Effects of Crown Position and Plant Age on Rooting of Jack Pine Long Shoot Cuttings

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Rooting success of long shoot cuttings of jack pine— Pinus banksiana Lamb*.— was found to be influenced by crown location effects among 5-to 30-year-old trees. Despite some variation in response, mid to lower crown cuttings generally rooted more readily than upper crown cuttings. The highest rooting frequencies observed for 4-, 5-, 6-, 7-, and 10-year-old trees ranged from 45 to 75%, whereas cuttings from 30-year-old trees rooted at 28%. These results are encouraging in light of the general perception that jack pine is difficult to propagate by rooted cuttings, and they suggest that operational asexual propagation may be feasible.* Tree Planters' Notes 47(3): 100-104; 1996.

Development of asexual propagation techniques for use in breeding programs or in larger operational scale situations is of interest for many forest species including jack pine— *Pinus banksiana* Lamb. (for example, Klein and others 1995). Richie (1991) has recently reviewed many of the potential uses of rooted cuttings in forestry. The goal of the present study was to examine the influence of crown and sampling location on the rooting success rate of jack pine long shoot cuttings from several age classes. Jack pine historically has been difficult to propagate by vegetative means (Chesick and Bergmann 1991), particularly from plants 5 years and older (Haissig 1982, 1983, 1989). Effects of crown sampling location on rooting of cuttings have been documented for several conifer species (Henry and others 1992; Roulund 1973; Tousignant and others 1995). In general, cuttings sampled from the lateral branches of the lower crown have rooted most readily. Previous investigations with jack pine have shown that there is a transitional period of decline in rooting capacity of central axis cuttings at 4 to 6 years of age (Browne and others 1997a). Agerelated declines in rooting frequency are expected to occur most rapidly and profoundly at the central leading axis, based on theories about the process of maturation in forest trees (Bonga 1982). Initially, there appears to be little difference in rooting capacity of central and lateral axis cuttings, based on trials with young seedlings (Browne 1996). It may be that as presumptive maturation-related losses in rooting capacity proceed, shoots in the lower crown regions retain some degree of juvenile potentiality, resulting in higher levels of regenerative capacity (Bonga 1982; Hackett 1985). This study is also part of a larger scale study on general aspects of asexual propagation of jack pine (see Browne and others 1997a, b; Klein and others 1995) aimed at developing improved methodologies.

Materials and Methods

Cuttings were sampled from different age classes of jack pine— *Pinus banksiana* Lamb.— originating from sites planted in the Sandilands Provincial Forest area of southern Manitoba, Canada. All sites had been planted with 2+0 seedlings (open-pollinated seed) from the Provincial Government Tree Seedling Nursery at Hadashville, Manitoba. Ages of plants at the time of sampling were confirmed by consultation with the Manitoba Department of Natural Resources planting records.

In an initial study, age-related changes in rooting potential had been identified and appeared to be most influential between ages 5 to 10 years (Browne and others 1997a). By 7 years, there was a marked decline in rooting potential. This change was explored in greater detail with respect to crown location. In the first trial, cuttings from only 7-year-old plants were used. A total of 10 trees were randomly selected and cuttings bulked according to crown sampling location (upper, mid, and lower). These positions were assigned based on ratios of lateral branch whorl number from the tip of the tree to total number of branch whorls. A score of <0.35 was considered upper crown, 0.35 to 0.65 mid crown, and >0.65 lower crown. The distal 20-cm lateral shoots were removed and bulked into 3 groups based on relative position within each tree. All sampling was done in late spring, just before to visible bud burst. Cuttings were kept in cold storage for 2 months before preparation for rooting experiments. Storage of harvested material has been shown not to influence subsequent rooting potential (Browne 1996). Rooting response of these cuttings was monitored at the end of an 8-week period under propagation conditions. A completely randomized block experimental design, with 18 replicates of 10 cuttings each for each crown position, was used. In addition, tissue nutrient contents by crown location were determined by a local commercial laboratory (NorWest Laboratories, Winnipeg, Manitoba). This material was equivalent to that used for cuttings in the rooting trial. All shoot samples were harvested and frozen at -20 /C (-4 /F) before analysis. Foliar and stem tissue were analyzed for N, P, K, and micronutrients.

