknown. It has been found that a low auxin:cytokinin ratio favors bud initiation; a high auxin:cytokinin ratio favors root initiation.

Synergistic effects of chemicals were noted by Larsen and Dingle (1969) who reported that kinetin alone was not effective in initiating roots, but when added with IBA it did have a positive influence on rooting. Gibberellic acid has been shown to inhibit root formation in several species (Bachelard 1965; Brian and others 1960; Hassig 1972), and recently Smith (1974) reported that gibberellic acid inhibited root formation in *Pinus radiata* if it was applied before initiation began. Hare (1974) found the growth retardant B-nine³ was effective in promoting rooting in pine.

When a cutting is severed, its supply of nutrients is cut off; therefore the supply of nutrients and carbohydrates must be adequate to supply energy and tissue-building requirements until a new root system can be established. Mergen and others (1958) concluded from the literature that rooting is augmented by endogenous food supply, although effects are confusing. Went and Thimann (1937) reported several sugars had a positive effect on root initiation. Thimann and Delisle (1939) advocated a sugar treatment for rooting most hard-to-root species. In addition, captan, a fungicide, augments rooting. Grigsby (1965) and others have reported benefits in using captan or other fungicides in rooting pines. Doran (1957) observed that captan with IBA or NAA increased rooting over either IBA or NAA alone. Van Elk (1969) found that treatment with captan increased rooting an average of about 20 percent. And finally, Lee and others (1977) advocated using an acid or base pretreatment to enhance rooting in certain species; acid pretreatment promoted rooting of plants native to neutral or alkaline soil, and a base pretreatment increased rooting ability of those plants native to acid soil.

The purpose of this paper is to report on three different studies designed to:

1. Ascertain the root-promoting influence of exogenously applied IBA, kinetin, and gibberellic acid alone and in combination on western white pine fascicles and cuttings.
2. Ascertain the root-promoting influence of IBA, sucrose, and the growth retardant B-nine alone and in combination on western white pine fascicles and cuttings.
3. Ascertain the root-promoting influence of several chemical treatments in combination with pretreatments of hot water, acid, or base on western white pine fascicles and cuttings.

²IBA is indolebutyric acid, IAA is indoleacetic acid, IPA is indolepropionic acid, and NAA is naphthaleneacetic acid.

³B-nine is N-dimethylaminosuccinamic acid.

MATERIALS AND METHODS

Experiment No. 1

The first study was set up to test the effects and interactions of three levels each of gibberellic acid (GA_3), indole-3-butyric acid (IBA), and kinetin (K) in all possible combinations on adventitious root development of adult and juvenile needle fascicles. Juvenile material was taken from 1-year-old seedlings and adult material was taken from two 20-year-old trees.

Cuttings were taken in early January from the lower lateral branches of the adult trees. Because the temperature was $-15^{\circ}C$, the cuttings were placed in plastic bags, brought into a cool greenhouse ($2^{\circ}C$) and thawed out overnight. The following morning fascicles were cut off the current year's growth with a razor blade, dipped in rooting powder, and planted in flats containing a sand:soil:peat moss (1:1:1) rooting medium. The investigations were carried out in growth chambers (55 cm x 125 cm inside dimensions) with 16-h photoperiods and a day/night temperature of $24^{\circ}/10^{\circ}C$. Light intensity was $1460 \mu W/cm^2$ (as measured with an ISCO model SR spectroradiometer) from cool white fluorescent plus incandescent lights. Cuttings were watered once each day with a mist nozzle on a hose. The experiment lasted 120 days at which time all of the fascicles were removed and checked for rooting.

Rooting powders used contained GA_3 (0, 0.2, or 1 mg/gm), IBA (0, 5, or 20 mg/g), and K (0, 5, 15 mg/gm) in all possible combinations. The powders were prepared by dissolving the chemicals in 100 percent ethyl alcohol to which talc powder was added to make a slurry. The slurry was dried, ground in a mortar, and sieved through a 100-mesh screen.

