Bud Removal Affects Shoot, Root, and Callus Development of Hardwood *Populus* Cuttings

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Abstract

The inadvertent removal and/or damage of buds during processing and planting of hardwood poplar (*Populus* spp.) cuttings are a concern because of their potential impact on shoot and root development during establishment. The objective of the current study was to test for differences in shoot dry mass, root dry mass, number of roots, length of the longest root, and callus dry mass among ten poplar clones subjected to three pre-planting bud removal intensities (0%, 50%, 100%). The ten clones and their genomic groups were: DM115 (*P. deltoides* Bartr. ex Marsh × *P. maximowiczii* A. Henry); DN34, I45-51 (*P. deltoides* × *P. nigra* L.); NC13446, NC13563, NC13649, NC13685, NC13747 (*P. trichocarpa* Torr. & Gray × *P. deltoides*); and NM2, NM6 (*P. nigra* × *P. maximowiczii*). Cuttings, 20 cm long, were processed from shoots collected January 2005 from stool beds established at Hugo Sauer Nursery in Rhinelander, Wisconsin, U.S.A. (45.6°N, 89.4°W). We measured the traits from harvested cuttings after 14 d of growth. The treatment x clone interaction governed shoot dry mass (*P < 0.0001*). In general, the top four clones (DM115, DN34, NM2, NM6) exhibited the best shoot dry mass with 0% and 50% of buds removed, while differences among treatments for the remaining clones were negligible. Clones differed for root dry mass...

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(P < 0.0001), while the treatment and clone main effects governed number of roots (P = 0.0126, P < 0.0001, respectively) and length of the longest root (P = 0.0077, P < 0.0001, respectively). Cuttings subjected to the 0% treatment exhibited the greatest number of roots, while cuttings of the 0% and 50% treatment exhibited the greatest length of the longest root. The treatment x clone interaction governed the presence of callus (P = 0.0161), while clones differed for callus dry mass (P < 0.0001). Bud removal did not affect root biomass but it did impact root initiation. Unlike shoot dry mass, the response to removing buds for all rooting traits was not clone-specific. From a practical standpoint, inadvertently damaging and/or removing ≤ 50% of the buds during processing and planting should not be a concern for establishment.

Key words: adventitious rooting, hybrid poplar, preplanting treatment, vegetative propagation, *Populus deltoides*, *P. nigra*, *P. maximowiczii*, *P. trichocarpa*.

**Introduction**

Short rotation intensive forestry systems have gained global credibility during the latter half of the 20th century and beginning of the 21st century because of the persistent need for fiber, wood products, and environmental services resulting from human population growth (Heilman, 1999; Tolbert and Wright, 1998; Joslin and Schoenholtz, 1997). The need for short rotation forestry in the North Central United States has become prevalent in past decades because of the loss of native forests to urbanization, the decrease in wood production from public forests, and the time required for native aspen (*Populus tremuloides* Michx. {quaking aspen}; *Populus grandidentata* Michx. {bigtooth aspen}) to reach marketable size following intensive harvesting (Gladstone and Ledig, 1990).

The use of species and hybrids within the genus *Populus* (hereafter referred to as poplars) have been developed, tested, and deployed in the North Central United States since the 1970’s (Zalesny et al., 2005b; Riemenschneider et al., 2001; Robison and Raffa, 1996; Farmer et al., 1989; Ying and Bagley, 1976). Poplars have proven to be useful alternatives to the region’s current utilization and growth potential in the North Central United States and their anticipated range of rooting abilities. The clones were: DM115 (*P. deltoides* Bartr. ex Marsh x *P. maximowiczii* A. Henry); DN34, 145-51 (*P. deltoides* x *P. nigra* L.); NC13446, NC13563, NC13649, NC13685, NC13747 (*P. trichocarpa* Torr. & Gray x *P. deltoides*) x *P. deltoides*; and NM2, NM6 (*P. nigra* x *P. maximowiczii*).