In the second trial, 6 uniformly aged stands of trees were sampled. Plants ranged from 4 to 30 years of age (4, 5, 6, 7, 10, and 30). Terminal shoots from lateral branches were sampled from 1 of 3 designated crown locations as previously outlined (upper, mid, and lower) and placed in rooting trials. A completely randomized block design with 12 replicates of 6 cuttings for each crown position and age class were used. Due to limited lateral branch development in the 4- and 5-year age groups, fewer cuttings were collected. Cuttings were kept in cold storage for 2 months before preparation for rooting experiments.

Treatment of cuttings. Terminal shoot segments were removed from the tree about 2 cm above the end of the previous season's growth, trimmed to 8 to 12 cm in length and the basal foliage from 1 to 2 cm (0.4 to 0.8 in) was removed. Cuttings were then dipped in a rooting hormone (5.4 µM naphthalene acetic acid dissolved in 95% ethanol) for 10 seconds, inverted for 30 seconds, and inserted into rooting medium. This medium consisted of 1:1 (v/v) Forestry Mix (Grace Horticultural Products, Edmonton, Alberta) and vermiculite (medium grade), filled into PBI-45 (110 ml cell volume) multipots (Plastiques Gagnon, Quebec, Quebec).

The rooting trials were all completed in a 15 (6 m (52.5 (19.7 ft) plastic-covered propagation greenhouse located at the Agriculture and Agri-Food Canada Research Centre at Morden, Manitoba. The containers were placed on raised beds filled with sand and misted from single lines of overhead anviltype nozzles spaced at 1-m (3.3-ft) intervals. Misting was delivered in 10second bursts every 8 minutes (18-hour cycle), maintaining a relative humidity of 50 to 90%. Air was drawn through the poly-house with a fan-louver system. Temperatures ranged from 20 to 38 /C (60 to 100 /F) during the day and 10 to 20 /C (50 to 68 /F) at night. Irradiances ranged from 1,030 to 1,980 µE/m^2/sec on sunny days and 270 to 770 µE/m^2/sec on cloudy days, under natural photoperiod.

Rooting assessments were made after 2 months under propagation conditions. The presence of roots >2 mm and total number of roots were counted and the length of longest roots were measured. Means and standard errors were calculated. Analysis of variance (ANOVA) was done using a SAS statistical program (SAS 1991) and least-squares-means comparisons to determine significant effects of age and crown location. Least-squares-means comparisons allows statistical comparisons among the various treatments to determine if differences are significant or not.

Results

In the first trial (table 1), rooting frequency was significantly affected by crown position (P=0.0064). Cuttings from the lower crown position rooted with the highest frequency, with progressively lower values from mid to upper crown positions. Crown position did not affect the numbers of roots produced on the rooted cuttings (P=0.4610). Similarly, root lengths observed did not vary among the various crown locations sampled (P=0.5479).

Nutrient analyses showed that nitrogen content was lower for mid and lower crown shoot tissue (table 2). In addition, boron content was lowest among lower crown cuttings, while copper and manganese were higher. No trends were found in the other elements with respect to descending crown position; contents appeared similar (data not presented).

In the second trial, both age (P<0.0001) and crown position (P= 0.0002) were found to significantly affect rooting frequency. As cutting age increased there was a general decline in rooting potential (table 3). Similarly; there was also a tendency for lower and mid-crown sampling locations to root at a higher frequency than upper locations, but this was not consistent throughout the trial.