The experimental design was a randomized block with 25 fascicles/replication and two replications. Statistical analysis was carried out with factorial analysis of variance using percentages transformed by $\arcsin \sqrt{\%}$ according to Snedecor (1956).

Experiment No. 2

In February 1974, a second study was initiated to investigate effects and interactions of two levels of IBA (0, 100 mg/liter), three levels of the growth retardant B-nine (0, 100, 200 mg/liter), and three levels of sucrose (0, 1, 2 g/liter) on both adult and juvenile needle fascicles. The same sources of material were used in this study as were used in the first study. On February 4, scions were collected from the adult ortets and handled the same as before. All conditions were the same as the first study except: (1) the rooting media was 1:1:2 (soil:sand:peat moss), (2) a 24-h soak was used instead of powder, and (3) following treatment all cuttings were dipped in 4 percent captan powder.

The experimental design was a randomized block with 25 fascicles/replication and four replications. Treatment design was a 3^3 factorial with two replications and 25 needle fascicles per treatment. Statistical analysis was carried out with factorial analysis of variance using percentages transformed by $\arcsin \sqrt{\%}$ according to Snedecor (1956).

Experiment No. 3

Experiment 3 was set up to test the effects and interactions of four pretreatments and five treatments on adventitious root development of juvenile branch cuttings. On February 1, 1978, a single scion was collected from each of 280 6-year-old seedlings in nursery beds at the Moscow laboratory. Scions were placed in plastic bags and stored in a refrigerator overnight. Scions were trimmed to a length of 8 to 10 cm, bases were cut diagonally with pruning shears, treated, and stuck in 65 cm³ Leach pine cell plastic containers containing a 2:2:1 (sand:peat:forest soil) rooting medium. The trial was conducted in a moist greenhouse rooting chamber with natural light and daylength. A spray-type watering system soaked the entire bench 1 minute out of each hour from 11:00 a.m. to 4:00 p.m. each day. The trial lasted 142 days, at which time the cuttings were removed and checked for roots. A split-plot design was used with 7 scions per treatment, replicated twice. All treatments were included in each pretreatment.

Pretreatments were: (1) 10-minute soak in cold water; (2) 10-minute soak in hot (50° C) water; (3) 10-minute soak in NaOH, pH 10.5; (4) 15-second dip in 2N H₂SO₄.

Treatments were:

1. 24-hour soak in water.
2. 24-hour soak in 50 ppm IBA + 1 g/liter sucrose.
3. 24-hour soak in 100 ppm IBA + 1 g/liter sucrose.
4. 0.8 percent IBA in 10 percent captan powder.
5. 0.8 percent IBA, 1 percent B-nine, 10 percent powdered sugar, 10 percent captan powder.

Treatments 1, 2, and 3 were dipped in 10 percent captan prior to sticking.

RESULTS AND DISCUSSION

Experiment No. 1

No adult needle fascicles rooted. Thus, only average rooting percentages for the juvenile fascicles are given in table 1. Analysis of variance indicate that GA₃ was the only growth substance treatment that was significant. The presence of GA₃ in the rooting powder significantly reduced rooting below that of non-treat cuttings. This is consistent with what Smith (1974) reported for *Pinus radiata*. However, IBA appears to be able to overcome some of the inhibition but not significantly. Kinetin was ineffective in counteracting the GA₃ effect.

Although effects of kinetin and IBA were not statistically significant (table 1), both appeared to enhance rooting either separately or together. When GA₃ was absent, all treatments except one exceeded the control. That treatment was a combination of the highest levels of both kinetin and IBA. This is in agreement with Larsen and Dingle (1969) who reported that kinetin enhanced rooting of *P. contorta*, but is inconsistent with Smith (1974) who reported that kinetin inhibited rooting of *P. radiata*. However, the concentration Smith worked with was selected due to its inhibitory effect. At lower concentrations he found no significant effects. Heide (1965) reported that 13 ppm kinetin promoted buds and inhibited rooting of begonia while 0.8 ppm stimulated the effect of IAA in root promotion.

