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ENHANCING THE ROOTING OF CANADA YEW STEM CUTTINGS WITH IBA TREATMENTS

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Abstract

Studies on rooting stem cuttings of *Taxus canadensis* Marsh., a shrub containing an anti-cancer chemical, paclitaxel, are reported. Dipping the stem base in a powder (0.8% IBA) improved neither the rooting nor the growth of adventitious roots (compared with controls) regardless of predetermined clone rooting abilities. Quick dips in concentrated IBA (0, 0.5, 1.0 1.5 and 2.0% in 50% ethanol) stimulated rooting, with 0.5% and 1.0% producing the best survival, while the 2% dip gave the best root growth. Clone ortets classified as good or recalcitrant (difficult to root) did not differ significantly in their Ingestad nutrient ratios and in foliar macronutrient concentrations, except for Ca, which had a greater concentration and larger ratio in the recalcitrant category. Concentrations of N and P were positively correlated with the rooting percentage, whereas K and Mg concentrations were negatively correlated.

Key words: concentrated-hormone solution, foliar nutrient concentration, indole-3-butyric acid (IBA), ingestad ratios, rooting, rooting powder, stem cuttings, *Taxus canadensis*

INTRODUCTION

The demand for the anti-cancer chemical paclitaxel, found in tissues of all yews (*Taxus* spp.) (Nandi et al. 1996, Zocher et al. 1996, Senneville et al. 2001) has increased biomass harvesting of Canada yew (*Taxus canadensis* Marsh.) (Senneville et al. 2001), a shrub common to southeastern Canada and the northeastern United States (Farrar 1995). Improved propagation methods for the species are needed to support a domestication program for Canada yew started in 1997 (Smith and Cameron 2002). Considerable variation has been found in the rooting ability of different clones (Smith and Cameron 2002). One objective of the current study was to test method(s) for improving the success of rooting stems of Canada yew, especially in difficult-to-root clones. Although various approaches were studied, we report here only on indole-3-butyric acid (IBA) applications. Auxins are commonly used to stimulate rooting of cuttings. Application methods vary (Hartmann et al. 2002, Blazich 1988a), several of which were tried in this study. Another objective was to examine the relationship between the nutritional status of the ortet (parent plant) and the success of rooting. Until stem cuttings produce roots, their nutritional status resembles that of the ortet. These nutrients and those absorbed through the stem base from the rooting medium may affect rooting ability (Haissig 1986).

MATERIALS AND METHODS

For all rooting trials, dormant stem cuttings 5-18 cm long (no more than 2 years of growth) were harvested in late fall (November 2004) from potted stock by cutting just below the annual bud scar. Clones were categorized as "easy rooting" [E] (60-100% rooting success), "moderate" [M] (40-59%), and "recalcitrant" [R] (<40%), based on earlier trials (Smith and Cameron 2002). Cuttings were promptly struck into 67-cell multipot trays (Ropak Multi-Pot© #1-67, Stuewe & Sons, Inc., Corvallis, Oregon) with a mix of 2 : 1 (v:v) peat moss : vermiculite predrilled with a 5 mm diameter rod to 2 cm depth. The trays were randomly arranged in a greenhouse and placed under ambient light (37 to 146 µmol sec^{-1} m^{-2}), 22/18°C (day/night), and relative humidity (RH) adjusted to 60/40% with misting. In mid-December, the temperature was reduced to 5°C and held there until March, when it was brought to 22/18°C and RH to 85%, at which point daylight ranged from 460-920 µmol sec^{-1} m^{-2}. Cuttings were fertilized with

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0.005% N (11-41-8, Plant Products Ltd., Brampton, ON) three times a week and periodically flushed with water until harvest in May. Cuttings that produced roots were counted at harvest. Except in the indole-3-butyric acid (IBA) powder treatment, live green cuttings were counted, even if without the roots. All cuttings were individually packaged and frozen until processing. The measurements taken included: initial length, base diameter, and dry weights of adventitious roots and new shoots.

IBA powder trial

Cuttings were collected from four easy rooting (E), two moderate (M), and four recalcitrant (R) clones and 40-67 cuttings per tray were placed into 20 trays (two trays per clone), depending on the number available from the ortet. Cuttings in one tray per clone had basal ends dipped in Stim-Root© No. 3 (0.8% IBA, Plant Products Ltd., Brampton ON, Canada); excess powder was tapped off. Those in the second tray were untreated (controls).