Cuttings from the lower crown of 4-year-old trees rooted at a 74%, frequency, and rooting frequencies from the other 2 crown locations were not significantly different (table 3). All rooting frequencies of cuttings from 5-year-old plants were significantly lower than those of

Table 1—*Effect of crown position on rooting of long shoot cuttings from 7-year-old jack pine* —Pinus banksiana *Lamb.*

Crown location	Rooting frequency (%)	*P* values	Mean total no. of roots	*P* values	Mean root length (cm)	*P* values
Upper	17.8	0.0017	5.4 ± 0.6	0.2837	16.4 ± 1.2	0.3958
Mid	24.4	0.0591	5.3 ± 0.6	0.2911	15.5 ± 1.2	0.8410
Lower	33.3	—	4.2 ± 0.4	—	15.9 ± 0.9	—

Note: Least square means comparisons were made with the lower crown location; P values indicate significant level of difference from lower location.

Table 2—*Mineral nutrient levels from foliar samples of 7-year-old jack pine*—Pinus banksiana *Lamb.*

Crown location	N (%)	B (ppm)	Cu (ppm)	M (ppm)
Upper	1.6	23.3	1.4	112.0
Mid	1.2	19.3	1.8	143.0
Lower	1.2	17.4	2.6	203.0

Table 3—*Effect of crown position on rooting of long shoot cuttings from 4-, 5-, 6-, 7-, 10-, and 30-year-old jack pine*
—Pinus banksiana *Lamb.*

Age (years)	Crown location	Rooting frequency (%)	*P* values	Mean total number of roots	*P* values	Mean root length (cm)	*P* values
4	Upper	62.5	0.1868	1.6 ± 0.2	0.0885	9.0 ± 1.4	0.1543
4	Mid	70.1	0.6711	2.5 ± 0.4	0.6562	11.3 ± 1.5	0.5107
4	Lower	73.8	—	2.7 ± 0.3	—	12.0 ± 1.2	—
5	Upper	23.6	0.0001	2.0 ± 0.3	0.4663	12.0 ± 2.0	0.8519
5	Mid	44.2	0.0006	2.8 ± 0.4	0.8795	12.3 ± 1.2	0.9921
5	Lower	45.0	0.0009	2.7 ± 0.3	0.9126	11.5 ± 1.4	0.8551
6	Upper	52.8	0.0144	3.9 ± 0.4	0.1198	13.2 ± 1.1	0.7352
6	Mid	72.2	0.8574	4.1 ± 0.3	0.0409	14.3 ± 0.8	0.2839
6	Lower	44.4	0.0007	3.5 ± 0.4	0.2264	12.5 ± 1.2	0.9953
7	Upper	45.8	0.0012	5.0 ± 0.6	0.0001	12.2 ± 1.1	0.6244
7	Mid	54.2	0.0222	5.5 ± 0.4	0.0001	14.9 ± 1.0	0.2184
7	Lower	48.6	0.0035	4.2 ± 0.5	0.0774	12.3 ± 1.5	0.6355
10	Upper	22.2	0.0001	3.6 ± 0.7	0.0626	17.5 ± 2.4	0.0040
10	Mid	43.1	0.0004	1.7 ± 0.2	0.1636	11.1 ± 1.4	0.7506
10	Lower	44.4	0.0007	1.5 ± 0.1	0.0636	10.1 ± 1.1	0.5029
30	Upper	16.7	0.0001	2.4 ± 0.4	0.6647	14.0 ± 1.7	0.4732
30	Mid	27.8	0.0001	2.6 ± 0.4	0.8241	12.7 ± 1.7	0.8063
30	Lower	18.1	0.0001	1.5 ± 0.2	0.0927	9.2 ± 2.2	0.2313

Note: Least square means comparisons were made with lower crown position of 4-year group; *P* values indicate significant level of difference from 4-year old plants.

4-year-old plants (table 3). Rooting frequencies for the other age classes (6, 7, 10, and 30 years) were also significantly lower than those of the 4-year-old plants, except for the 72% rooting frequency for the mid crown cuttings of 6-year-old trees, which were not significantly different than the lower crown cuttings of 4-year-old plants (table 3). Based on results from this experiment, rooting frequency as high as 44 to 54% can be obtained from 7- and 10-year-old trees, and 28% for 30year-old trees grown in the forest with no additional cultural treatments.