Table 1.--Average percentage rooted needle fascicles of 1-year-old western white pine from two replications with 25 fascicles per replication

Treatment			Average rooting ¹
Kinetin	IBA	GA ₃ ²	
mg/g			Percent
0	0	5	2
0	0	20	2
1	0	20	2
1	5	20	2
0.2	0	20	4
.2	5	20	4
.2	15	20	4
.2	0	5	6
0	5	20	8
0	15	20	8
1	0	5	8
1	15	20	8
.2	5	5	12
1	5	5	12
1	15	5	16
0	5	5	22
.2	15	5	22
1	15	0	26
0	0	0	32
0	15	5	32
1	5	0	40
0	5	0	46
.2	0	0	48
0	15	0	50
.2	5	0	50
1	0	0	58
.2	15	0	60
			Control

¹Averages not connected by the same line are significant at the 5-percent level according to Duncan's new multiple range test.

²Analysis of variance showed significant difference for GA₃ treatments at 1-percent level.

Thus, the results here indicate that kinetin at least is not inhibitory to rooting of western white pine and could possibly be a stimulant.

Experiment No. 2

Because the rooting response of juvenile and adult needle fascicles varied so much, this study was analyzed as two separate experiments. The overall rooting success was considerably lower with the adult needle fascicles than with juvenile fascicles (tables 2 and 3).

Table 2.--Treatments and average rooting percentages for adult western white pine needle fascicles; ranked in order of increasing rooting success

IBA ³	B-nine	Sucrose	Average rooting ¹
--- mg/l ---		g/l	Percent
0	0	0	2 0
100	100	1	11
100	100	0	12
100	100	2	13
100	200	1	14
100	200	0	15
100	200	2	16
0	200	2	17
100	0	1	18
0	200	0	22
100	0	2	22
0	100	2	23
0	0	2	24
0	200	1	24
0	0	0	26
100	0	0	27
0	100	1	28
0	0	1	32
0	100	0	33
			Control

¹Averages not connected by the same line are significant at the 5-percent level according to Duncan's new multiple range test.

²Needle fascicles of all treatments except this control were dipped in 4-percent captan prior to sticking.

³Analysis of variance showed significant difference for IBA treatments at 1-percent level.

Table 3.--Treatments and average rooting percentages for juvenile western white pine needle fascicles; ranked in order of increasing rooting success

IBA ³	B-nine	Sucrose ⁴	Average rooting ¹
--- mg/l ---		g/l	Percent
0	0	0	2 10
100	200	2	13
100	200	0	25
100	100	2	29
100	100	0	32
100	0	2	34
0	0	0	39
0	100	2	39
0	200	2	39
0	0	2	41
100	200	1	41
0	200	0	42
100	100	1	52
0	100	1	54
100	0	1	57
100	0	0	58
0	100	0	63
0	200	1	66
0	0	1	84
			Control

¹Averages not connected by the same lines are significantly different at the 1-percent level according to Duncan's new multiple range test.

²Needle fascicles of all treatments except this control were dipped in 4-percent captan prior to sticking.

³Analysis of variance showed significant difference in IBA treatments at 5-percent level.

⁴Analysis of variance showed significant difference in sucrose treatments at 1-percent level.

According to the analysis of variance for the adult needle fascicles, IBA was the only significant treatment, but further analysis with Duncan's new multiple range test (Duncan 1955) (table 2) indicates some other differences also. IBA in general is significantly poor, especially in combination with B-nine. No IBA treatment significantly improved rooting, and in combination with B-nine and/or sucrose it had an inhibiting effect as compared to the captan treated control. On the other hand, B-nine and sucrose, individually or together, enhanced rooting at the intermediate level.

