Early rooting of dormant hardwood cuttings of *Populus*: analysis of quantitative genetics and genotype × environment interactions

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Abstract: Rooting of hardwood cuttings is under strong genetic control, although genotype × environment interactions affect selection of promising genotypes. Our objectives were (1) to assess the variation in rooting ability among 21 *Populus* clones and (2) to examine genotype × environment interactions to refine clonal recommendations. The clones belonged to five genomic groups ((*Populus trichocarpa* Torr. & Gray × *Populus deltoides* Bartr. ex Marsh.) × *P. deltoides* 'BC'; *P. deltoides* 'D'; *P. deltoides* × *Populus maximowiczii* A. Henry 'DM'; *P. deltoides* × *Populus nigra* L. 'DN'; and *P. nigra* × *P. maximowiczii* 'NM'). Cuttings, 20 cm long, were planted in Iowa and Minnesota in randomized complete blocks at 1.2 m × 2.4 m spacing, across three planting dates during 2001 and 2002. We measured nine belowground and aboveground traits from harvested cuttings after 14 days of growth. Percent rooting ranged from 22% to 86%. Broad-sense heritability for root and top dry mass ranged from 0.09 to 0.11 and 0.31 to 0.38, respectively. There were genotype × environment interactions for most traits, with belowground growing degree-days accounting for >54% of environmental variation. Clonal rooting was stable, except at Westport, Minnesota, during 2002, when root growth was relatively poor.

Résumé : L'enracinement des boutures d'essences feuillues est une caractéristique très héritable, bien que les interactions génotype × environnement puissent affecter la sélection des génotypes prometteurs. Les objectifs de cette étude étaient (1) d'évaluer la variabilité de l'aptitude à l'enracinement parmi 21 clones de *Populus* et (2) d'évaluer les interactions génotype × environnement afin de préciser les recommandations de sélection clonale. Les clones provenaient de cinq groupes génomiques distincts ((*Populus trichocarpa* Torr. & A. Gray × *Populus deltoides* Bartr. ex Marsh.) × *P. deltoides* 'BC', *P. deltoides* 'D', *P. deltoides* × *Populus maximowiczii* A. Henry 'DM', *P. deltoides* × *Populus nigra* L. 'DN', *P. nigra* × *P. maximowiczii* 'NM'). Les boutures de 20 cm de long ont été plantées en Iowa et au Minnesota, aux États-Unis, selon un dispositif en blocs aléatoires complets avec un espacement de 1,2 m × 2,4 m et à trois dates de plantation en 2001 et 2002. Les auteurs ont mesuré neuf caractères sur les parties aériennes et souterraines de boutures récoltées après 14 jours de croissance. Le pourcentage d'enracinement variait de 22 % à 86 %. L'héritabilité au sens large pour le poids sec des racines ou de la partie aérienne variait respectivement de 0,09 à 0,11 et de 0,31 à 0,38. Des interactions génotype × environnement ont été remarquées pour la plupart des caractères, alors que le nombre de degrés-jours de croissance dans le sol expliquait >54 % de la variation d'origine environnementale. L'enracinement des clones était stable excepté à Westport au Minnesota en 2002, où la croissance racinaire est apparue relativement modeste.

[Traduit par la Rédaction]

Introduction

The ability of cottonwoods and their hybrids (*Populus* spp., excluding the aspens, and colloquially known as poplars) to form adventitious roots from a dormant hardwood cutting is critical for economic and biological viability of poplar plantations. First, rapid and extensive adventitious rooting reduces establishment costs by permitting the use of unrooted cuttings as commercial propagules, rather than rooted stock. Unrooted cuttings are less expensive to produce and plant than any other propagule. Second, given concomitant and well-balanced shoot development, rapid and extensive rooting promotes early growth, thereby reducing the time to crown closure and vegetation management costs. Third, rapid and extensive rooting that is stable in the face of varying environmental conditions can increase operational flexibility by lengthening the period during which successful planting can occur.

These assertions, taken collectively, suggest that adventitious rooting of poplar cuttings merits substantial, detailed

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study. Breeding for enhanced rooting ability has been a key component of poplar clonal development for nearly three quarters of a century (Stout and Schreiner 1933), because the ability of poplars to form adventitious roots from hardwood cuttings is important for the commercial deployment of intensively cultured plantations (Heilman et al. 1994*b*; Riemenschneider and Bauer 1997). Current breeding efforts in the north central United States primarily focus on the following four poplar species: *Populus deltoides* Bartr. ex Marsh. (eastern cottonwood), *Populus trichocarpa* Torr. & Gray (western black cottonwood), *Populus nigra* L. (European black poplar), and *Populus maximowiczii* A. Henry (Japanese poplar).

Studying the quantitative inheritance of rooting ability should provide information for the development of improved cultural technologies. Such practices may enhance the potential for success of commercial plantation deployment, because rooting is the first biological requirement in stand establishment. In addition, rooting is important because it affects stocking levels and ultimately rotation yield. The influence of rooting on survival supports the use of greater-rooting clones on relatively stressful sites. Greater rooting affects carrying capacity, competition control, and the cost of harvesting at rotation age.

Certain relevant knowledge of rooting exists. For example, Wilcox and Farmer (1967) conducted rooting experiments of *P. deltoides* based on root dry mass, number of roots, and root length and found ranges in broad-sense heritabilities (H) of 0.36–0.58, 0.44–0.56, and 0.33–0.52, respectively. Ying and Bagley (1977) estimated H for number of roots in *P. deltoides* to be >0.80. Riemenschneider and Bauer (1997) reported H of 0.20–0.33, 0.15–0.18, and 0.23–0.28 for root dry mass, number of roots, and root length in *P. trichocarpa*, respectively. In addition, Cunningham (1953) reported that the proportion of cuttings that rooted in a population of 30 *P. deltoides* clones and 30 hybrid clones ranged from 3% to 100%.

The aforementioned studies showed rooting of poplars was highly variable, and the extensive genotypic variation could result in substantial realized selection gains. However, high and unpredictable variation makes it difficult to predict how clones will perform across sites. Prediction is especially difficult because genotype × environment interactions may govern rooting (Haissig et al. 1992; Heilman et al. 1994*a*).

