SEASONAL ROOTING RESPONSE OF 6-YEAR-OLD LOBLOLLY PINE CLONES TO DIFFERENT CONCENTRATIONS OF INDOLE-3-BUTYRIC ACID AND DIMETHYL SULFOXIDE

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Abstract.--Cuttings from seven clones of loblolly pine (Pinus taeda L.) were divided into five treatment groups (Hare's powder, 4000 ppm IBA + 1% DMSO, 4000 ppm IBA + 0.5% DMSO, 4000 ppm IBA and 0.5% DMSO) and rooted in a greenhouse in February, May, and September. Rooting was highest in the IBA + 1% DMSO, 0.5% DMSO and Hare's powder treatments. Highest rooting percentages occurred in February (64%) followed by September (39%) and May (29%) setting dates. Results indicate significant differences among clones and treatments in percent rooting and callus development. Significant clone*treatment and season*treatment interactions were present and suggest the need to alter rooting treatments according to the time of year.

Additional keywords: Pinus taeda, DMSO, IBA.

The focus of operational and research oriented vegetative propagation has been to develop cultural pre-treatments to promote year-round rooting of field and greenhouse cuttings or to characterize those best "rooting windows" where highest rooting percentages are realized a few months each year.

The application of dimethyl sulfoxide (DMSO) to enhance rooting of woody horticultural varieties has been summarized by Edwards (1979). DMSO, which increases the permeability of plant tissues to exogenous auxins, has been used to propagate some varieties of Juniperus (McKinniss 1969). The potential of DMSO to enhance the uptake of exogenous auxins and increase rooting in species such as loblolly pine appears to be promising. However, DMSO has not been routinely used to propagate forest tree species.

The objectives of this study with loblolly pine cuttings were: 1) to determine the effects of IBA and DMSO treatments compared to "standard" rooting treatments, and 2) to evaluate the rooting response of the selected treatments during three seasons of the year.

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The use of trade names does not imply endorsement of products named nor criticism of products not named.

METHODS

In March, 1981, seven clones were vegetatively propagated from seedlings germinated from open-pollinated seeds in July, 1980. Rooted cuttings were transferred to 5.5 liter pots and kept hedged to a 0.25 to 0.5 m height in a greenhouse before planting in the field at the NCSU Genetics Gardens in spring 1983. Three ramets of each clone were planted and thereafter hedged annually to a height of 0.5 to 1 m.

Dormant cuttings were collected February 21, 1986. Succulent shoo were available from the trees for the May 19, 1986 collection period. moth (<u>Rhyacionia</u> sp.) infestation limited the number of cuttings collected September 26, 1986.

Approximately 105 cuttings were collected per clone. Each cutting was submerged in a Benomyl solution (0.3 gm/1 water) for one-half hour and stuck the same day in a rooting medium of a 2:2:1 ratio of perlite, vermiculite, and peat. Cuttings from the seven clones were randomly divided into five treatment groups:

(1) Hare's powder (Hare 1978)
(2) 4000 ppm IBA + 1% DMSO
(3) 4000 ppm IBA + 0.5% DMSO
(4) 4000 ppm IBA
(5) 0.5% DMSO

The IBA and/or DMSO treatments were prepared using a 50% ethyl alcohol base. Because of the limited number of cuttings available from the September collection and preliminary rooting results from the February and May experiments, some September cuttings were omitted from the 4000 ppm IBA + 0.5% DMSO treatment. Generally, there were 21 cuttings per clone per treatment for a total of 735 cuttings per experiment.

The cuttings were placed in a greenhouse where the humidity was controlled by a MEE II Cloudmaker®. Fog was maintained at a level to keep the foliage wet but without saturating the rooting medium. All cuttings were fertilized weekly with a foliar application of 15-30-15 N-P-K plus micronutrients (Peter's High Phos Special®) at 200 ppm N concentration.

Callus score (0 = no basal swelling; 1 = basal swelling, no callus; 2 = minimal callus, no roots; 3 = moderate callus with roots; and 4 = maximal callus with roots) and shoot elongation (cm) were assessed at 7 weeks. Rooting was assessed at week 12.

A two-way ANOVA was used to test for differences among clones and treatments for each rooting period. All data were analyzed on a clone-treatment-mean basis. Because clone-treatment means for percent rooting covered a wide range of values (0% - 100%), both non-transformed percentages and arc sine percentages were analyzed. No differences were noted in significance levels for the F-tests, therefore, only non-transformed percentages are presented. The three-way ANOVA used to test differences among seasons was limited to those treatments and clones common to all three rooting

Significant treatment differences were evaluated using the Waller--Duncan k--ratio t-test (SAS 1985).

