Effects of IBA and NAA treatments on rooting Douglas-fir stem cuttings

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Abstract. The effectiveness of six IBA and four NAA concentrations, four combinations of IBA and NAA concentrations, and control were tested for their ability to enhance rooting frequency (%) of Douglas-fir cuttings. Two IBA and one NAA treatments were also compared to the control for quality of root system. Between 1984 and 1998, six independent studies were conducted in mist or fog environments with the same 11 or 14 clones. Auxin concentrations tested ranged from 0 to 123 mM IBA and 0 to 10 mM NAA. Auxin, clone, and auxin by clone effects were significant in every study, although individual clone analyses showed only two clones to differ significantly for auxin. All auxin treatments but the 10 uM NAA treatment induced significantly greater rooting than the control, but no single auxin, auxin concentration or combination of auxins was clearly superior in every study. The 10 mM NAA concentration was the only concentration tested that reduced rooting percentage to less than the control. Both NAA and IBA appeared to have broad ranges of root-enhancing activity. However, within the effective range of IBA evaluated, 24.6 mM produced the greatest rooting percentage in four of five studies testing IBA. NAA solutions with concentrations between 2.5 and 7.4 mM NAA generally resulted in similar rooting success. Rooting responses to increased IBA and NAA were both nonlinear; rooting decreased with both too little and too much auxin. Combinations of IBA and NAA in the same solution did not increase rooting percentage above what was achieved with one auxin. For root system quality, auxin treated cuttings in one study, had significantly better root systems than control, but there was no difference in the other study in which root quality was estimated.

Introduction

Operational reforestation with rooted stem cuttings of Douglas-fir (*Pseudot-suga menziesii* (Mirb.) Franco) is currently limited because rooted cuttings cost considerably more than seedlings. Various biological factors contribute to this situation; one factor is the decline in rooting ability that increases with plant age (Black 1972). Propagators avoid this problem by limiting cutting propagation to very young seedlings (Franclet 1985; Ritchie et al. 1993; Ross 1975). Annual hedging of Douglas-fir is an effective treatment for stopping or

retarding the loss of rooting associated with aging (Copes 1983, 1992), but the process must be initiated when the stock plants are young. Another limiting factor for Douglas-fir cuttings is the amount and persistence of plagiotropism (Copes 1976). Plagiotropism is also reduced by rooting young seedling, but there is no treatment to eliminate or reduce the severity of the condition in cuttings from older trees. Discovery of new or improved techniques or procedures that decrease propagation costs by increasing rooting frequency or root quality will reduce the cost differential between seedlings and rooted cuttings and allow managers to select from a greater array of planting stock.

Exogenous auxins are commonly used to improve rooting efficiency and quality of stem-cuttings. Both IBA (indole-3-butyric acid) and NAA (1-napthaleneacetic acid) stimulate adventitious rooting; nevertheless, published results for Douglas-fir are confusing when the best auxin(s) or concentration(s) are sought for inducing maximum rooting. Many rooting investigations have been conducted with Douglas-fir cuttings, but only a few have included planned studies of different auxins or different auxin concentrations. Proebsting (1984) found a 5-second dip in 7.4 mM NAA was superior to IBA and other NAA concentrations. His auxin treatments range from 1 to 100 mM. Griffith (1940) tested both IBA and NAA between 0 and 1.0 mM in 24-hour soaks and found 0.246 mM IBA to be more effective than any of the NAA concentrations. Cornu (1973) evaluated three concentrations of IBA between 24.6 mM and 98.6 mM in talc and reports 24.6 mM IBA to be the best treatment. Bhella and Roberts (1974) had satisfactory rooting after a 5-second dip in a one in ten dilution of a commercial rooting product containing 24.6 mM of both NAA and IBA. No studies compared solutions containing different combinations of IBA and NAA.

This report describes results from six rooting investigations made between 1984 and 1998. Almost 27,000 cuttings from 14 Douglas-fir clones were studied. Most of the IBA and NAA concentrations tested were reported in the literature to be effective. NAA and IBA were tested both singly and in combination treatments. The primary objective of this investigation was to more accurately identify the optimum concentrations or the most effective range of concentrations of IBA and NAA. Such knowledge will aid propagators, geneticists, and tree-improvement specialists in selecting treatments that reduce propagation costs by maximizing rooting success.