Therefore, existing knowledge is inadequate for our regional needs. Adventitious rooting clearly depends on specific genetic lineage and environmental conditions that lie, respectively, within any given set of optional breeding strategies and within any given geographic zone of commercial deployment. Our set of options includes the varying environments of the north central United States and any poplar that is adapted to that region. Overall, we seek (1) knowledge of any factor that promotes or constrains rooting ability; and (2) guidelines that allow us to incorporate rooting ability as a selection criterion in strategic (selection of parental species) or operational (selection within specified genomic groups) breeding plans. Relevance of any genetic knowledge is further constrained because of the potentially interacting and main effects of regional environmental conditions. Therefore, the objectives of our current study were (1) to assess the variation in rooting ability among 21 *Populus* clones planted in a field setting and (2) to examine genotype × environment interactions to refine clonal recommendations. We believe that selection within the range of the aforementioned variation is important for acquiring knowledge from which sound genotypic selections can be made, given specific deployment and utilization needs.

Materials and methods

Clone and site selection

Twenty-one clones (Table 1) were selected across the current range of clones from five *Populus* genomic groups in December 2000, on the basis of their growth potential and anticipated range of rooting abilities. Clones were treated as random in the analysis to calculate variance components, which were used for estimates of H. Shoots were collected from stool beds established at Hugo Sauer Nursery in Rhinelander, Wisconsin (45.6°N, 89.4°W). Cuttings, 20 cm long, were prepared during December and January of 2001 and 2002. Cuttings were stored in polyethylene bags at 5 °C until each planting date in May and June of 2001 and 2002. Cuttings were soaked in water for 3 days before planting. There were three planting dates for each combination of year and site. Test plots were established at Ames, Iowa (42.0°N, 93.6°W); Waseca, Minnesota (44.1°N, 93.5°W); and Westport, Minnesota (45.7°N, 95.2°W). Sites were chosen because of their inclusion in a *Populus* regional testing program (Riemenschneider et al. 2001*a*) and because they represented a latitudinal gradient from central Iowa to central Minnesota and a range of soil types typical of poplar plantations (Hanlon fine sandy loam at Ames; Clarion loam – Webster clay loam at Waseca; and Estherville sandy loam at Westport). The experimental design comprised randomized complete blocks, with 12 blocks per planting date and one ramet per each of 21 clones per block. Blocks were placed perpendicular to slope gradients, where possible. The spacing between cuttings was 1.2 m × 2.4 m. Two border rows containing clones DN34 and NM2 were established outside the blocks, except at Westport in 2002, where only one border row of clone NM2 was planted because of spatial constraints.

Measurements

Environmental data were collected at 15-min intervals throughout each growing season. HOBO® H8 Pro Series data loggers (Onset Computer Corporation, Bourne, Mass.) were used to record relative humidity, soil temperature at a depth of 20 cm, and air temperature in the shade at 1 m above the ground.

Individual trees were harvested 14 days after planting. A soil mass up to 65 cm in diameter and 40 cm deep was excavated around each developing cutting. Roots were isolated by washing and were photographed with a computerized image-capturing system. Images were stored on high-resolution (high-8 format) videotape and subsequently converted to 8-bit grayscale tagged image file format (TIFF). The TIFF images were analyzed with Optimas™ 6.2 image analysis software (Optimas Corporation, Bothell, Washington) to determine dimensions and numbers of leaves and roots. Leaves, stems, lateral roots, callus, and callus roots were dissected from each

Table 1. Genomic groups, clones, and their origin in an experiment testing the ability of poplar (*Populus* spp.) clones to develop roots from dormant hardwood cuttings.

Genomic group	Clone	Origin
(*P. trichocarpa* × *P. deltoides*) × *P. deltoides* (BC)	NC13563	D. Riemenschneider, USDA Forest Service
	NC13570	
	NC13624	
	NC13649	
	NC13686	
	NC14042	
P. deltoides (D)	D110	C. Mohn, University of Minnesota
	D105	
	D117	
	D133	
P. deltoides × *P. maximowiczii* (DM)	25	V. Steenacker, Belgium;
	DM105	C. Mohn, University of Minnesota, and
	NC14103	D. Riemenschneider, USDA Forest
	NC14105	Service
	NC14106	
P. deltoides × *P. nigra* (DN)[a]	DN17	France ('Robusta')
	DN34	Europe ('Eugenei')
	DN5	Netherlands ('Gelrica')
	DN70	Europe
P. nigra × *P. maximowiczii* (NM)	NM2	Germany
	NM6	Germany

[a]Euramerican hybrids with the common designations of *Populus* × *euramericana* Guin. and *Populus* × *canadensis* Moench.

cutting, bagged, and dried at 70 °C for dry mass determination. Callus roots, which differentiate from callus at the base of the cutting in response to wounding, were considered negligible, because only 15 callus roots were produced over the entire experiment. Thus, subsequent rooting variables refer to lateral roots only. Lateral roots differentiate from latent primordia distributed throughout the length of the cutting. A rooted cutting was defined as any cutting that exhibited a root >1 mm in length, that is, any root clearly distinguishable from a nodule. The dry mass of cuttings was tested as a potential covariate in the analysis and was negligible for each dependent variable ($P > 0.05$).

Nine dependent variables were evaluated: root dry mass (milligrams), callus dry mass (milligrams), top dry mass (dry mass of stem and leaves, milligrams), total leaf area (sum of individual leaf areas, square centimetres), mean leaf area (total leaf area / number of leaves, square centimetres), number of roots, total root length (sum of individual root lengths, centimetres), mean root length (total root length / number of roots, centimetres), and percentage of rooting.

Data analysis

Data were subjected to analysis of variance according to PROC GLM and PROC VARCOMP (SAS Institute Inc. 2000) on multiple-year (model I) and single-year (model II) bases, assuming all random effects (Table 2). Interaction terms with $P \geq 0.25$ from the original all-effects model were pooled with the residual error term to increase precision of F tests.

Planting date was used for logistical efficiency and acted as a surrogate measure of soil temperature. Planting date served the purpose of creating a classification variable associated with belowground growing degree-days (GDD). We used soil temperature instead of air temperature because belowground temperature had a greater influence on rooting. The plant sciences use GDD to predict growth, harvest dates, insect outbreaks, and other biological phenomena (Eisensmith et al. 1980; Dunn et al. 1996; Zalesny et al. 2004). The GDD value is the sum of the average temperature in a 24-h period minus a base temperature; the base temperature equals a threshold that supports adequate plant growth over a specified period. A commonly accepted base temperature in the north central region is 10 °C (Hansen et al. 1983; Hansen 1986; Wan et al. 1999). We converted our soil temperature data to belowground GDD, assuming a base temperature of 10 °C, and used it as a factor in models I and II.