RESULTS

A large and significant ($p < 0.05$) portion of the variation in percent rooting and callus score was attributed to clones (table 1). Differences among treatments were seen in percent rooting in February and May and in callus development in February. Generally, clone and treatment differences important for shoot elongation.

Table 1.— Analysis of variance with significance levels for rooting percent, callus score, and shoot elongation, by month.

		Mean Square[1]		
Source of Variation	df	Percent Rooting	Callus Score	Shoot Elongation
February				
Clone	6	0.33**	0.36**	17.49**
Treatment	4	0.05**	0.48**	0.63NS
Error	24	0.01	0.07	0.28
May				
Clone	6	0.32**	2.26**	<0.01NS
Treatment	4	0.04*	0.22NS	<0.01NS
Error	24	0.01	0.12	<0.01
September				
Clone	3	0.21**	0.79*	3.56NS
Treatment	3	0.02NS	0.23NS	0.57NS
Error	9	0.03	0.22	1.38

[1] NS = non-significant
* = $0.01 < p \leq 0.05$
** = $p \leq 0.01$

Rooting was greatest in February, followed by September and May (figure 1). The standard rooting treatments, Hare's powder and 4000 ppm IBA, were not different from the two IBA + DMSO treatments. The addition of IBA or IBA + DMSO applied in May and September inhibited rooting, yet DMSO alone yielded the best rooting percentages at these times.

Callus development was not affected by treatment or by season when treatment was applied (figure 2). Although the presence of callus is usually necessary for root initiation in pines (Cameron 1968 and Haissig

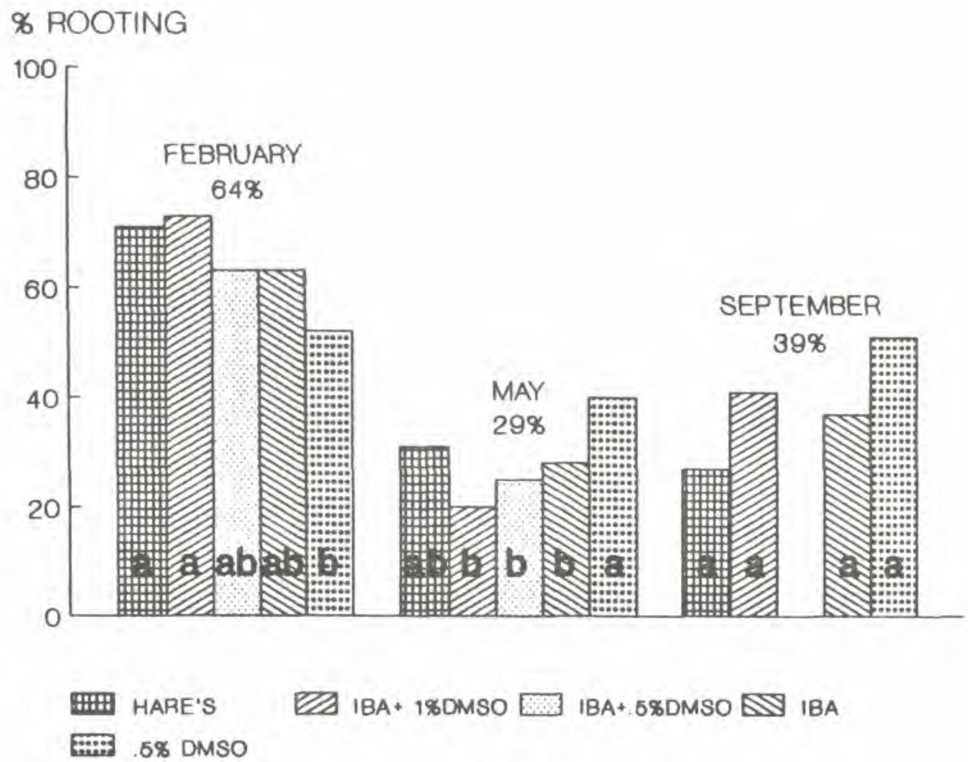

Figure 1. Seasonal trends in percent rooting of loblolly pine cuttings, by treatment. (Treatment means within a season with the same letter are not significantly different, $p < 0.05$).

1982), the best rooting treatments (figure 1) did not necessarily have the largest callus development at week seven (figure 2).

The treatment differences in shoot elongation were largely seasonal and were neither inhibited nor promoted by either DMSO or IBA + DMSO (figure 3). Virtually no shoot elongation was observed during May.