Quick dips in concentrated IBA

IBA crystals (Sigma–Aldrich Co., Oakville ON Canada) were dissolved in 95% ethanol and then diluted with water to 50%. Trial 1 consisted of three treatments using two E, five M, and four R clones: (i) control (50% ethanol only), (ii) 1.0% IBA, and (iii) 2.0% IBA. Trial 2 used five M clones in five treatments: (i) control and 0.5, 1.0, 1.5, and 2.0% IBA in treatments ii-v, respectively. Basal ends of cuttings (34-67 and 42-67 per clone and treatment in Trials 1 and 2, respectively) were dipped for 10 sec before striking (Copes and Mandel 2000).

Foliar nutrient analyses

Foliar nutrient analyses were conducted at the Canadian Forest Service laboratory in Fredericton on two E and two R clones selected from those used in the IBA quick-dips Trial 1. Two samples of current-year and two of 1-year old foliage were collected from each of 10 stock plants per clone, and oven dried at 60°C. The samples were analyzed for P, K, Ca, and Mg with an AA-200 Atomic Absorption Spectrophotometer (Varian Inc., Sugar Land, TX, USA.) and for N with the LECO CNS-2000 elemental analyzer (LECO Corporation, St. Joseph, MI, USA) assuming equal leaching from foliage of all sampled ortets.

Analyses of variance (ANOVA) were performed using the General Linear Models Procedure of SAS version 8.2 for Windows (SAS Institute, Cary, NC, USA). The rooting trials were split-plot designs, with clone category as the main plot nesting clones (random factor) and with treatment as the split plot. In survival analyses, clone was the experimental unit, but in analyses of weights, a cutting was the experimental unit. The nutrient study was a split-split plot with the year of growth being the split-split plot. The SAS Univariate procedure was used to check the data, with the Box-Cox option of SAS's Transreg procedure used to choose the most suitable transformation, if required. All reported means are untransformed least-square (ls) means compared using the lsmean option of the GLM. The Correlation procedure was used to examine correlations.

RESULTS

In all trials, all cuttings, which rooted survived until harvest and no abnormal growth of roots or shoots was noted in any treatment.

IBA powder trial

Rooting was similar in both treatments (55% ± 4.8 and 58% ± 4.8), and ranged between 50-59% among clone categories, without significant effects from any source (Table 1). Less than half the rooted cuttings flushed (62%, 39%, and 31% in E, M, and R clones, respectively; 43% in controls vs. 45% in IBA treatment). Only clone category affected flushing ($p \leq 0.05$), where each category differed from the others. There was no significant difference in dry weights of adventitious roots between the treated cuttings and controls (0.18-0.19 g), but clone category was significant (0.24 ± 0.016 g, 0.18 ± 0.024 g, and 0.14 ± 0.017 g in E, M, and R clones, respectively) as were differences among clones (Table 2). New shoot dry weights were not significantly affected by any source of variance. Although the cutting size variables were significantly (all $p < 0.0001$) and positively correlated with dry weights of adventitious roots and of new shoots, Pearson's correlation coefficients (r) were low (below 0.4), and including these variables as covariates

Table 1. Results of analysis of variance on percentage of rooted cuttings of Canada yew after treatment (T) with IBA powder or without. C = Clone category (easy rooting, moderate, and recalcitrant), MS = mean square, Df = degrees of freedom, F = F-ratio, p = probability.

Source	Df	Mean square	F	p	Error term
T	1	34.8	0.17	0.69	MS Error
C	2	169.3	0.81	0.43	MS Error
T x C	2	0.7	1.00	0.36	MS Error
Error	14	208.4			

Table 2. Results of analysis of variance on dry weight of adventitious roots that developed on stems of Canada yew after treatment (T) with IBA powder or without. Nesting factors are bracketed; Cl = Clone, C = clone category (easy rooting, moderate, and difficult), MS = mean square, Df = degrees of freedom, F = F-ratio, p = probability.

Source	Df	Mean square	F	p	Error term
T	1	0.55	1.3	0.25	MS T x Cl(C)
C	2	11.84	28.4	<0.0001	MS T x Cl(C)
T x C	2	0.42	1.0	0.36	MS T x Cl(C)
Cl (C)	7	8.10	19.4	<0.0001	MS T x Cl(C)
T x Cl (C)	7	0.73	1.7	0.09	MS Error
Error	625	0.42			

did not affect the identification of significant sources of variance (results not shown).

