VEGETATIVE PROPAGATION OF *PINUS STROBUS* BY NEEDLE FASCICLES[1]

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Abstract. Studies were conducted during 1974 and 1975 to determine the influence of chemical growth regulators for increasing bud development in dwarf shoots of *Pinus strobus* and also to determine effect of rooting of dwarf shoots as influenced by clonal response and sampling date. Results indicate that both single and multi-applications of N^6-BA (N^6-benzyladenine) increased bud development, while PBA (Pyranybenzyladenine), Atrinal (kidegulac-sodium) and MBR 12325 showed no effect. Rate of application of N^6-BA was significant with 1000 ppm being more effective than 500 ppm. Results on rooting of dwarf shoots indicate that no differences in percent rooting of dwarf shoots could be attributed to sampling date, but substantial variation occurred between individual clones.

Research in silviculture throughout the world by both geneticists and physiologists during recent years has been directed toward discovering new methods to improve superior quality clones in forest trees. Particularly in pine, retaining superior clones is important in selection for pulp, paper, lumber, and ornamental value. Commercial methods used by foresters to retain desirable qualities in forest trees have been by sexual reproduction through selection of seed from superior trees and by vegetative propagation. Unlike sexual reproduction, vegetative propagation provides genetically uniform material, multiplication of selected germ plasm for progeny testing and, potentially, could provide large numbers of propagules (9).

Graftage and cuttage have been used to vegetatively propagate hardwood and softwood pines, but stock-scion incompatibility, expense, and limited numbers of plants which can be produced have reduced its practicability as a commercial method of propagation. Although some success in rooting stem cuttings has been achieved, only one species, *Pinus radiata*, has been commercially propagated by stem cuttings in large numbers for distribution (1, 7).

A new method of vegetative propagation which shows promise in the genus *Pinus* is the use of dwarf shoots or needle fasicles. The dwarf shoot consists of 1 to 5 needles and contains a diminutive shoot apex which is formed in the axil of either a terminal or lateral branch (8). When this dwarf shoot is propagated, adventitious roots arise at the base of the needles and a plant genetically identical to its parent is produced. Rooting of dwarf shoots or needle fascicles has been demonstrated in a number of species (4, 5, 6, 8, 10, 11). Al-

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[1]Paper No. 4919 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh. Use of trade names does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named nor criticism of similar ones not mentioned.

though this method of reproduction has shown promise, difficulty in stimulating development of the fascicular bud within the dwarf shoot after rooting into a growing apex has been successful only in a few cases. As a result, death of the rooted dwarf shoot frequently occurs within several months (11, 14).

The major objective of this study was to evaluate the effect of growth regulators and a cultural treatment as a method to enhance bud development in dwarf shoots of *Pinus strobus*. Number of applications as well as rate of application were studied. Another objective was to investigate the effect of clonal response and sampling date in rooting of *Pinus strobus*.

MATERIALS AND METHODS

Experiment 1. Two separate groups of 3-year-old plants of *Pinus strobus* were root-pruned and placed in 3.78 liter containers containing 3:1:1 bark, sand, and peat mixture. Plants were then transferred on March 8, 1975 to the greenhouse where they received approximately 11 hr of natural light followed by 3 hr of 100 ft-c of incandescent light. From March through May plants received ample water and fertilizer to stimulate new shoot development.

On June 20, as the sheath of the dwarf shoots began to shed, plants were selected for uniform terminal stem length. Each terminal stem was tagged and plants of these two separate groups were arranged in RCBD (randomized complete block design). Three replications of each treatment were used in the first group and four in group two.

Foliar applications of N^6-BA, Atrinal, MBR 12325, and PBA were applied to drip point on June 20 to Group 1 and June 20 and July 18 to Group 2. Application was made with a compressed air stainless steel sprayer applying 50 ml of chemical/plant. Applications of chemicals were made in the early morning when temperatures ranged from 65-75°F.

Buds measuring greater than 2 mm in length were used as a criteria for buds which developed into a growing apex. Number of buds developed within the dwarf shoot on the terminal stem were recorded after 8 weeks following last treatment.

Experiment 2. Five-year-old field-grown *Pinus strobus* were selected for uniform stem and needle growth on January 5, 1974 and 1975. Each of these groups of trees during both years had been pruned the previous summer to assist in stimulating bud development within the dwarf shoot. From each tree during 1974, 30 dwarf shoots were removed from the uppermost terminal whorl on January 15 and February 5, while in 1975 60 dwarf shoots were removed on January 15.

After samples were collected each year, they were placed in plastic bags and submerged in water overnight to prevent evapora-

tion of moisture from the dwarf shoots. Following sampling, dwarf shoots from each tree were divided into equal parts and randomized with other samples from other trees. As a result, during 1974 and 1975, each sample plant was replicated three times, but the number of samples of a particular plant varied between 1974 and 1975.

On January 16 and February 16, 1974 and January 16, 1975, samples were dipped in 0.8% Hormodin talc and placed in a perlite medium under intermittent mist. A plastic tent was also utilized to cover this structure to assist in keeping a high humidity. Temperature in the propagation structure ranged from 80-120°F during the day and 65-70°F during the evening.

Cuttings of these dwarf shoots were evaluated after 120 days for percent rooting and effect of date on rooting.