Methods

Studies testing the effectiveness of IBA and NAA in promoting rooting efficiency and root quality were conducted in 1984, 1985 and 1998. The same propagation facilities and basic propagation procedures were used for all six

studies. Cuttings were collected in January and stored until February at 2 °C in plastic bags containing moist paper towels. Ramets used as sources of cuttings were 14 clones grown in hedges; their rooting characteristics had been studied annually from 1974 (Copes 1983, 1992). In 1984, the stock plants of each clone were 10- and 13-year-old rooted stecklings (20 and 23 years from seed) hedged annually since 1978. The cuttings, primarily third-order lateral shoots, were trimmed to about 15-cm lengths in February, immediately preceding auxin treatment.

IBA (Sigma Co.) and NAA (J.T. Baker Chemical Co.) were the auxins evaluated for their capacity to enhance adventitious rooting. The mention of trade or firm names here and elsewhere does not imply endorsement by the USDA. The NAA crystals were dissolved in 5 ml of 28 to 30% ammonium hydroxide (NH_4OH) or 99% acetone (C_2H_2) and then diluted with 95% ethanol (C_2H_5OH) and water to make 500 ml of 50% ethanol/water solutions. The IBA treatments were made by dissolving IBA crystals in 95% ethanol and then diluted with water to make a 50% ethanol/water solution. The solutions of auxins were stored in darkness at 2 °C in brown bottles. The time between solution preparation and use did not exceed 5 days.

For the growth regulator treatment, the basal 4 to 5 cm of each cutting was dipped in auxin solution for 5 to 10 seconds. Needles were not removed from the base of each cutting before or after auxin treatment. No stickers or carriers were used with either auxin. Cuttings for the control treatment were dipped into a solution consisting solely of 50% ethanol/water. Within 15 minutes of auxin treatment, the cuttings were placed into the rooting beds in clonal rows of 8 to 14 cuttings.

The rooting medium was a mixture of peat moss and fine sand (2:1 v/v). During rooting, the cuttings were in intermittent mist or fog during the daylight hours and allowed to dry at night. The rooting medium was heated to about 21 °C with electric soil cables (Copes 1983). The outdoor mist house was covered with a single layer of vinyl chloride. The indoor fog environment was produced in a conventional glasshouse containing a vinyl-chloride chamber enclosing a Spraying Systems Co. automatic, self-contained humidifying unit (Model 3404). During the day, relative humidity was maintained at 80%. The fogging system was not operated at night. Each week the cuttings were given foliar applications of 20:20:20 ($N:P_2O_5:K_2O$) fertilizer and a fungicide (Captan, Benlate, Bravo, Banrot, or Subdue). The fertilizer was not applied until some cuttings had formed visible roots. Fungicide treatment was done every week. Cuttings remained in the rooting environments until October.

Rooting success was denoted as the percentage of cuttings of each clone or tree that rooted. Root quality was classified as good (cuttings suitable for

transplanting (three or more primary roots with well-developed secondary-root structure), weak (one or two primary roots with poorly developed secondary root structure), and those that rooted but died before root assessment in October. The percentage of cuttings with good root systems was calculated as a percentage of all rooted cuttings.

Studies all used a split plot design with clones on the major plot and auxin treatments on the sub-plots. The experimental unit of each study was the percentage of cuttings that rooted per clonal row of 8 to 14 cuttings. Each clonal row was a replication of a specific clone-by-auxin treatment. No transformation of data was required. The $P \leq 0.05$ significance level was used for all studies. Clones were considered random effects in most analyses because the 14 clones had originally been selected for graft compatibility, not for rooting traits. Auxins were fixed effects.

Rooting data of each study were subjected to analysis of variance (ANOVA) by using SAS procedures for general linear models (SAS 1990b). The type III mean square of block by clone was used as the error term for clone, while type III mean square of clone by auxin was used as the error term for auxin (Steel and Torrie 1980).

The SAS Catmod procedure (SAS 1990a) for categorical analysis was used for root quality because of extreme variation in size of denominators (number of rooted cuttings) used to calculate percentage of cuttings with good roots.

Results and discussion

Results of all six studies for percentage rooting, including two of studies in which root quality was evaluated, are presented in Table 1. The mist and fog environments were compared for NAA in 1985 and for IBA in 1984 and 1985. There was no significant difference between the environments and there was no clone by environment interaction for either auxin. Thus, the effect of rooting environment is not given further consideration. The 10 mM NAA concentration was the only treatment that reduced rooting below the value of the control. Auxin, clone, and auxin by clone effects were significant in all studies. The study by clone interaction was not significant for IBA nor NAA. This effect was evaluated for all fourteen clones in the four studies that used 24.6 mM IBA and in the three NAA studies that evaluated 7.5 mM NAA.