Concentrated IBA solution dips

In Trial 1, rooting ranged from 3% to 61% among clones. Only treatment effect was significant ($p \leq 0.009$), with best rooting in the 1.0% IBA treatment (Table 3). Although the R category had the fewest rooted cuttings (Table 3), neither category ($p \leq 0.12$) nor its interaction with treatment ($p \leq 0.76$) were significant. The control had the greatest percentage of green cuttings (Table 3), and treatment effect was significant ($p \leq 0.001$), but effects of other sources were not. Only treatment affected ($p \leq 0.05$) the dry biomass of adventitious roots, which was greatest in the 2.0% IBA treatment and lowest in the control (Table 3). Dry weights of new shoots (Table 3) were not significantly affected by any factor. Cutting length and diameter were significantly (both $p < 0.0001$, Pearson's r between 0.4-0.5) and positively correlated with dry weights of roots and shoots but, as in the previous trial, these variables did not decisively change the outcome of the analyses when used as covariates.

Trial 2 used only M clones. Although the two lower IBA concentrations had more cuttings, which rooted than the other treatments (Fig. 1), the difference due to treatment was not statistically significant ($p \leq 0.19$). The control had the most of green cuttings and their percentage declined (treatment effect $p \leq 0.04$) with increasing IBA concentrations (Fig. 1). The concentrated dip treatments had a significant effect ($p \leq 0.03$) on the dry weights of adventitious roots, with means of the control and 2% IBA treatment being most different (Table 4). However, when the data were analyzed with the original cutting length as a covariate, the effect of the treatment on root dry weight was not significant ($p \leq 0.16$). On average, cuttings that flushed produced about 0.3 g of dry biomass, and only clone had a significant effect ($p \leq 0.02$), whether cutting-size related covariates were used or not.

Stock plant nutritional status

For all examined macronutrients but Ca, there were significant interactions between clone and the year of growth, but no consistent pattern was evident. Ca was the only element whose concentration differed significantly ($p \leq 0.02$) between the E and R rooting categories regardless of the year of growth. In current-year foliage, it was 15 143 ± 203 and 16 797 ± 2880 mg kg^{-1}, whereas in last-year foliage, it was 13 289 ± 304 and 14 163 ± 413 mg kg^{-1} for the E and R categories, respectively. Ingestad ratios (Ingestad and Kähr 1985) were very

Table 3. Means and standard errors (bracketed) for survival and dry biomass of adventitious roots and new shoots produced by Canada yew cuttings quick-dipped in concentrated IBA solutions of 0 (control), 1%, or 2%. Rooted cuttings produced adventitious roots whereas green cuttings remained green at the end of the first growing season, but only some produced roots. Means (in columns) marked with the same letter are not significantly different from each other (at α = 0.05).

Factor	% rooted	% green	Dry weight (g)	
Treatment			roots	new shoots
control	22 ± 3.9 b	92 ± 4.5 a	0.018 ± 0.004 b	0.17 ± 0.016 a
1%	39 ± 3.9 a	81 ± 4.5 b	0.027 ± 0.002 ab	0.16 ± 0.009 a
2%	24 ± 3.9 b	65 ± 4.5 c	0.032 ± 0.003 a	0.14 ± 0.013 a
Clone category				
easy rooting	33 ± 4.9 A	81 ± 3.6 A	0.024 ± 0.003 A	0.15 ± 0.012 A
moderate	30 ± 3.1 A	80 ± 5.6 A	0.029 ± 0.003 A	0.15 ± 0.012 A
recalcitrant	22 ± 3.4 A	77 ± 4.0 A	0.024 ± 0.004 A	0.17 ± 0.015 A

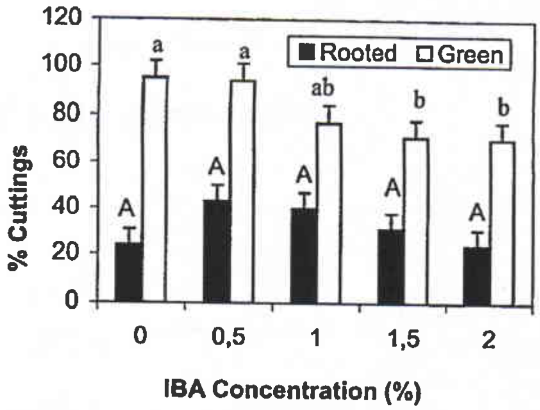

Fig. 1. Mean percentages of rooted Canada yew cuttings and those that remained green whether they produced roots or not in the IBA quick-dip trial using different concentrations of the hormone. Capped bars are standard errors, means marked with the same letters are not significantly different from each other at $\alpha = 0.05$.