Analysis of data for juvenile needle/fascicles shows significant differences due to levels of IBA and sucrose. Also, there was a significant interaction of the three substances. Further analysis with Duncan's new multiple range test (table 3) shows that when the level of IBA used was combined with higher levels of either B-nine or sucrose, there was no increase in rooting and in some cases rooting was reduced. However, when IBA was added to moderate levels of B-nine and sucrose there was a significant increase in rooting, but the best treatments did not include IBA. The best treatment was 1 g/liter sucrose by itself.

Although the magnitude of response to the various treatments is much smaller in the adult than juvenile needle fascicles, there are several similarities between the two groups. For example, the least rooting in either juvenile or adult fascicles occurred in the control without captan. In both cases the survival of needle fascicles was good; therefore, it was concluded that captan does enhance rooting of western white pine, and the fungicidal effect may be minimal. Needle fascicles in both categories rooted best without auxin treatment, and either sucrose or B-nine effectively replaced IBA for enhancing root initiation.

Deuber (1942) reported that cuttings from 6-year-old eastern white pine treated with 1.5 percent sucrose rooted about the same as those given IBA alone, but those treated with IBA plus sucrose rooted about the same as the controls. The results here show a similar response to sucrose, i.e., sucrose alone is better than sucrose plus IBA. However, the results of this study vary for both juvenile and adult material. As stated above, the best treatments for both categories was without IBA. Also, sucrose alone was better than IBA in both cases, but IBA alone enhanced rooting of juvenile but not adult fascicles. The effect of IBA plus sucrose was the same as IBA alone for juvenile but considerably lower for the adult fascicles.

These results indicate that western white pine needle fascicles may contain enough endogenous auxin for root initiation but initiation is limited due perhaps to gibberellin inhibition or lack of energy. Thus, when sucrose and/or B-nine are added at or near the proper level root initiation can occur.

Experiment No. 3

The effects of hormone treatments on the rooting of western white pine stem cuttings were significant at the 0.5 percent and pretreatment effects were significant at the 12 percent level (table 4). The interaction of treatments with pretreatments was nonsignificant.

Table 4.--Analysis of variance for data in table 5

Source of variation	Degrees of freedom	Mean squares	F
Blocks	1	422.50	2.97 ns
Pretreatments (A)	3	696.90	4.90*
Error (a)	3	142.23	
Treatments (B)	4	1,446.19	42.92**
Interaction (AB)	12	260.34	0.77 ns
Error (b)	16	336.55	
Total error	19	305.87	
Total	39		

* Significant at the 12-percent level.

** Significant at the 0.5-percent level.

ns=Nonsignificant.

Treatments 3 (24-h soak in 100 ppm IBA + 1 g/liter sucrose), 4 (0.8 percent IBA in 10 percent captan powder), and 5 (0.8 percent IBA, 1 percent B-nine, 10 percent powdered sugar, 10 percent captan powder) gave the best response (table 5). The best pretreatments were H_2SO_4 (15-second dip in 2N H_2SO_4) and NaOH (10-minute soak in NaOH, pH 10.5). The best combinations were H_2SO_4 pretreatment followed by treatment 5 or 4, respectively. This enhancing effect of H_2SO_4 is contrary to the findings of Lee and others (1977). They reported that in general an acid pretreatment promoted rooting of plants native to near neutral or alkaline soil and base pretreated cuttings increased rooting of those native to acid soil. Since western white pine is native to acid soil, they would have predicted the base to be best, which is what happened when hormone treatment 3 was used. Therefore, it appears that the pretreatment interacts with the hormone treatment and specific combinations should be selected for optimum rooting.

CONCLUSIONS

Western white pine juvenile material, at least up to 6-year-old trees, can be rooted with sufficient frequency to propagate clonal lines for research. However, except for needle fascicles, we have not been able to root adult material. Results from rooting adult needle fascicles may give some clues as to what is required for rooting adult branch cuttings.