**Materials and Methods**

**Clone selection**

Ten poplar (*Populus* spp.) clones were selected from four genomic groups during January 2005 based on their current utilization and growth potential in the North Central United States and their anticipated range of rooting abilities. The clones were: DM115 (*P. deltoides* Bartr. ex Marsh x *P. maximowiczii* A. Henry); DN34, 145-51 (*P. deltoides* x *P. nigra* L.); NC13446, NC13563, NC13649, NC13685, NC13747 (*P. trichocarpa* Torr. & Gray x *P. deltoides*) x *P. deltoides*; and NM2, NM6 (*P. nigra* x *P. maximowiczii*).

**Cutting preparation and treatment application**

Shoots were collected on January 11, 2005 from stool beds established at Hugo Sauer Nursery in Rhinelander, Wisconsin, U.S.A. (45.6°N, 89.4°W). Hardwood cuttings, 20 cm long, were processed immediately following shoot collection, with cuts made to position at least one primary bud not more than 2.5 cm from the top of each cutting. Cuttings were sealed in polyethylene bags and stored at 5 °C for 28 d. Before planting, treatments consisting of 0%, 50%, and 100% bud removal were applied to the cuttings. The 0% treatment served as the experimental control. The 50% treatment consisted of removing the uppermost bud and then removing every other bud in a basipetal direction until half of the buds were removed. Bud removal consisted of using a razor blade...
and slicing the base of the bud at a plane perpendicular to the cutting so as not to damage cutting tissue or the axillary buds.

**Planting**

Cuttings were soaked in water to a height of 15 cm for 3 d at a daytime and nighttime temperature of 24°C and 20°C, respectively, before planting in book planters containing equal parts of sand, peat, and vermiculite (v:v:v). Planting took place on February 8, 2005 in a greenhouse at the Forestry Sciences Laboratory in Rhinelander with a 16-h photoperiod and air temperatures equal to those used for soaking. The cuttings were irrigated with overhead irrigation at a frequency of 15 s h⁻¹ d⁻¹, a watering schedule that provides good growing conditions.

**Data collection and analysis**

Shoot, root, callus, and cutting dry mass, number of roots, and length of the longest root were determined 14 d after planting. Trees were excavated and washed prior to dissection of shoots, roots, and callus. Following dissection, number of roots and length of the longest root were recorded, and individual plant components were bagged and dried at 70°C for 72 h for dry mass determination.

Shoot and root dry mass, number of roots, and length of the longest root data were subjected to analyses of variance according to SAS® (PROC GLM; SAS INSTITUTE, Inc., 2004) assuming a split-plot design arranged in randomized complete blocks, with six blocks, three treatments (0%, 50%, 100% bud removal), and ten clones. Blocks were considered random in the analysis, while treatments were fixed whole plots and clones were fixed sub plots. Clones were arranged in randomized complete blocks in order to minimize effects of any potential environmental gradients in the greenhouse, and clones were treated as fixed in the analysis in order to analyze means rather than variances. The significance of the interaction between block and clone from the original all-effects model was tested to evaluate potential pooling with the residual error term (the three-way interaction between block, treatment, and clone) to increase precision of F-tests, assuming a probability level for pooling of $P \geq 0.25$ (ZALESNY et al., 2005b).

Probability levels associated with the interaction between block and clone for shoot dry mass, root dry mass, number of roots, and length of the longest root were $P = 0.4040$, $P = 0.9607$, $P = 0.1305$, and $P = 0.0385$, respectively. Therefore, the block x clone interaction was pooled with the residual error term for shoot dry mass and root dry mass, but not for the other two rooting traits. Thus, the following linear additive model was used for shoot dry mass and root dry mass:

$$ Y_{ijk} = \mu + B_i + T_j + BT_{ij} + C_k + TC_{jk} + BTC_{ijk} + \text{Pooled Error} $$