Variance components were determined for both models by using restricted maximum likelihood (REML) estimation in PROC VARCOMP (SAS Institute Inc. 2000). These variance estimates were used to estimate H, the percentage of phenotypic variation among genomic groups due to combined genetic effects, on an individual-tree basis (Wilcox and Farmer 1968).

The specific equations used to estimate H were as follows:

$$H_{\text{MODEL I}} = \frac{\sigma^2_C}{\sigma^2_C + (\sigma^2_{YC} + \sigma^2_{SC} + \sigma^2_{GC}) + \sigma^2_E}$$

$$H_{\text{MODEL II}} = \frac{\sigma^2_C}{\sigma^2_C + (\sigma^2_{SC} + \sigma^2_{GC}) + \sigma^2_E}$$

Table 2. Degrees of freedom and expected mean squares in an experiment testing for clonal differences in poplar rooting.

(A) Multiple-year analysis (model I).

Source of variation	df	Expected mean squares[a,b]
Year	1	$\sigma^2 + 12\sigma^2_{GC} + 108\sigma^2_{YC} + 21\sigma^2_B + 252\sigma^2_G + 756\sigma^2_{YS} + 268\sigma^2_Y$
Site	2	$\sigma^2 + 12\sigma^2_{GC} + 72\sigma^2_{SC} + 21\sigma^2_B + 252\sigma^2_G + 756\sigma^2_{YS} + 1512\sigma^2_S$
Year × site	2	$\sigma^2 + 12\sigma^2_{GC} + 21\sigma^2_B + 252\sigma^2_G + 756\sigma^2_{YS}$
GDD/year × site	12	$\sigma^2 + 12\sigma^2_{GC} + 21\sigma^2_B + 252\sigma^2_G$
Block /(GDD/year × site)	198	$\sigma^2 + 21\sigma^2_B$
Clone	20	$\sigma^2 + 12\sigma^2_{GC} + 72\sigma^2_{SC} + 108\sigma^2_{YC} + 216\sigma^2_C$
Year × clone	20	$\sigma^2 + 12\sigma^2_{GC} + 108\sigma^2_{YC}$
Site × clone	40	$\sigma^2 + 12\sigma^2_{GC} + 72\sigma^2_{SC}$
(GDD/year × site) × clone	280	$\sigma^2 + 12\sigma^2_{GC}$
Error	3960	σ^2
Total	4535	

(B) Single-year analysis (model II).

Source of variation	df	Expected mean squares[a,b]
Site	2	$\sigma^2 + 12\sigma^2_{GC} + 36\sigma^2_{SC} + 21\sigma^2_B + 252\sigma^2_G + 756\sigma^2_S$
GDD/site	6	$\sigma^2 + 12\sigma^2_{GC} + 21\sigma^2_B + 252\sigma^2_G$
Block/(GDD/site)	99	$\sigma^2 + 21\sigma^2_B$
Clone	20	$\sigma^2 + 12\sigma^2_{GC} + 36\sigma^2_{SC} + 108\sigma^2_C$
Site × clone	40	$\sigma^2 + 12\sigma^2_{GC} + 36\sigma^2_{SC}$
(GDD/site) × clone	120	$\sigma^2 + 12\sigma^2_{GC}$
Error	1980	σ^2
Total	2267	

Note: GDD, belowground growing degree-days.

[a]Degrees of freedom and type III expected mean squares generated by using the RANDOM statement in PROC GLM (SAS Institute Inc. 2000).

[b]σ^2 is the variance attributed to the term in the model. Model I: σ^2_Y, year; σ^2_S, site; σ^2_{YS}, year × site; σ^2_G, GDD within year and site; σ^2_B, block within GGD; σ^2_C, clone; σ^2_{YC}, year × clone; σ^2_{SC}, site × clone; σ^2_{GC}, GDD × clone; σ^2, error. Model II: σ^2_S, site; σ^2_G, GDD within site; σ^2_B, block within GDD; σ^2_C, clone; σ^2_{SC}, site × clone; σ^2_{GC}, GDD × clone; σ^2, error.

where σ^2_C is the genotypic variance attributed to genetic differences among clones; σ^2_{YC} is the variance attributed to the interaction between year and clone; σ^2_{SC} is the variance attributed to the interaction between site and clone; σ^2_{GC} is the variance attributed to the interaction between belowground GDD and clone; and σ^2_E is the environmental variance attributed to experimental error.

Principal components analyses (PROC PRINCOMP; SAS Institute Inc. 2000) were used to assess genotype × environment interactions on multiple-year and single-year bases (Manly 1986).

Results

Multiple-year analysis

The percentage of cuttings that rooted after 14 days of growth was highly variable, ranging across years and sites from 22% to 86% (Fig. 1). The NM (81%) and DM (66%) genomic groups exhibited the greatest percentage of rooting, followed by the DN (61%), BC (57%), and D (33%) genomic groups. Clones of the BC and D genomic groups exhibited the greatest variation in rooting percentage (38%–73% and 22%–47%, respectively).

Genotypic effects

Phenotypic correlations for all traits ranged from 0.11 to 0.89, whereas genotypic correlations ranged from –0.14 to 1.00 (Table 3). Clones varied greatly in root dry mass and top dry mass, accounting for 8% and 27% of the total variation in each of these traits, respectively (Table 4). Estimates of H for root dry mass and top dry mass were 0.09 and 0.31, respectively.

Clones differed for root dry mass and top dry mass (Table 4): root dry mass ranged from 1.0 to 11.5 mg (Fig. 2); clones NC13563 and NC13649 exhibited at least twice as much root dry mass as all other clones. The BC genomic group expressed the most variation among clones. Trends in top dry mass differed from those observed for root dry mass. The DM and NM genomic groups had almost twice and more than twice the aboveground production, respectively, of the other groups. Top dry mass ranged from 32.3 to 145.8 mg. Clonal variation within genomic groups was low, except among DM clones (92.3–134.1 mg).

Similar rooting trends existed for number of roots ($P < 0.0001$), total root length ($P < 0.0001$), and mean root length ($P = 0.0016$). Number of roots ranged from 0.7 (D105) to 6.7 (NC13563). Number of roots for NC13563 was almost

Fig. 1. Percent rooting after 14 days of growth in dormant hardwood poplar cuttings of clones belonging to five genomic groups (two groups in A, three groups in B) at three sites in 2 years. Standard error bars represent 1 SE (n = 216 cuttings for each clone).