Because of the tip moth damage to the shoots, the overall three-way ANOVA was limited to four clones and excluded the 4000 ppm IBA + 0.5% DMSO treatment. Significant ($p \leq 0.05$) season*clone interactions were found for callus score and shoot elongation. Most importantly, season*treatment interactions were present for rooting percent and callus score (table 2).

DISCUSSION

The most important finding from this study was the seasonal trends in rooting. Rank changes in clones and treatment means were found in February and May, contributing to the clone*treatment and season*treatment interactions. The increased rooting percentages during early spring followed by early fall and late spring agree with similar treatments applied to 2- and 3-year-old slash (P. *elliotti* Engelm.) and loblolly pine cuttings (Reines and Bamping 1960), and 1- to 5-year-old Monterey pine (P. *radiata* D. Don)

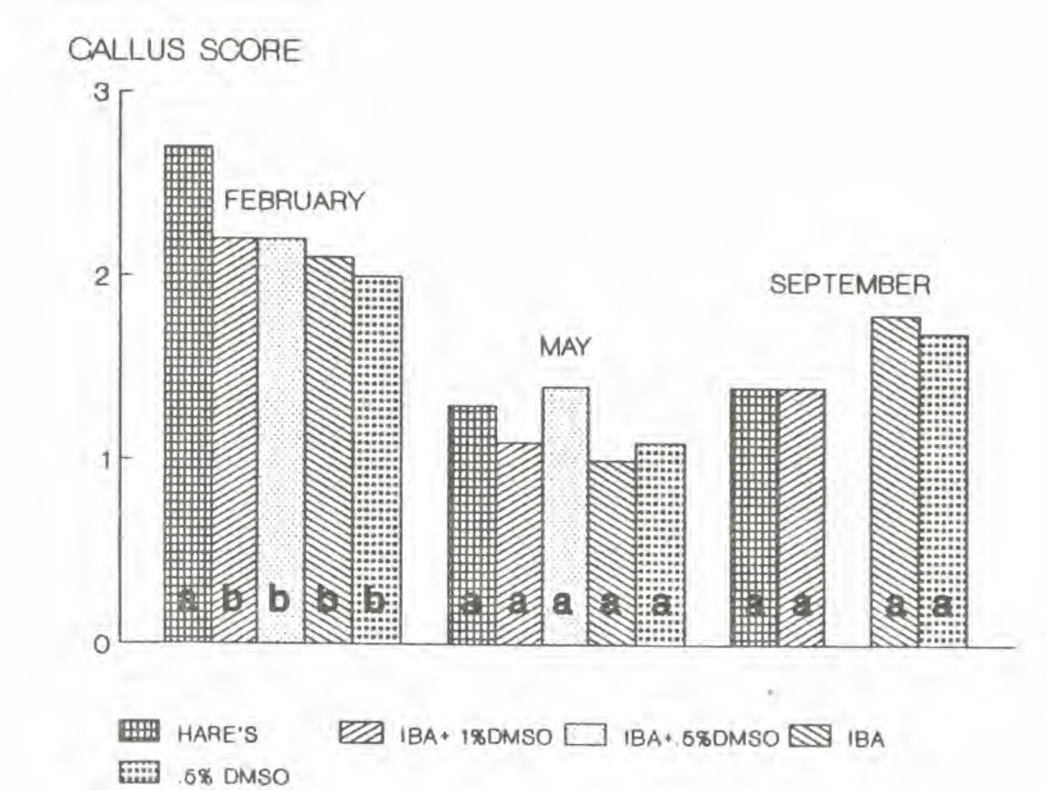

Figure 2. Mean callus score of loblolly pine cuttings by treatment. (Treatment means within a season with the same letter are not significantly different, $p < 0.05$.)