Both NAA and IBA appeared to have fairly broad ranges of root-enhancing activity. Enhanced rooting percentages occurred at IBA concentrations between 12.3 and 123 mM (Table 1). While the results from the 24.6 mM IBA treatment were not always significantly different from other IBA treatments, they were numerically greater in four of five studies

Table 1. Results for rooting percentage and of rooted cuttings with good root systems.

Year Environment	Auxin (mM)		Percentage rooting and significance of comparisons[1]	Percentage good roots and significance of comparisons[2]
	IBA	NAA		
1984 Mist	0		14	67
	24.6		46	69
	49.3		40	68
1984 Fog	0		27	
	24.6		44	
	49.3		41	
	98.6		38	
1985 Mist	0		51	53
	24.6		66	76
	49.3		76	77
		7.5	68	83
1985 Fog	0 +	0	39	
	" +	2.5	59	
	" +	7.5	68	
	24.6 +	0	68	
	" +	2.5	64	
	" +	7.5	58	
	49.3 +	0	60	
	" +	2.5	60	
	" +	7.5	59	
1998 Mist	0		26	
	12.3		57	
	24.6		66	
	61.5		63	
	123.0		51	
1998 Fog		0	31	
		2.5	50	
		5.0	44	
		7.5	39	
		10.0	10	

[1] * Signifies indicated orthogonal comparison was significant at $P \leq 0.05$; ns indicated the comparison was not significant.

[2] * Signifies indicated maximum-likelihood comparison was significant at $P \leq 0.05$; ns indicated the comparison was not significant.
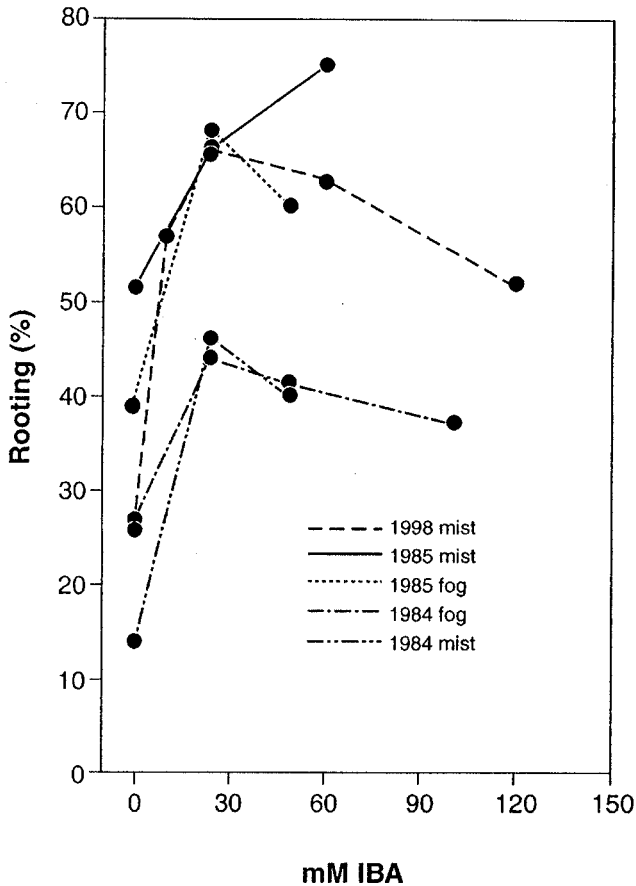
Figure 1. Percentage of rooting achieved in mist and fog environments with IBA.

(Figure 1). The relation between IBA concentration and rooting percentage was nonlinear in 4 of the 5 studies. Rooting percentages of the 12.3 mM and 123.0 mM treatments were significantly lower than from 24.6 to 98.6 mM IBA. Rooting success of the various IBA concentrations was similar to the results of Cornu (1973) and contrary to the linear IBA relation reported by Proebsting (1984).