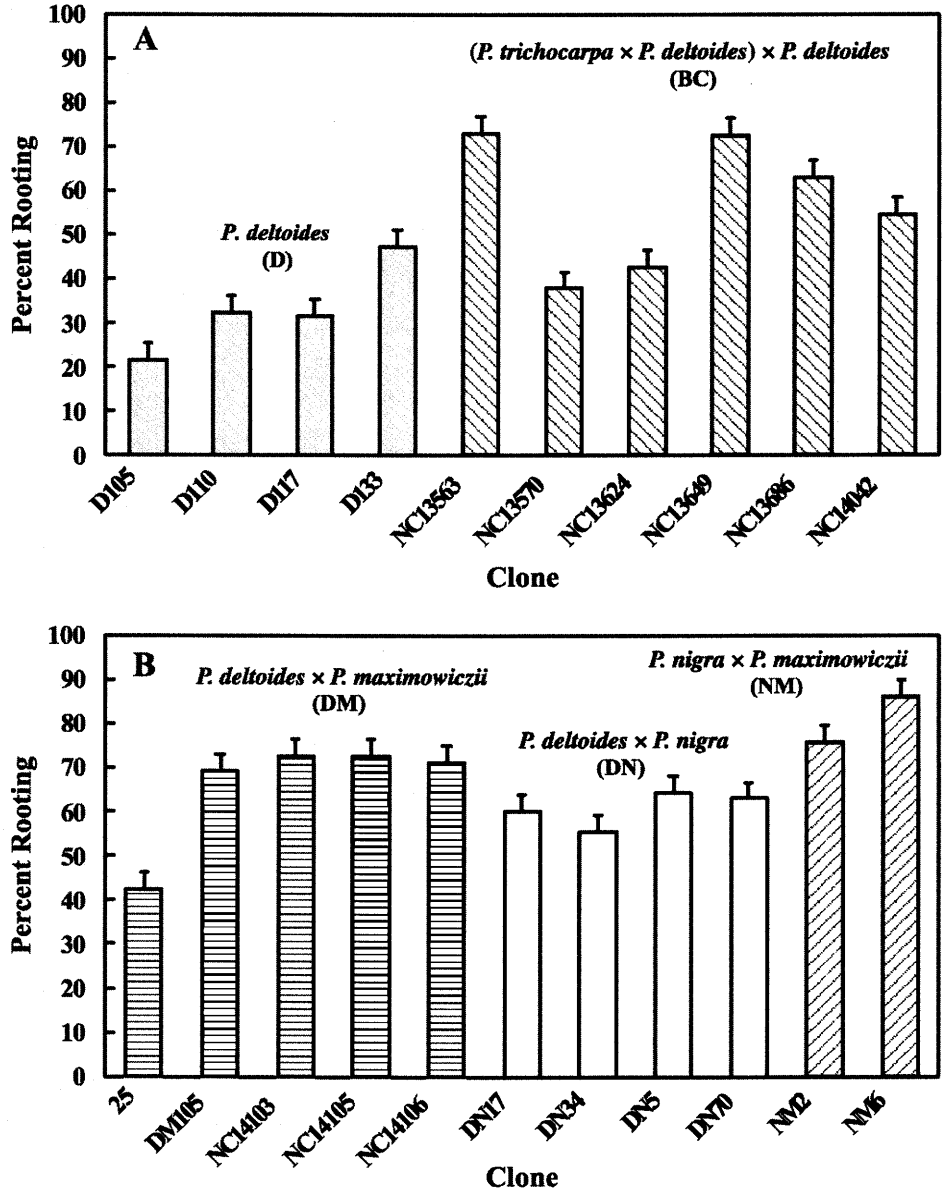


Table 3. Phenotypic correlations (above diagonal) and genotypic correlations (below diagonal) among aboveground and belowground traits in an experiment testing for differences in poplar rooting.

	Cutting dry mass	Root dry mass	Callus dry mass	Top dry mass	Total leaf area	Mean leaf area	No. of roots	Total root length	Mean root length
Cutting dry mass		0.24	0.22	0.60	0.46	0.45	0.24	0.20	0.22
Root dry mass	–0.14		0.13	0.38	0.41	0.40	0.80	0.89	0.61
Callus dry mass	0.57	0.30		0.29	0.24	0.19	0.11	0.11	0.14
Top dry mass	0.94	–0.02	0.66		0.86	0.77	0.40	0.36	0.41
Total leaf area	0.97	0.12	0.55	1.00		0.88	0.44	0.41	0.42
Mean leaf area	0.94	0.14	0.48	0.96	1.00		0.44	0.41	0.43
No. of roots	–0.03	1.00	0.36	0.10	0.25	0.25		0.87	0.52
Total root length	–0.14	1.00	0.25	–0.02	0.13	0.14	1.00		0.67
Mean root length	0.36	0.88	0.44	0.49	0.56	0.55	0.95	0.88	

Note: All phenotypic (Pearson) correlation coefficients were generated using PROC CORR (SAS Institute Inc. 2000) and were significant at $P < 0.0001$. Genotypic correlations estimated according to Falconer and Mackay (1989).

Table 4. Analyses of variance, variance components, and derived statistics in an experiment testing for differences in root dry mass and top dry mass among 21 poplar clones during 2001 and 2002 at Ames, Iowa (42.0°N, 93.6°W); Waseca, Minnesota (44.1°N, 93.5°W); and Westport, Minnesota (45.7°N, 95.2°W).