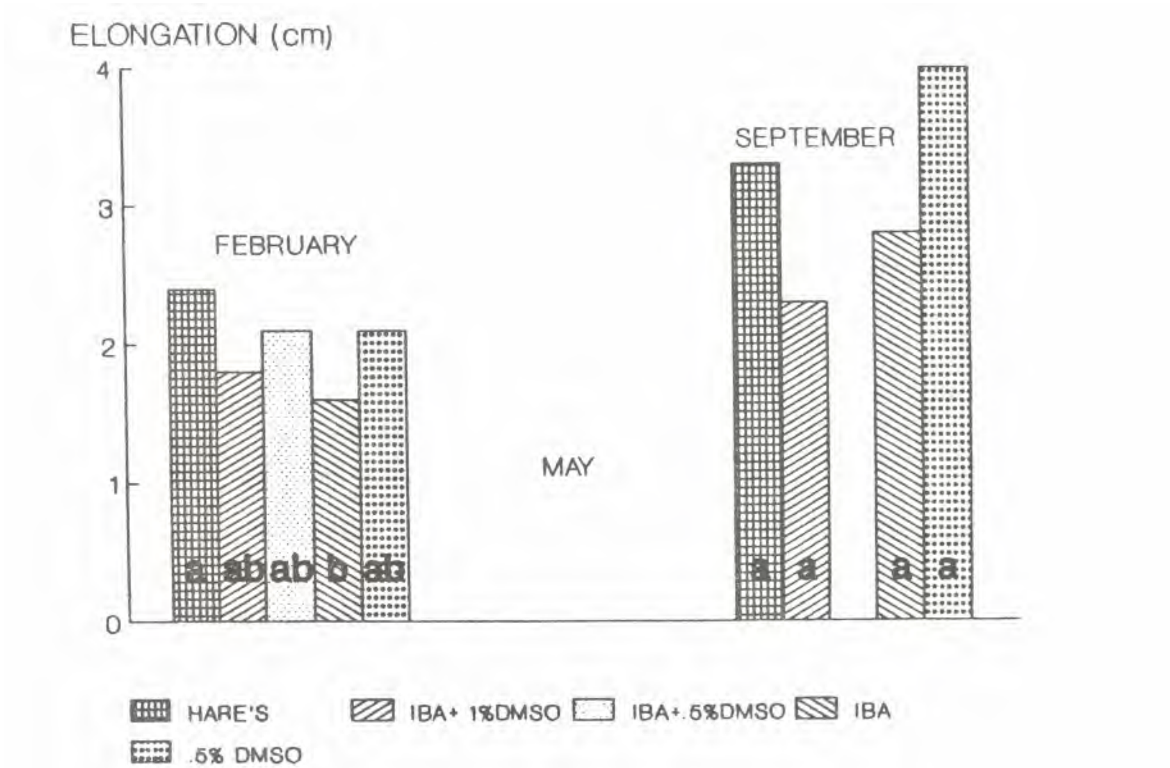

Figure 3. Mean shoot elongation of loblolly pine cuttings by treatment. (Treatment means within a season with the same letter are not significantly different, $p < 0.05$.)

Table 2.--Three-way analysis of variance with significance levels for percent rooting, callus score, and shoot elongation

		Mean Square[1/]		
Source of Variation	df	Percent Rooting	Callus Score	Shoot Elongation
Season	2	0.55**	4.43**	14.58**
Clone	3	0.44**	2.29**	14.27**
Season*Clone	6	0.02NS	0.80**	5.23**
Treatment	3	<0.01NS	0.05NS	1.32NS
Season*Treatment	6	0.03*	0.03*	1.26NS
Clone*Treatment	9	0.03*	0.17NS	0.68NS
Error	18	0.01	0.12	0.74

[1/] NS = non-significant
* = $0.01 < p \leq 0.05$
** = $p \leq 0.01$

and ponderosa pine (*P. ponderosa* var. *arizonica* (Engelm.) Shaw.) cuttings (Mahalovich, unpublished data). These results do not agree with the seasonal rooting success of slash pine (Bower and van Buijtenen 1977, Hare 1978, and Mergen 1955), where late spring and summer showed the highest rooting percentages. It is likely that the above differences can be due to species, geographic location, cultural pre-treatments, and donor age, all which affect rooting of stem cuttings.

The importance of characterizing "rooting windows" and appropriate treatments for research and operational rooting of stem cuttings cannot be ignored. As an example, percent rooting for one clone treated with IBA + 1% DMSO was 100, 52, and 100% for the February, May and September rooting periods, respectively. Rooting was poorest in May, but that clone rooted at 90% in May when 0.5% DMSO alone was used. Identifying favorable clone-treatment-season combinations will increase the number of healthy rooted cuttings which can be produced.

DMSO as an agent to transport IBA across plant tissues did not consistently enhance rooting percentages over that of Hare's powder or IBA alone. In May and September 0.5% DMSO alone had the highest rooting response. The lower percentages of the other treatments containing IBA may have been due to some IBA toxicity. High concentrations of IBA have been shown to inhibit rooting in some species (Bowen et al. 1975, Larsen and Dingle 1969, and Whatley et al. 1966).

The role of DMSO in stimulating the rooting of pine requires further study with lower concentrations of IBA. The potential exits to use DMSO as a "carrier" of IBA into mature, woody cuttings to enhance rooting. In the absence of exogenous auxin application, pre-treatment with DMSO and ethyl alcohol may increase permeability and/or mobilize endogenous auxins thereby promoting higher rooting percentages.

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