RESULTS

Growth Regulator and Cultural Treatment Studies. Results indicated that N^6-BA was the only effective treatment for increasing the number of dwarf shoots with developed buds greater than 2 mm in length on a given shoot (Table 1). Differences in rate of application of N^6-BA was not significant. Treatments of other chemicals and removal of terminal buds did not show any difference in the number of buds developed within the dwarf shoot. All chemicals and rates tested indicated no phytotoxicity symptoms.

Results of multi-spray applications indicated that both 500 and 1000 ppm were the best treatments (Table 2). Differences in application rate of N^6-BA was highly significant, with 1000 ppm being more effective than 500 ppm. All other treatments, as in the previous experiment, showed no difference in number of buds developed within the dwarf shoot. All chemicals and rates tested indicated no phytotoxicity symptoms.

Table 1. *Effect of removal of terminal buds and the foliar application of* N^6BA, PBA, MBR 12325, and Atrinal on number of buds developed within dwarf shoots of *Pinus strobus* after 8 weeks (1975).

Treatment	Rate (ppm)	No. of buds formed within dwarf shoots Terminal Stem[1]
PBA	100	0
PBA	200	1
N^6BA	500	37
N6BA	1000	48
MBR 12325	120	0
MBR 12325	240	0
Atrinal	1000	3
Atrinal	3000	0
Pinched		7
Control		0
LSD .05		12.3

[1]Each value is the mean of 3 plants.

Table 2. Effect of removal of terminal buds and the foliar applications (June 20 and July 18) of N⁶BA, PBA, MBR 12325, and Atrinal on number of buds developed within dwarf shoots of *Pinus strobus* after 8 weeks (1975).

Treatment	Rate (ppm)	No. of buds formed within dwarf shoots Terminal Stem[1]
N⁶BA	500	27
N⁶BA	1000	70
PBA	75	10
PBA	150	13
MBR 12325	120	12
MBR 12325	240	13
Pinched		9
Control		10
Atrinal	1000	9
Atrinal	3000	10
LSD .05		17.7

[1]Each value is the mean of 4 plants.

Rooting Studies. Results from 1974 indicate that there was only a slight difference in percent rooting of dwarf shoots as influenced by sampling date (Table 3). Results also showed that differences among clones did exist, and that those variations among clones were substantial. Best overall rooting was attained with clone No. 5, with 36% rooting.

Results of 1975 support the previous years' study in that rootability was largely affected by individual clonal response (Table 4). Average rooting percentages were noted to be higher during the 1975 testing period with an average of 22% rooting of all clones. Of the 45 clones tested, 42% of these had an average percent rooting of 34. Best overall rooting was attained with clone No. 4 with 68% rooting.

Table 3. Percent rooting of dwarf shoots as influenced by clone and sampling date in *Pinus strobus* (1974).

Clone	**January** Percent Rooted	**February** Percent Rooted
1	7	7
2	—	—
3	56	33
4	3	—
5	36	10
6	13	10
7	3	7
8	13	23
9	10	3
10	16	20
11	3	10

Table 3. (continued)

Clone	January Percent Rooted	February Percent Rooted
12	3	3
13	—	3
14	10	20
15	7	—
16	3	10
17	—	3
18	—	13
19	13	3
20	16	16
21	7	10
22	13	20
23	1	3
24	—	7
25	3	10
26	13	3
27	—	3
28	23	23
29	23	30
30	16	3
Avg.	10.4	10.2

Table 4. Effect of clone on percent rooting of dwarf shoots in *Pinus strobus* (1975).

Clone	Percent Rooted	Clone	Percent Rooted
1	2	24	5
2	27	25	3
3	13	26	10
4	13	27	17
5	37	28	10
6	25	29	10
7	15	30	22
8	3	31	47
9	30	32	7
10	20	33	17
11	15	34	5
12	3	35	13
13	30	36	0
14	15	37	50
15	33	38	18
16	40	39	45
17	7	40	12
18	38	41	68
19	30	42	32
20	15	43	45
21	28	44	33
22	10	45	32
23	30		
Avg.			22

DISCUSSION

Results from the growth regulator studies support the theory (12, 13) that cytokinins play an essential role with auxins in bud development as well as altering apical dominance. Early work conducted by Wickson and Thimann (15) reported that auxins inhibit axillary bud growth, but that kinetin could overcome the auxin effect. They also noted that the higher the auxin concentration, the higher the rate of kinetin needed to remove this inhibition. Concha and Montaldi (3) in their study reported that N^6-BA could release axillary bud inhibition of dwarf shoots of *Pinus elliottii*. Cohen and Shanks (2) also have reported that foliar applications of N^6-BA stimulated bud development in dwarf shoots of *Pinus ponderosa* even though terminal buds of long shoots were present.

From these studies, it appears that a similar mechanism of apical dominance via lateral bud inhbibition exists in *Pinus strobus* also. It is speculated that the level of cytokinin and auxin within the bud during the growing season plays an essential role in controlling bud development, and once the level of either compound is "unbalanced," axillary bud development ceases. Only N^6-BA overcame apical dominance and resulted in a reduction in axillary bud inhibtion. Rate was also important, further substantiating the fact that a given level must be present of a cytokinin-like substance if axillary bud development is to occur.

Results of the studies on rooting support the concept that propagation of dwarf shoots was influenced by clonal types. These studies during 1974 and 1975 indicated variation among clones even though, 1) trees were the same age, 2) culturally treated in the same manner, and 3) sampling techniques were the same for all trees. The data also confirmed that there was no difference in overall rooting during January or February. Reports (6, 10) of other investigations support the theory that best rooting occurs during early winter.

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