Differences in root length were generally not significant within any age class (table 3). However, root number varied somewhat with crown position ($P=0.0409$) and with age ($P<0.0001$). Numbers of roots in any age class or crown location were not significantly different than those from lower crown cuttings of 4-year-old plants, with the exception of significantly higher values obtained from mid crown cuttings of 6-year-old plants and upper/mid crown cuttings of 7-year old plants (table 3). In the 4-year age class, the number of roots and root length tended to be greater for mid lower crown cuttings. In 10- and 30-year groups, the number of roots tended to be lower for mid and for lower crown locations, and the roots tended to have shorter lengths. Trends were less apparent in other age classes.

Discussion

Within each age class, the highest rooting frequency tended to originate from the lower and/or mid crown, but differences appeared marginal in some cases. Notably, there was a crown-location effect observed in the first trial on 7-year-old trees, but none seemed to exist within the 7-year-old age group in the second trial. In this latter trial, cuttings from 7-year-old trees rooted at relatively high frequencies compared to the first and no clear positional effect was observed. These 2 groups of plants originated from different locations, and results may have been affected by genotypic and environmental influences. Selby and others (1988) found that upper crown cuttings from average (rated on vigor) Sitka spruce— *Picea sitchensis* (Bong.) Carriere— rooted at a significantly lower frequency than lower crown cuttings, but there were no significant influences of crown location on rooting of cuttings from elite trees.

There is a general lack of physiological evidence to explain crown location effects on rooting of cuttings from forest trees. One possibility is that there are regional differences in nutrient status, which secondarily affects rooting capacity. Results from analyses of 7-year-old jack pine indicated that lower N content may be associated with higher root capacity. Fertility studies have shown that N levels affect root initiation and lower levels are sometimes beneficial (Blazich 1988). Both manganese and boron are known to influence endogenous auxin levels, via activation of IAA oxidase (Blazich 1988; Jarvis and others 1984). The implications for this study are unclear, but perhaps there was a combined effect on the balance of auxin levels. There appears to be a lack of information for possible copper effects on adventitious root initiation (Blazich 1988). In general, interpretation of nutrient effects on adventitious rooting is constrained by a lack of information on their role at the cellular level during the early critical stages of root initiation (Blazich 1988).

During the course of this study, it became apparent that there were differences in shoot characteristics with respect to crown position. Preliminary measurements indicated that shoots from the lower and mid crown tended to have lower stem caliper, lower new--growth increments, and needles with higher length to width ratios (Browne, data not presented). The significance of these characteristics as a means of determining rooting potential (morphological markers) remains to be determined. Clearly; there appeared to be less vigor among lower crown shoots, particularly for the 10- and 30-year-old trees. This may, in part, account for differences in root number and length in comparison to upper crown cuttings.

This study successfully demonstrated that improvements in rooting of jack pine cuttings from 4- to 30-year-old plants can be realized through selection of mid to lower crown cuttings. The highest rooting frequencies obtained from these age classes exceeded those reported in other studies from jack pine of similar age (Haissig 1983, 1989; Zsuffa 1974). In light of these current findings, the feasibility of developing an operational rooted cutting program for jack pine appears more tenable. Populations of rooted cuttings from the various age classes were established in pots under greenhouse conditions and have now been out-planted for continued assessment in field trials.

Conclusions

The rooting of jack pine cuttings is affected by a variety of factors. Plant age and sampling location in relation to crown position appear to play an important role in the rooting process. Younger plants root more readily than older ones. Lower crown to mid crown cuttings typically root at a higher frequency than upper crown cuttings. However, this may be a tenuous conclusion, due to observed variations in rooting responses among age classes. It is apparent that rooting response of jack pine cuttings can likely be enhanced by judicious shoot selection, and that results reported here demonstrate a marked improvement over those of previous studies. The relative success of this study may not solely be due specifically to crown location effects, but also to other aspects of rooting methodology (for example, rooting environment, date of sampling). Further investigations may delineate possible effects of other factors on the rooting of jack pine cuttings.

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