Table 5.--Average percent rooting of cuttings of 6-year-old western white pine

Pretreatment	Hormone treatment ¹					Average
	1	2	3	4	5	
Cold water	0	7.0	28.5	14.0	35.5	17.0
Hot water	14.5	14.0	36.0	21.5	29.0	23.0
NaOH	14.0	21.5	50.0	43.0	28.5	31.4
H ₂ SO ₄	7.0	21.5	28.5	57.0	64.0	35.6
Average	8.9	16.0	35.8	33.9	39.2	26.75

¹Treatments were:

1. 24-hour soak in water.
2. 24-hour soak in 50 ppm IBA + 1 g/liter sucrose.
3. 24-hour soak in 100 ppm IBA + 1 g/liter sucrose.
4. 0.8 percent IBA in 10 percent captan powder.
5. 0.8 percent IBA, 1 percent B-nine, 10 percent powdered sugar, 10 percent captan powder.

Treatments 1, 2, and 3 were dipped in captan prior to sticking.

Gibberellins have been implicated as being involved in the aging and (or) maturing process in plants. Experiment 1 showed that GA₃ inhibits rooting of very young western white pine, which agrees with what Smith (1974) reported for *Pinus radiata*. The second experiment showed that the growth retardant B-nine (which acts as an antigibberellin) promotes rooting in adult and juvenile needle fascicles. In Experiment 3, one of the best hormone treatments for inducing rooting in branch cuttings from 6-year-old trees included B-nine. Therefore, it appears that gibberellins may be inhibiting rooting in western white pine and the use of B-nine may help produce a hormonal balance favorable for rooting. Hare (1974) has reported similar results with southern pines.

The effect of kinetin on rooting western white pine was inconclusive. Although it appeared to enhance rooting of juvenile needle fascicles it was not significantly different from the control. However, the results of Experiment 1 would indicate that at least at the levels used kinetin does not inhibit rooting.

Although IBA is effective in promoting rooting in juvenile white pine and most other plants, it has not been effective by itself in rooting cuttings in older western white pine. Results of Experiment 2 with adult needle fascicles indicate that the endogenous auxin level may be adequate and that the limiting factor may be inhibition by gibberellins or low available energy reserves, or both. Sucrose, at moderate levels, promotes rooting in both needle fascicles and stem cuttings, but at higher levels it appears to be inhibitory. At 1 g/liter, it stimulated rooting of both juvenile and adult needle fascicles more than the IBA treatments, but at 2 g/liter rooting was less than the control. Sucrose alone was a better rooting stimulant than sucrose plus IBA. These results are similar to those of Deuber (1942), who found that cuttings of 6-year-old eastern white pine treated with 1.5 percent sucrose rooted about the same as IBA alone, but IBA plus sucrose rooted about the same as the controls. The balance of auxins:gibberellins:carbohydrates and other growth substances necessary for root initiation is critical and only slight alteration of any one may induce or inhibit rooting.

Lee and others (1977) found that cuttings from acid loving plants rooted better when pretreated with a base. The results here were not as conclusive. The two best combinations of pretreatments: treatments included an acid pretreatment, but one base pretreatment plus hormone treatment was almost as good. Therefore, specific combinations should be selected for optimum rooting.

An added observation in Experiment 2 was the benefits of using captan fungicide. The use of captan increased rooting 26 and 29 percent, respectively, for adult and juvenile needle fascicles over that of controls without captan. This effect had previously been reported by Van Elk (1969) who noted a 20 percent increase with captan.

The following treatments are recommended for rooting cuttings of western white pine up to 6 years old: (1) pretreat for 15 seconds with 2N H₂SO₄; (2) treat with a rooting powder containing 0.8 percent IBA plus 10 percent captan, or 0.8 percent IBA, 1 percent B-nine, 10 percent powdered sugar, and 10 percent captan powder. Either powder should give good results and the 0.8 percent IBA 10 percent captan is much simpler to make up. No recommendation can be made for rooting adult branch cuttings; however, Toda and Isikawa (1971) reported that needle fascicles show the same response in rooting in relation to age of the ortet as stem cuttings. Thus, from the experiment with adult needle fascicles, the best treatment for adult cuttings may be B-nine, sucrose, or both without auxin.

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