Source of variation	df[a]	Mean square	*F* variance ratio	*P* value	Variance component[b]	Percentage of total variation	*H*[c]
Root dry mass (mg)							
Year	1	0.60	0.03	0.8858	0	0	σ^2_G = 58.36
Site	2	40.98	1.83	0.3504	14.8	1.94	σ^2_P = 642.24
Year × site	2	22.16	1.16	0.3459	0	0	*H* = 0.09
GDD/year × site	12	19.09	7.86	**<0.0001***	65.1	8.55	
Block/(GDD/year × site)	198	1.36	2.57	**<0.0001***	39.5	5.18	
Clone	20	14.78	6.77	**<0.0001***	58.4	7.66	
Year × clone	20	1.90	1.77	**0.0233***	7.6	1.00	
Site × clone	40	1.35	1.26	0.1451	3.9	0.51	
Clone × (GDD/year × site)	280	1.07	2.04	**<0.0001***	45.5	5.97	
Error	3960	0.53			527.0	69.18	
Total	4535				762.0	100.00	
Top dry mass (mg)							
Year	1	81.27	0.34	0.6130	0	0	σ^2_G = 1.34
Site	2	63.69	0.28	0.7791	0	0	σ^2_P = 4.32
Year × site	2	223.96	2.19	0.1551	0.04	0.76	*H* = 0.31
GDD/year × site	12	102.49	14.12	**<0.0001***	0.38	7.67	
Block/(GDD/year × site)	198	6.84	2.46	**<0.0001***	0.19	3.92	
Clone	20	309.70	14.71	**<0.0001***	1.34	27.11	
Year × clone	20	20.14	6.29	**<0.0001***	0.16	3.17	
Site × clone	40	4.13	1.29	0.1246	0.01	0.26	
Clone × (GDD/year × site)	280	3.20	1.15	**0.0471***	0.04	0.71	
Error	3960	2.78			2.78	56.40	
Total	4535				4.93	100.00	

Note: *, significant at $P < 0.05$ (shown in bold); GDD, belowground growing degree-days.

[a]Degrees of freedom generated by using the RANDOM statement in PROC GLM (SAS Institute Inc. 2000).

[b]Restricted maximum likelihood estimates of variance components.

[c]Broad-sense heritability (*H*), estimated on an individual-tree basis: σ^2_G, genotypic variance attributed to combined genetic effects of clone (σ^2_C); σ^2_P, variance attributed to phenotypic effects; phenotype = genotype + (genotype × environment) + environment; that is, $\sigma^2_C + (\sigma^2_{YC} + \sigma^2_{SC} + \sigma^2_{GC}) + \sigma^2_E$.

twice as much as for all other clones, except NC13649 (5.8), NM6 (4.7), and NC13686 (4.1). Clone D133 (3.1) had more than twice the number of roots of D110 (1.2) and D117 (1.2) and more than three times the number of roots of D105 (0.7). The BC genomic group expressed the most variation among clones (1.2–6.7). Total root length ranged from 1.2 (D105) to 15.5 cm (NC13563). Clones NC13563 and NC13649 had almost twice the total root length of all other clones. Clones NC13570 (2.4 cm) and NC13624 (2.3 cm) exhibited very poor root growth. Clone D133 (6.1 cm) had excellent root growth, despite belonging to an erratic-rooting genomic group. Mean root length ranged from 0.4 (D105) to 1.5 cm (NC13563). Mean root length of NC13563 and NC13649 (1.4 cm) was at least 50% greater than for all other clones except NM2 (1.3 cm) and NM6 (1.4 cm). Once again, clones of the BC genomic group expressed the most variation (0.7–1.5 cm).

Clones differed also for callus dry mass, total leaf area, and mean leaf area ($P < 0.0001$). Callus dry mass ranged from 0.0 (D105, NC13624) to 3.8 mg (NM2). Total leaf area ranged from 1.4 (D105) to 11.1 cm^2 (NM2). The NM clones had three times the total leaf area of all other clones, except those belonging to the DM genomic group. The DM genomic group had the most variation (1.6–9.6 cm^2). Mean leaf area ranged from 0.2 (D105) to 1.9 cm^2 (NM6). NM and DM clones expressed the greatest mean leaf area. Other trends were similar to those shown for total leaf area.

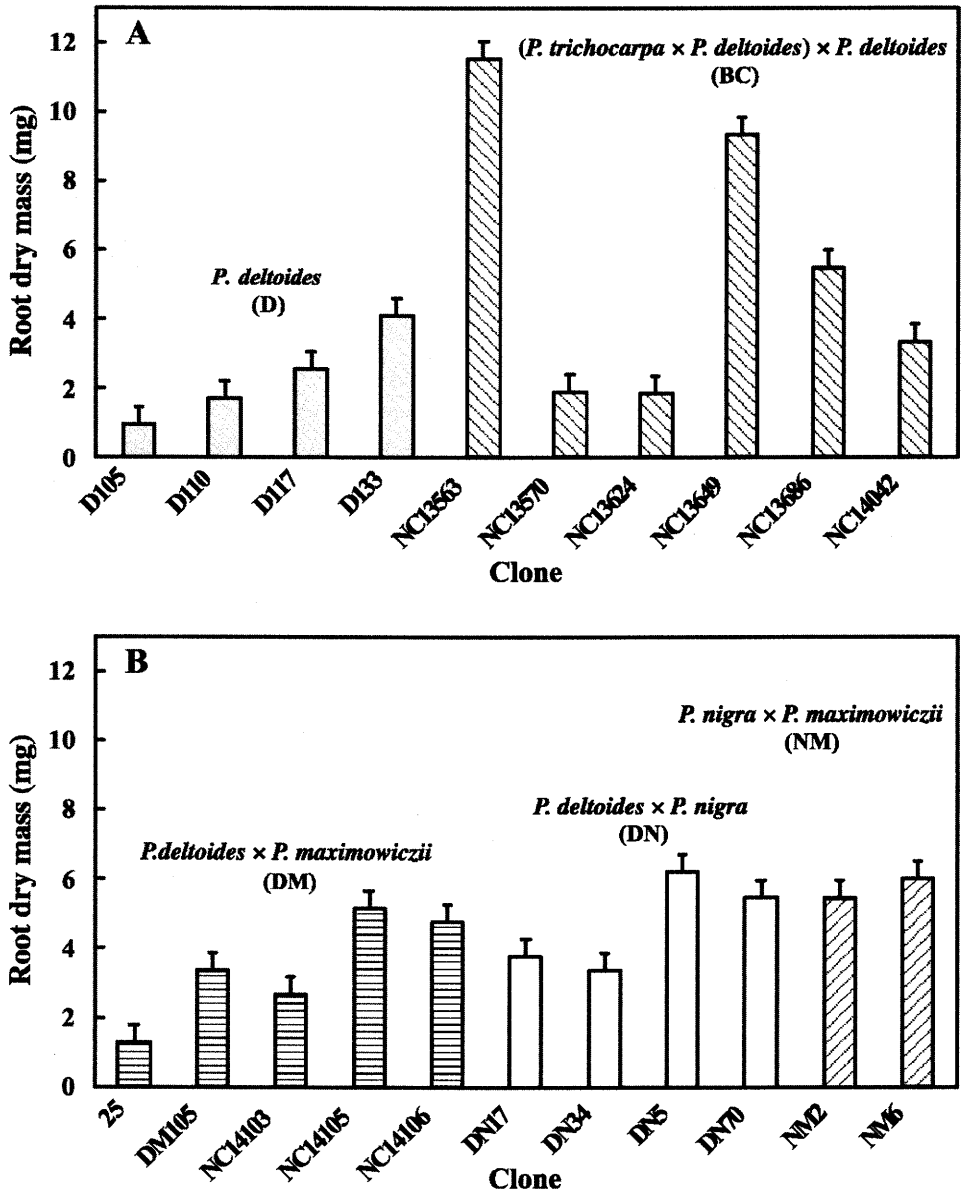
Site effects

Site effects accounted for <2% of the total variation in root dry mass and top dry mass (Table 4). Differences between sites were negligible for all traits ($P > 0.05$).