similar in both categories (Holloway 2007) except for Ca whose ratio to N was 57.4 and 65.2 ($p \leq 0.05$) in E and R categories, respectively. Some elements and rooting success were significantly correlated. Averaged across both years (n = 80), N ($p \leq 0.03$) and P ($p < 0.0001$) concentrations were positively (Pearson's $r = 0.24$ and 0.48) correlated with rooting percentage, and more so for the current-year concentration (not shown). There were negative correlations between concentrations of K in current-year foliage and the rooting ($p \leq 0.02$, Pearson's $r = -0.36$) and Mg concentration in previous-year foliage and rooting ($p \leq 0.03$, Pearson's $r = -0.33$).

DISCUSSION

Across our trials, rooting success for untreated cuttings was 36%, similar to Mitchell's (1997) results with Pacific yew (*Taxus brevifolia* Nutt.). Using the same IBA powder as we did, Mitchell (1997) increased rooting from 30.6% to 50%. Based on this information, IBA powder has been routinely applied to Canada yew stem cuttings (Yeates et al. 2005), but it did not improve rooting in our study. The concentrated dips were more effective, especially in the lower range of concentrations, where the rooting percentage almost doubled. A high concentration of IBA stimulated rooting in *Tsuga* and *Rhododendron* (Cooper 1944) and in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Copes and Mandel 2000). Quick dips in concentrated IBA + α-naphthaleneacetic acid (NAA) solution were successful in promoting rooting of stems in some woody angiosperms (Beeson 2000).

Although the rooting in our study varied greatly among individual clones, quick dips of Canada yew cuttings in IBA at 0.5%-1.0% is recommended over the use of commercial rooting powder. The IBA powder dip did not enhance the growth of adventitious roots, but quick dips in concentrated IBA did. This is inconsistent with the findings of Copes and Mandel (2000), who found no stimulation of root growth in Douglas-fir by IBA at the concentrations they applied. In our study, adventitious roots grew best after a quick dip in the most concentrated IBA solution, and better in easy-rooting than in recalcitrant clones. The same was true for bud flushing, which tended to follow the categorization for rooting ability. The poor flushing of cuttings is of concern because it delays production of shoot biomass. The survival of green but non-rooted cuttings was best in the controls and declined after IBA application. It is uncertain whether green cuttings without roots would eventually produce them or perish. Prolonging their greenhouse culture would certainly increase their production cost, which may not be prudent.

Little is known about the importance of specific nutrients to root initiation (Blazich 1988b). Moe and Andersen (1988) generalized that fertilization just suboptimal to that resulting in greatest growth of stock plants yields cuttings that root the best. In eastern red cedar (*Juniperus virginiana* L.), there was no correlation between foliar N concentration and rooting (Henry et al. 1992). In our study, there were positive correlations between rooting and foliar N and P concentrations, and negative correlations with K and Mg concentrations. Positive correlations may reflect the vigor of the stock plants, but the significance of the negative correlations is unknown. In eastern red cedar, K supply was positively correlated with rooting and growth of roots (Henry et al. 1992). The greater concentration and ratio of Ca in the R category may have to do with the apparent accumulation of this element in plants that tend to be less vigorous (Timmer and Armstrong 1987). Svenson and Davies (1995) reported a decline in P, K, Ca, and Mg during initiation of roots in poinsettia cuttings and

Table 4. Mean dry weights (g) of adventitious roots, in cuttings that produced them, in the IBA quick-dip trial using five clones of the moderate (M) rooting category. Standard error = SE, means in rows marked with the same letter are not significantly different from each other at $\alpha = 0.05$.

	IBA quick dip concentration				
	Control (0)	0.5%	1.0%	1.5%	2.0%
Dry weight ± SE (g)	0.017 ± 0.005 c	0.026 ± 0.003 ab	0.031 ± 0.003 ab	0.023 ± 0.004 bc	0.039 ± 0.005 a

low concentrations of these elements during subsequent root elongation. Our stock plants were intensively managed, so it is not surprising that there were no significant differences among rooting categories in their nutrient concentrations and in Ingestad ratios (except for Ca), although the latter were lower than those optimal for many conifers (Ingestad 1979). It appears that foliar nutrient concentrations in intensively managed stock plants are not factors that radically influence the rooting success of Canada yew. Choosing large (long and thick) cuttings may improve rooting because both these variables were positively correlated with growth of the rooted plants.

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