Activity of various NAA concentrations was investigated in only two studies: the 1985-fog and 1998-mist environments. Results from those studies conflicted. In 1998, NAA concentrations of 2.5 mM and 5.0 mM were superior to 7.5 mM NAA, but in 1985, 7.5 mM NAA was superior to 2.5 mM NAA (Table 1). The 10 mM NAA concentration in 1998 rooted significantly less than both the control treatment and the other auxin treatments.
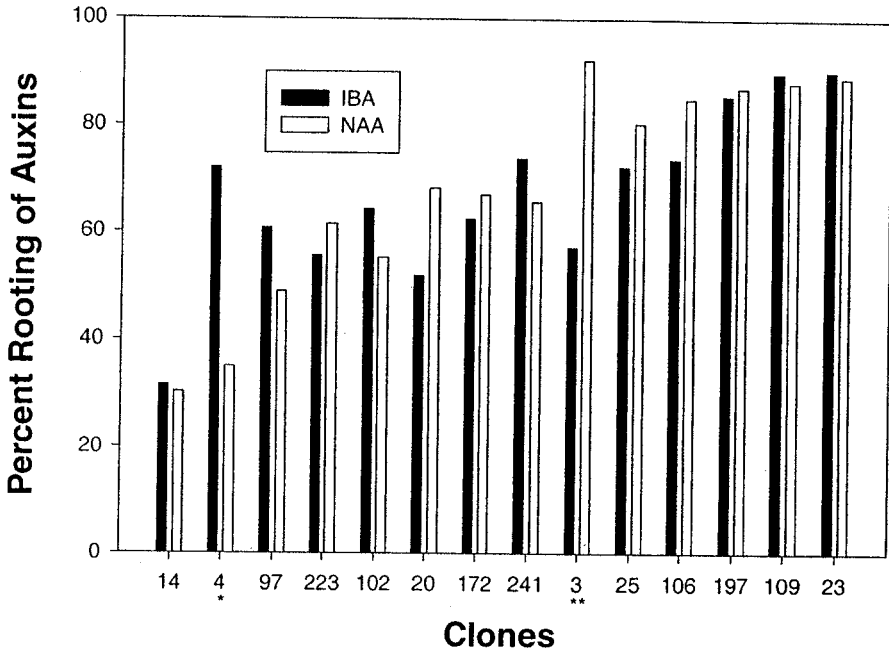
Figure 2. Percentage rooting of fourteen clones treated with 7.5 mM NAA or 24.6 mM IBA.

The NAA concentration and rooting percentage relation was nonlinear in the study with the widest range of concentrations. The nonlinear relationship for NAA supports Proebsting's (1984) results.

The two studies in 1985 had three auxin treatments in common, 7.5 mM NAA and 24.6 and 49.3 mM IBA (Table 1). There were no significant differences among these treatments, but there was a significant clone by auxin interaction. This suggests that average rooting percentage might be increased by selectively treating each clone with the auxin that gave the greatest rooting response. Individual analysis of the rooting of each of the fourteen clones revealed only clone-4 and clone-3 to have significant clone by auxin effects (Figure 2). Clone-3 rooted best with NAA, while clone-4 rooted best with IBA. For the 14 clones, little overall gain in rooting percentage would result from selectively treating one clone with IBA and the other 13 clones NAA.

Treatments containing both IBA and NAA in the same solution were evaluated in the 1985-fog study. This study was designed as a factorial (Table 1) but was not analyzed as a factorial because such analysis could not answer any of the questions of interest. Two NAA and two IBA concentrations were tested in all four combinations and alone. The selected concentrations of auxins were within assumed optimum range of each auxin when used alone.

Combination treatment did not significantly enhance rooting above what was achieved with 24.6 or 49.3 mM IBA or the 2.5 or 7.5 mM NAA used alone. The favorable rooting reported by Bella and Roberts (1974), after treating cuttings with a commercial product containing a combination of IBA and NAA, was probably a response to 2.5 mM NAA and not to 2.5 mM IBA.

Results for root-system quality were less definitive than results for rooting percentage because root quality was only estimated in two studies. Their results were not consistent. In the 1984 mist environment, root quality of the control treatment was not significantly different from the 24.6 and 49.3 mM IBA treatments, but in the 1985 mist environment, the percentage of cuttings with good root systems was significantly greater for NAA and IBA treated cuttings than for the control. Proebsting (1984) reported that root quality was weakly related to auxin concentration.

Conclusions

Five- to 10-second dips into 12.3 to 123 mM IBA or 2.5 to 7.5 mM NAA working solutions enhanced percent rooting with Douglas-fir stem cuttings. One should note that rooting percentage from the 24.6 mM IBA concentration was numerically greater than the other IBA concentrations in four of five studies. Minor variation in rooting percentage among studies was likely the result of uncontrolled environmental variation. Only two of fourteen clones exhibited significant differences between NAA and IBA treatments, thus the auxin by clone interaction will not enable propagators to significantly increase rooting by treating some clones with NAA and others with IBA. The relative difference in root quality following NAA or IBA treatment was not conclusive and requires further study. No additional enhancement of rooting percentage resulted with both NAA and IBA in the same solution. Combination treatments are not recommended because they increase propagation costs without increasing rooting frequency.

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