Genotype × environment interactions

Genotype × environment interactions were highly variable across all traits (Table 4). The interaction of year and clone was significant for root dry mass and top dry mass, along with number of roots ($P = 0.0070$), mean root length ($P = 0.0002$), callus dry mass ($P < 0.0001$), total leaf area ($P < 0.0001$), and mean leaf area ($P < 0.0001$) but negligible for total root length ($P = 0.1592$). The interaction of site and clone was significant for total root length ($P = 0.0551$) and callus dry mass ($P = 0.0026$) but negligible for all remaining traits ($P > 0.05$). The interaction of belowground GDD and clone was significant for root dry mass and top dry mass, along with all remaining traits ($P < 0.0001$).

Fig. 2. Root dry mass after 14 days of growth of dormant hardwood poplar cuttings of clones belonging to five genomic groups (two groups in A, three groups in B) at three sites in 2 years. Standard error bars represent 1 SE (n = 216 cuttings for each clone).



Phenotypic correlations among all sites for root dry mass, number of roots, total root length, and mean root length were similar and uniformly significant (Fig. 3). Correlations among sites for root dry mass were the most variable, ranging from 0.81 (between Ames and Waseca) to 0.94 (between Ames and Westport). All correlations suggested stable clonal performance across sites. Principal component analyses corroborated our univariate interpretation of variance in the four rooting traits. Principal component 1 loaded highly uniformly on all traits at each site, with associated eigenvalues accounting for at least 92% of the variation.

Single-year analysis

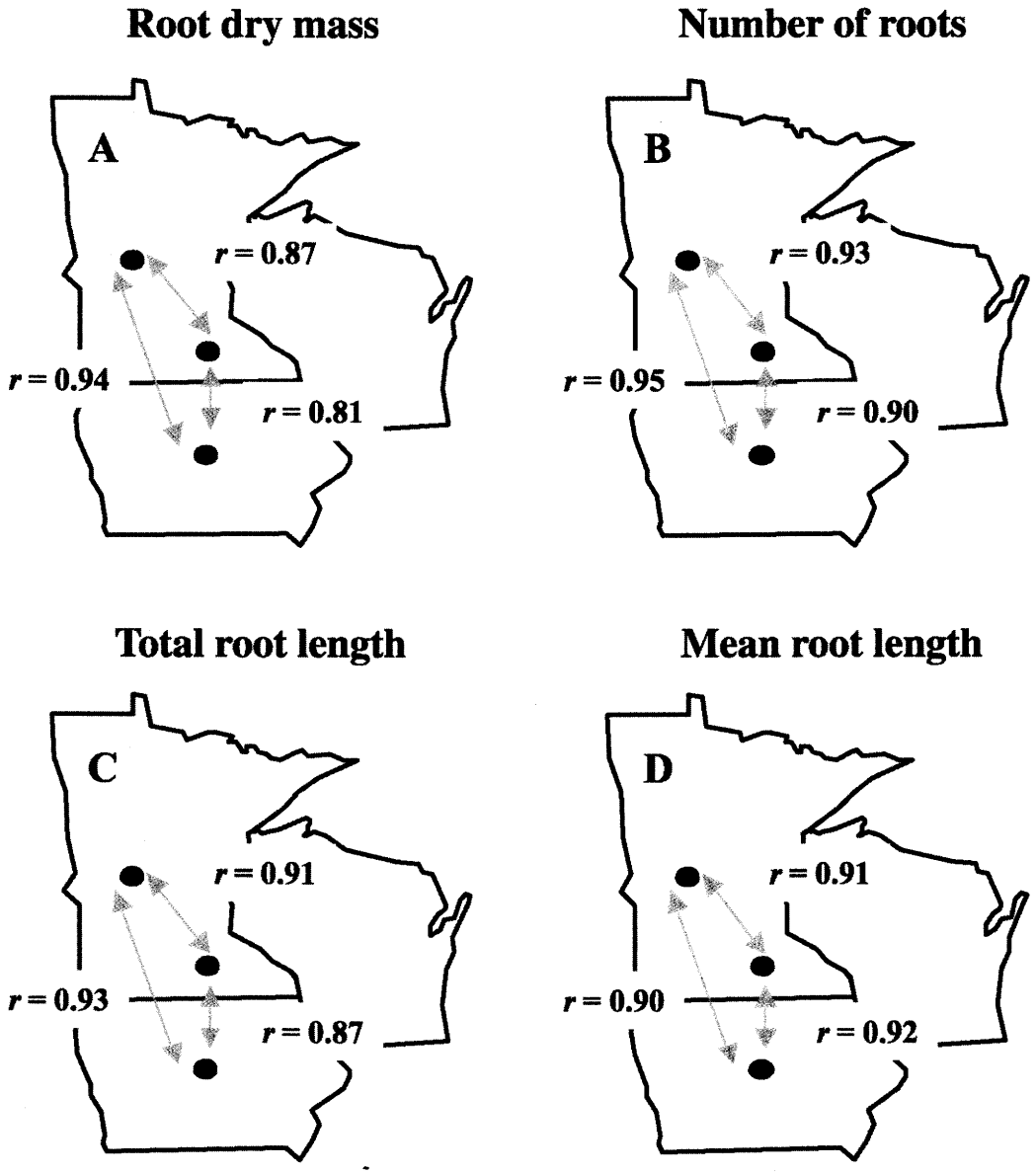
Genotypic effects

Clones varied greatly in root dry mass and top dry mass during 2001 and 2002. In 2001, the percentages of total variation of root dry mass and top dry mass attributable to clones were 10% and 31%, respectively; and in 2002, 7% and 30%, respectively. In 2001, H estimates for root dry mass and top dry mass were 0.11 and 0.32, respectively; and in 2002, 0.09 and 0.38, respectively.