where: $Y_{ijk}$ = response variable to be analyzed, $\mu$ = overall mean, $B_i$ = main effect of $i^{th}$ block, $T_j$ = main effect of $j^{th}$ treatment, $BT_{ij}$ = effect of interaction between $i^{th}$ block and $j^{th}$ treatment, $C_k$ = main effect of $k^{th}$ clone, $TC_{jk}$ = effect of interaction between $j^{th}$ treatment and $k^{th}$ clone, and pooled error = error term resulting from pooling of $BC_k$ and $BTC_{jk}$ terms, defined as: effect of interaction among $i^{th}$ block and $k^{th}$ clone, and effect of interaction between $i^{th}$ block, $j^{th}$ treatment, and $k^{th}$ clone, respectively.

In contrast, the following linear additive model was used for number of roots and length of the longest root:

$$ Y_{ijk} = \mu + B_i + T_j + BT_{ij} + C_k + BC_{ik} + TC_{jk} + BTC_{ijk} $$

where variables were as defined above.

Analyses of covariance were conducted to test for the effect of cutting size on all traits because of broad variation in cutting dry mass at 14 d after planting (1.23 to 7.55 g). Cutting dry mass was a significant covariate for shoot dry mass ($P < 0.0001$), root dry mass ($P = 0.0042$), and length of the longest root ($P = 0.0031$); however, cutting dry mass did not have an effect on number of roots ($P = 0.7146$). Therefore, all means except for number of roots were adjusted for the variation in cutting dry mass. Fisher’s protected least significant difference (LSD) was used to compare adjusted and unadjusted means (CARMER and WALKER, 1985; 1982).

The data for callus dry mass were left-skewed with 29% of the cuttings failing to initiate callus formation. Therefore, non-parametric tests were used to evaluate callus formation across treatments and clones. First, a Chi-square ($\chi^2$) test from frequency counts was used to analyze differences for the presence of callus (CODY and SMITH, 1997). However, these data must be interpreted with caution because most expected values in the $\chi^2$ table were estimated with a number of observations less than five. Second, the Kruskal-Wallis Test (an ANOVA on ranks) was used to test for differences in callus dry mass using the all-effects split-plot model described above. A Bonferroni adjustment ($\alpha’$) of $[\alpha/(k(k–1)/2)] = 0.0011$ ($\alpha = 0.05$ and $k = 10$) was used to limit the experiment-wise error rate to $\alpha \leq 0.05$. Significant effects were differentiated according to Fisher’s protected LSD using $\alpha’ = 0.0011$ in lieu of $\alpha = 0.05$.

**Results**

Treatment and clone main effects were significant for shoot dry mass ($P = 0.0004$, $P < 0.0001$, respectively), but the treatment x clone interaction governed this trait ($P < 0.0001$). There was broad variation among clonal responses to treatments, with a general trend for the top four clones (DM115, DN34, NM2, NM6) of cuttings subjected to 0% and 50% of buds removed exhibiting greater shoot dry mass than those with 100% of buds removed (Fig. 1). Cuttings of the 0% and 50% treatments were superior to those of the 50% treatment for clones DM115 and NM6. Differences among treatments for the remaining six clones were negligible. Overall, shoot dry mass across treatments and clones ranged from 0.00 ± 0.00 to 160.27 ± 15.06 mg, with a mean of 45.05 ± 9.01 mg.

The main effect of treatment and the treatment x clone interaction were negligible for root dry mass ($P = 0.8557$, $P = 0.2165$, respectively), but clones differed for this trait ($P < 0.0001$). There was extensive genotypic variation among clones belonging to the backcross
genomic group [(P. trichocarpa x P. deltoides) x P. deltoides; the NCxxx clones] (Fig. 2), which was expected given the potential variation in allele distribution during backcross breeding. Clone 145-51 exhibited the least root dry mass that was not different from any backcross clone except NC13446. Overall, root dry mass across clones ranged from 7.12 ± 11.56 to 137.78 ± 12.49 mg, with a mean of 58.59 ± 14.44 mg.

Treatment and clone main effects were significant for number of roots (P = 0.0126, P < 0.0001, respectively), while the treatment x clone interaction was negligible for this trait (P = 0.1903). Cuttings subjected to the 0% treatment exhibited the greatest number of roots that was significantly greater than the 50% and 100% treatments (7.62 ± 0.92, 5.43 ± 0.67, 4.82 ± 0.74, respectively), which were not different from one another (α = 0.05, n = 60, LSD = 1.75). The genotypic response for number of roots was similar to that of root dry mass with respect to clonal ranks and variation among the backcross clones; however, other genotypic differences varied for each trait (Fig. 2). Overall, number of roots across clones ranged from 0.3 ± 0.2 to 12.7 ± 0.6, with a mean of 6.0 ± 1.5.

Similarly, treatment and clone main effects were significant for length of the longest root (P = 0.0077, P < 0.0001, respectively), while the treatment x clone interaction was negligible for this trait (P = 0.7577). Cuttings subjected to the 0% and 50% treatment were not different from one another and exhibited greater length of the longest root than those of the 100% bud removal treatment (5.95 ± 0.34 cm, 5.56 ± 0.34 cm, 4.57 ± 0.34 cm, respectively; α = 0.05, n = 60, LSD = 0.80). Clones segregated into four response groups for length of the longest root (Fig. 2). Clone NM6 was superior to all other clones, while clones DM115 and NM2 exhibited the second best length. All other clones responded similarly and less.
favorably than those in the aforementioned response groups, with the exception of clone I45-51 that exhibited the shortest roots. Overall, length of the longest root across clones ranged from 0.5 ± 0.5 to 11.2 ± 0.6 cm, with a mean of 5.4 ± 1.0 cm.

The main effect of treatment was negligible for the presence of callus \((P = 0.8986)\), while clones differed for this trait \((P < 0.0001)\). However, the treatment x clone interaction governed the presence of callus \((P = 0.0161)\). Nevertheless, the frequency of cuttings developing or failing to develop callus was stable \((± one cutting)\) across treatments for all clones except clone I45-51. In this case, five cuttings of the 100% bud removal treatment developed callus and one failed to develop callus, compared to three cuttings developing versus three cuttings failing to develop callus for the 0% and 50% treatments. Moreover, according to an ANOVA on ranks, the main effect of treatment and the treatment x clone interaction were negligible for callus dry mass rank \((P = 0.2975, \ P = 0.02975\), respectively), but clones differed for this variable \((P < 0.0001)\). Similar to root dry mass and number of roots, there was broad variation among backcross clones for callus dry mass (Table 1). Overall, callus dry mass ranged from 0.00 to 89.20 mg, with a mean of 16.11 ± 3.91 mg.

**Discussion**

In general, removing less than or equal to 50% of the buds from the poplar cuttings in the current study was not detrimental to cutting establishment. However, our first hypothesis was upheld. That is, the intensity of bud removal was inversely related to shoot, root, and callus development. In general, cuttings subjected to 0% and 50% bud removal exhibited better growth than those with complete bud removal. Thus, we speculate a threshold intensity of bud removal exists between 50% and 100%, above which substantial impacts to shoot, root, and callus development begin. Unfortunately, the methodology of the current study did not allow testing for the identification of this intensity of substantial impact. However, future studies are needed to address this issue. Moreover, our second hypothesis was not upheld for all traits. That is, the interaction between treatment and clone was negligible for all rooting traits and callus dry mass, despite varying clonal responses to bud removal intensities for shoot dry mass and the presence of callus. Once again, further studies are needed to address the issue of clone-specificity towards different intensities of bud removal. Testing a greater number of clones that represent more genomic groups also is needed.