Clones differed in 2001 and 2002 in root dry mass and top dry mass ($P < 0.0001$). In 2001 root dry mass ranged from 0.4 to 12.2 mg, whereas in 2002 it ranged from 0.2 to 24.4 mg. Clone NC13563 exhibited at least twice as much root dry mass as all other clones, given very high productivity in Ames 2002. However, root dry mass of NC13649 was comparable at all other sites and dates. The BC genomic group expressed the most variation among clones (root dry mass, 1.4–12.2 mg in 2001; 0.2–24.4 mg in 2002).

Top dry mass in 2001 ranged from 13.0 to 161.9 mg, whereas in 2002 it ranged from 14.4 to 163.9 mg. The DM and NM genomic groups exhibited the greatest top dry mass. Clone NC13563 expressed top dry mass similar to D clones.

Fig. 3. Phenotypic correlations among Ames, Iowa (42.0°N, 93.6°W); Waseca, Minnesota (44.1°N, 93.5°W); and Westport, Minnesota (45.7°N, 95.2°W) for root dry mass (A), number of roots (B), total root length (C), and mean root length (D) in an experiment testing the ability of 21 poplar clones to develop roots from dormant hardwood cuttings (n = 21). All correlations significant at $P < 0.0001$.



Clones differed in 2001 and 2002 in number of roots, total root length, mean root length, callus dry mass, total leaf area, and mean leaf area ($P < 0.0001$). Similar rooting trends existed for number of roots, total root length, and mean root length as for root dry mass, and similar aboveground trends existed for total leaf area and mean leaf area as for top dry mass.

Site effects

Sites accounted for no more than 1% of the total variation in root dry mass and top dry mass in 2001, and no more than 6% in 2002. Thus, site effects were negligible for all traits in 2001 and 2002 ($P > 0.05$).

Genotype × environment interactions

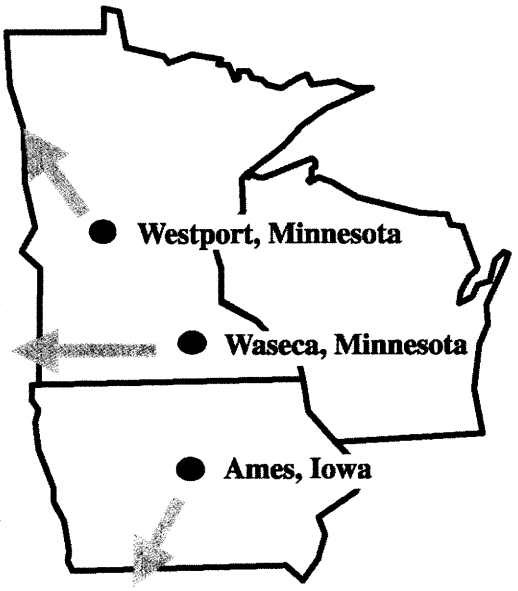
Genotype × environment interactions were highly variable across all traits. The interaction of site and clone during 2001 was negligible for all traits ($P > 0.05$). There was a significant interaction between site and clone for root dry mass ($P = 0.0098$) and total root length ($P = 0.0121$) during 2002, but this interaction was negligible for all remaining traits ($P > 0.05$).

The interaction of belowground GDD and clone during 2001 was significant for root dry mass, number of roots, total root length, mean root length, and callus dry mass ($P < 0.0001$) but negligible for top dry mass, total leaf area, and mean leaf area ($P > 0.05$). In addition, the interaction of belowground GDD and clone during 2002 existed for all traits: root dry mass, top dry mass, number of roots, total root length, mean root length, total leaf area, and mean leaf area ($P < 0.0001$), and callus dry mass ($P = 0.0130$).

Changes in rank and scale of genotypic production across sites defined the genotype × environment interactions in 2001

Fig. 4. Root dry mass after 14 days of growth of the best five clones at three sites in 2 years in an experiment testing the ability of 21 poplar clones to develop roots from dormant hardwood cuttings (n = 108 cuttings for each clone).

Clone	2001	Clone	2002
NC13563	11.1	D133	5.2
NC13649	9.4	DN5	5.0
NC13686	7.0	NC13649	4.6
DN5	4.5	NC13563	4.3
NM2	4.4	NM6	3.0



Clone	2001	Clone	2002
NC13649	12.2	NC13563	7.9
NC14105	9.9	NM6	7.4
NC13563	9.4	NC13649	7.0
DN70	7.6	NM2	5.7
NM6	7.1	D133	5.4

Clone	2001	Clone	2002
NC13563	12.1	NC13563	24.4
NC13649	11.2	NC13649	11.8
NC14106	8.1	DN5	11.7
NC13686	7.5	NC13686	9.8
NC14105	6.8	NM6	9.2

and 2002. Clonal ranks for root dry mass of the top five clones at each site in 2001 and 2002 are given in Fig. 4. Clone NC13563 illustrates the rank and scale change, as NC13563 ranked first in both Ames and Westport in 2001 and third in Waseca in 2001. The difference in root dry mass of NC13563 and the second-ranked clone increased from 0.9 mg in Ames in 2001 to 1.7 mg in Westport in 2001.

The greatest change in magnitude of scale occurred in Ames in 2002, when NC13563 ranked first, with a 12.6 mg advantage over the second best clone (Fig. 4). This advantage decreased to 0.5 mg in Waseca in 2002. NC13563 ranked fourth in Westport in 2002. Clonal performance at Westport in 2002 suggests that clones may have responded differently to that site. Principal component analyses supported this interpretation. Despite loading highly uniformly on number of roots for each combination of site and year (0.40–0.42), principal component 1 loaded highly on root dry mass, total root length, and mean root length for each combination of site and year, except for Westport in 2002 (0.34–0.43, 0.33–0.43, and 0.28–0.45, respectively). Principal component 2 loaded highly on root dry mass, total root length, and mean root length at Westport 2002, but weakly on all other combinations of site and year.

Discussion

Genotypic and environmental main effects, along with genotype × environment interactions, governed rooting of the genotypes tested. Clones accounted for >8% of the variation in all traits, and H for root dry mass ranged from 0.09 to 0.11. Genotype × environment interactions were significant for most traits, with belowground GDD accounting for >54% of the environmental variation. Clone-mean correlations and principal component analyses showed that root dry mass, number of roots, total root length, and mean root length were stable across sites. Thus, the variability in rooting traits was due to a combination of broad genetic variation between and within genomic groups, changing environmental conditions, and genotypic responses to conditions at contrasting field sites.