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**Table 1.** Callus dry mass of ten clones in an experiment testing the effects of bud removal on above- and below-ground growth of dormant hardwood cuttings of *Populus*. Clones were differentiated using the Kruskal-Wallis Test (an ANOVA on ranks), with a Bonferroni adjustment \((α')\) of \([α / (k(k−1)/2)] = 0.0011 (α = 0.05 and k = 10)\) to limit the experiment-wise error rate to \(α ≤ 0.05\). Clones with the same letter are not different according to Fisher’s protected least significant difference (LSD) \((α' = 0.0011; \ n = 18; \ LSD = 44.09)\).

<table>
<thead>
<tr>
<th>Clone</th>
<th>Mean rank</th>
<th>Minimum</th>
<th>Mean (± standard error)</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM2</td>
<td>32.81 a</td>
<td>2.2</td>
<td>40.87 ± 5.38</td>
<td>89.2</td>
</tr>
<tr>
<td>NM6</td>
<td>49.03 a</td>
<td>13.8</td>
<td>26.34 ± 2.14</td>
<td>47.5</td>
</tr>
<tr>
<td>DM115</td>
<td>52.97 a b</td>
<td>0.0</td>
<td>28.41 ± 3.85</td>
<td>59.3</td>
</tr>
<tr>
<td>NC13446</td>
<td>74.11 a b c</td>
<td>0.0</td>
<td>19.74 ± 3.81</td>
<td>49.3</td>
</tr>
<tr>
<td>DN34</td>
<td>95.75 b c d</td>
<td>0.0</td>
<td>12.59 ± 2.62</td>
<td>35.8</td>
</tr>
<tr>
<td>NC13649</td>
<td>109.19 c d</td>
<td>0.0</td>
<td>10.86 ± 4.08</td>
<td>65.0</td>
</tr>
<tr>
<td>I45-51</td>
<td>115.25 c d</td>
<td>0.0</td>
<td>7.52 ± 3.14</td>
<td>52.0</td>
</tr>
<tr>
<td>NC13747</td>
<td>117.78 c d</td>
<td>0.0</td>
<td>5.85 ± 1.97</td>
<td>30.1</td>
</tr>
<tr>
<td>NC13563</td>
<td>126.50 d</td>
<td>0.0</td>
<td>5.39 ± 2.10</td>
<td>28.8</td>
</tr>
<tr>
<td>NC13685</td>
<td>131.61 d</td>
<td>0.0</td>
<td>3.51 ± 1.63</td>
<td>22.4</td>
</tr>
</tbody>
</table>

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The interaction between treatment and clone for shoot dry mass is intuitive, given reported data illustrating clonal differences among these genomic groups for aboveground traits (Zalesny et al., 2005b; 2004; Riemenschneider et al., 2001) and that removal of buds should have an impact on the capability of the newly developing cutting to produce a shoot. Although poplars have two axillary buds at the base of each primary bud (Larson and Pizzolato, 1977), 14 d may be too short of a time period for axillary bud initiation and subsequent shoot growth for some genotypes. For example, despite extensive genotypic variability within our backcross population, there were no differences in the response of the backcross clones to treatments. This could be the result of relatively greater axillary bud initiation among the backcross clones compared with those of other genomic groups. There is one exception. Clones DN34 and I45-51 belong to the same genomic group, yet clone I45-51 appeared to exhibit a similar level of axillary bud initiation as the backcross clones, whereby clone DN34 was not able to respond as quickly when all of the buds were removed. Thus, an important practical implication for shoot dry mass is that clones such as DM115, DN34, NM2, and NM6 were more sensitive to complete bud removal, where clones such as I45-51 and the backcross clones were robust to 100% bud removal.

The lack of treatment effects for root dry mass and presence of such effects for number of roots and length of the longest root also are intuitive, given that root dry mass is proportional to storage capacity and the other traits are indicative of meristematic activity and subsequent growth. Researchers have reported root development (i.e. biomass) and root initiation (i.e. number of roots) are regulated by different mechanisms (Friend et al., 1994; Haissig and Davis, 1994; Lux, 1978). For example, DesRochers and Thomas (2003) reported negligible differences for root mass between 5 cm cuttings with a single bud and 10 cm cuttings with multiple buds, despite differences between the cutting groups for root initiation (represented by percent rooting). Consequently, they speculated different mechanisms were responsible for root development and root initiation (DesRochers and Thomas, 2003). Traditionally, root dry mass has been the most common parameter for rooting studies because of its ease of estimation before the advent of modern equipment (Bohm, 1979). However, we believe the lack of treatment effects for root dry mass most likely was the result of cuttings being grown for 14 d, during which meristematic activity, root initiation, and subsequent growth in length were more prevalent than storage functions. Nevertheless, the treatment response for number of roots of our cuttings corroborated the results of previous studies (Smith and Wareing, 1974). For example, Eggens et al. (1972) reported single root initiation from cuttings without buds and numerous roots from cuttings with buds. Smith and Wareing (1972) reported 1.9 times the number of roots from cuttings with buds versus debudded cuttings, both grown for 21 d at 20°C. In the current study, cuttings of the 0% treatment exhibited greater than 1.5 times the number of roots as those of the 100% treatment. In addition to the lack or presence of treatment effects, clonal differences for all rooting traits corroborate findings of previous studies (Zalesny et al., 2005a; 2005b; 2003; Riemenschneider and Bauer, 1997; Farmer et al., 1989; Ying and Bagley, 1977; Wilcox and Farmer, 1968).

The interaction between treatment and clone for the presence of callus must be interpreted with caution, given the small sample sizes used for the expected values in the $\chi^2$ test. The frequency counts were consistent across treatments for all genotypes other than clone I45-51. However, differences among clones existed, regardless of treatment. Similarly, there was broad variation in callus dry mass across clones, according to the ANOVA on ranks. Although treatments were negligible for callus development, the practical implication of these results was that callus production was under strong genetic control and, therefore, should be responsive to selection. Furthermore, selection of clones with great levels of callus dry mass offers a means of potentially increasing the capability of successful cutting establishment, because callus root development is one of two rooting ontogenies present in the genus Populus (Luxova and Lux, 1981a; 1981b).

**Conclusion**

Early establishment of poplar cuttings depends upon successful rooting and subsequent shoot growth. External factors affecting cutting production and establishment include but are not limited to: insects, diseases, herbivores, environmental damage, and mechanical activities. Regardless of the extent of impact from such factors, it is important to consider what effects the damage and/or removal of buds has on early shoot and root development of hardwood poplar cuttings. Results from the current study must be interpreted considering that the cuttings were grown in a greenhouse with minimal stress, in contrast to a field setting where the aforementioned external factors are prevalent. Nevertheless, the two primary lessons from this study are important for short rotation intensive forestry. First, bud removal did not affect root biomass but greater intensities did impact root initiation. Second, unlike shoot dry mass, the response to removing buds for all rooting traits was not clone-specific. Overall, from a practical standpoint, inadvertently damaging and/or removing some buds during processing and planting should not be a concern for establishment. However, if greater than 50% of the buds on any individual cutting are damaged and/or removed, that cutting should not be used, regardless of genotype.

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**References**


Book Review


Trotz der kleinen Mängel ist die Diplomarbeit eine gelungene Zusammenstellung, die die Variation der Behandlungsmethoden aufzeigt und den Baumschulbetrieben Hinweise zur Optimierung gibt. Der Fachhochschule, dem VuB und der LWK Schleswig-Holstein ist zu danken, dass sie diese Diplomarbeit der Öffentlichkeit zugänglich machen. Die Ringbindung und die gewählte Papierqualität sind äußerst praxisfreundlich.

Title: “Conifer seed treatment”

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