The genetics and physiology underlying the ability of cuttings to root are poorly understood (Haissig et al. 1992; reviewed by Haissig and Davis 1994). However, genetic differences between genotypes in their ability to root have outweighed genotype × environment interactions (Cunningham 1953; Ying and Bagley 1977). Current molecular genetic technologies provide tools for studying the plausible mechanisms controlling rooting, which aid in identification of promising genotypes

(Cervera et al. 2001; Yin et al. 2001). Although we did not apply molecular techniques in this study, the variation in rooting success of our BC genomic group most likely was an expression of broad genetic variation resulting from backcross breeding. Depending on recombination, genotypes of the first generation following backcrossing may have many alleles from the recurrent parent for a particular trait or only half of the alleles from the recurrent parent and half of the alleles from the donor parent. Thus, clones such as NC13563 that exhibited >70% rooting may have acquired alleles associated with rooting from the *P. trichocarpa* parent. Likewise, NC13570, an erratic-rooting clone, may have more alleles from the *P. deltoides* parent, which is known for excellent growth rates and disease resistance in the north central United States.

Differences among clones in their ability to develop preformed root primordia throughout the parent shoot during the preceding growing season likely contributed to the variation in number of roots (Smith and Wareing 1974; Farmer et al. 1989). Well-rooting genotypes, such as BC and NM clones, may have developed an abundance of preformed primordia, whereas poor-rooting genotypes, such as D clones, may have developed fewer preformed primordia. *Populus deltoides* does not root well from dormant hardwood cuttings in the north central United States. With the exception of D133, D clones were well below the overall mean for number of roots produced from the clones tested. Therefore, most D clones may not have as many preformed primordia. Root initiation and growth from D cuttings may be the result of induced primordia, which emerge as a result of influence from environmental stimuli (Haissig 1974; reviewed by Haissig and Davis 1994). In a related study, the June survival rate of D clones (78% at 30 days of growth) was comparable to survival of clones belonging to other genomic groups (Zalesny 2003). Thus, rooting of the D genomic group in the current study likely resulted from induced primordia that developed after 14 days.

Another consideration is the distribution of primordia along the parent shoot. More primordia may be located near the base of the parent shoot than near the apex, and therefore differences in rooting may be related to the location where the cutting originated on the parent shoot. A general trend of increasing survival and root growth from cuttings that originated closer to the base of the original stool plant has been reported (Bloomberg 1959; Hansen and Kristensen 1990; Zalesny et al. 2003).

Genotypic differences in utilization of soluble carbohydrates available in the cutting likely contributed to variable rooting responses (Bloomberg 1963; Okoro and Grace 1976; Tschaplinski and Blake 1989). Clone NC13563 exhibited high rooting, yet height growth at the end of the season was moderate (Zalesny 2003). Thus, NC13563 and similar clones may initially utilize more reserves for rooting and subsequently invest more photosynthate in rooting than in aboveground growth. These results agree with those of Wu et al. (1998), who reported that an inbred F_2 generation of poplar (*P. trichocarpa* × *P. deltoides*) exhibited a broad amount of variation resulting in variable rooting success. Those genotypes with the best rooting consistently did not have the best growth rates. Likewise, differences in carbohydrate metabolism among DN hybrids and hybrids between *P. balsamifera* and *P. deltoides* have been reported (Tschaplinski and Blake 1989). Although cutting dry mass was a negligible covariate in our analysis, cuttings of larger diameter made from the base of the parent shoot may have had more available reserves than smaller cuttings made from the apex of the parent shoot (Fege and Brown 1984). Thus, early rooting may be attributed to the quantity of available reserves and how fast such reserves are utilized. The availability of reserves is important for deployment because smaller cuttings with fewer reserves may have a decreased ability to survive in stressful environments.

Increased initial rooting supports higher rates of plantation establishment and subsequent survival (Heilman et al. 1994*b*). Therefore, rooting must be used as an initial selection criterion for clonal performance (Tschaplinski and Blake 1989; Riemenschneider et al. 2001*b*). Likewise, highly significant phenotypic correlations among traits suggested that a few dependent variables could be used to explain most of the variation in rooting ability among genotypes (Wilcox and Farmer 1968). Biological validity likely will not suffer if the number of variables evaluated is decreased. Our results corroborated those of Riemenschneider and Bauer (1997) and Tschaplinski and Blake (1989), who also related root initiation and growth to early plant production. However, the percentage rooting (22%–86%) among clones in our study is lower than other poplar studies reported. Farmer et al. (1989) reported that 53 of 100 clones belonging to four provenances of *P. balsamifera* exhibited 100% rooting, whereas only 2 clones showed <50% rooting. Cunningham and Farmer (1984) reported 13%–100% rooting of *P. balsamifera* cuttings, with an overall mean of 70%. We attribute lower percentage rooting in our study to the cuttings' being planted at three contrasting field sites, where the cuttings were subjected to different soil and air temperature, soil moisture, solar radiation, wind, and soil physical properties. In contrast, most previous studies were conducted under near-ideal conditions in growth chambers or greenhouses, where environmental conditions were controlled.

Genotypic responses to different environmental conditions at our three field sites were also highly variable. Genotype × environment interactions are thought to have a major role in governing rooting responses (Zsuffa 1976). In the current study, significant genotype × environment interactions existed for most traits. However, our interpretation of univariate and multivariate analyses showed that, despite such genotype × environment interactions, clonal performance for rooting traits was relatively stable across sites. Clones performed similarly for all combinations of sites and years, except for Westport, Minnesota, during 2002, when rooting was relatively poor.

Overall, from an operational perspective, we recommend selecting generalist genotypes that perform well over a broad geographic range. Also, from a research standpoint, we recommend additional testing for specific genotype × environment interactions across regional scales to improve the reliability of recommendations for genotypes adapted to regional environmental conditions.

The importance of acquiring knowledge about early rooting of poplars from dormant hardwood cuttings is highlighted by decreased establishment costs, time to crown closure, and vegetation management costs, along with the increase in operational flexibility that comes with broadened planting win-

dows. The results of this study lead us to assert that the broad amount of variation among the genomic groups currently utilized in the north central United States promises exceptional gains from further breeding, testing, and selecting of superior rooting clones.

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