Growth performance of hybrid poplar clones on two agricultural sites with and without early irrigation and fertilization

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Abstract

Growth, clone rank, genotype x environment interaction, and effects of early fertilization and irrigation were evaluated for 56 hybrid poplar clones after three years' growth on two agricultural sites in Indiana, USA. Forty-eight percent of the clones were *Populus deltoides* x *P. nigra* (DxN) crosses made with a female parent of Minnesota provenance, with the hybrids or female parent screened in Minnesota for survival, growth rate, and disease tolerance or resistance. Forty-one percent of the clones had at least one parent from provenances that are more southerly and/or have more moderate climates of provenance origin than Minnesota. Eleven percent of the clones were screened in Minnesota but were either not DXN crosses or did not have parents from Minnesota provenances in their parentage. Height growth averaged 1.78 m per year for all clones over all treatments and 2.02 m per year for the fastest-growing six clones (top 10th percentile). Tree bole volume for the fastest-growing 10 % of the clones was 70 % larger than the average of two commercial standard clones. The clonal effect was dominant in comparison to site, treatment, and interaction effects. The fertilizer, irrigation, and fertilizer x irrigation treatments tended to increase growth, but the statistical significance of the treatment effects differed by site, and the treatments explained only a small portion of the variance. Clone rank was the same on both sites, regardless of treatment, except for the fertilizer x irrigation treatment. DxN clones linked to Minnesota parentage out-performed most clones of more southerly or other more moderate climatic origins, in these tests conducted far south of Minnesota. The data provide further evidence of broad adaptability of DxN hybrids with female *P. deltoides* parents of Minnesota provenance, possibly eliminating the need for narrow breeding zones and reducing the number of screening tests needed at different latitudes, saving time and money. Tests are planned to further analyze and possibly extend this inference.

Keywords: *hybrid poplars, Populus, irrigation, fertilization, genotype x environment interaction, breeding*

Introduction

The potential for achieving one billion dry tons of cellulosic biomass per year nationally by 2030 as specified in the DOE 2016 Billion-Ton Report will require the production of more than 239 million dry tons from dedicated energy crops (US DOE 2016). Poplar (*Populus* spp.) culture has significant potential to help meet this target, and the Midwestern United States has the capacity to be a major contributor due to adequate rainfall and land availability.

The realization of this potential may require improved hybrid poplar genetic material that has superior and robust growth over a variety of geographic regions, soils, and climates (Nelson et al., 2018) due to the large capital requirement for narrow breeding zones. A low genotype x environment interaction would be cost efficient and operationally favorable. A hybrid poplar clonal trial established on agricultural soils on two Midwestern USA sites (northern and southern Indiana) in 2011 provides another opportunity to identify broadly adapted clones that exhibit superiority in growth rates on dissimilar sites. This study also can contribute to a better understanding of the relative strengths of clone and clone x environment

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(GxE) effects for improved populations of poplar. Early fertilization and irrigation treatments were tested on both sites to determine whether clone ranks and GxE interaction changed within or between test sites in response to these treatments. A comparison of the results obtained in this pair of trials was made with similar trials in Minnesota, USA.

The fertilizer, irrigation, and fertilizer x irrigation treatments in this study were designed to test nutrient and water stress amelioration on clonal performance and were not intended to represent commercial operations. Although fertilizer applications in poplar production plantations are sometimes done early in the rotation as in this study, the preferred method is application at crown closure (Coleman et al., 2006). Irrigation has been used in commercial poplar plantations in semi-arid areas of Oregon, USA (Stanton et al., 2002), but irrigation is unlikely to be used in the eastern and central portions of the Midwestern United States, where rainfall is plentiful.

Studies within *Populus* and other tree genera show a broad pattern of growth being limited by nutrient availability, with response to nutrient amelioration dependent on adequate soil water availability (Linder, 1989). Fertilizer application in poplar plantations can increase growth from 20 % to 60 % on poorer sites, but it has little or no effect on some better sites (Coleman et al., 2006 and citations within). Significant growth benefits from irrigation of poplars have been demonstrated for sites in humid temperate climates (Hansen 1988; Dickmann et al., 1996; Moffat et al., 2001). Coyle and Coleman (2005) and Samuelson et al. (2007) reported positive effects of fertilization and irrigation on growth of *P. deltoides* but no interaction of fertilization and irrigation. A positive interaction of fertilization and irrigation on poplar growth was documented by van den Driessche et al. (2003).

Twenty-one of the DxN clones in our study were bred by the Natural Resources Research Institute (NRRI) Poplar Program, which is based at the University of Minnesota Duluth, and tested and selected in northern Minnesota at 46 to 48 degrees north latitude. The *P. nigra* parents of the NRRI DxN clones were from populations screened in Ontario, Canada. Six of the DxN clones derived from other breeding programs also had female *P. deltoides* parents of Minnesota provenance, with the female parent but not the hybrids screened in Minnesota. The Indiana sites were at 41.4 and 38.7 degrees north latitude, a difference of 5-9 degrees latitude and 7-9 degrees longitude from the sites used to initially test and select the NRRI DxN clones.

The objectives of this study were to: (1) analyze the effects of site, clone, and clone x site interaction with and without early fertilization and irrigation on the growth performance of hybrid poplar clones of different origins; (2) determine of the effects of site, fertilization, and irrigation on clone rank; (3) investigate the effect of parental origins on clone rank; and (4) seek inferences on GxE interactions and the strength of genetic control of growth rate by comparing the results with other studies involving the same clones.

Materials and Methods

Plant material

Clone selection for tests—

Fifty-six clones were included in the tests (Table 1). Forty-eight percent of the clones were *Populus deltoides* x *P. nigra* (DxN) crosses made with a female parent of Minnesota provenance, with the hybrids or female parent screened in Minnesota for survival, growth rate, and disease tolerance or resistance. Most *P. deltoides* parents from Minnesota provenances were originally from the C.A. Mohn selection and breeding program, University of Minnesota. The NRRI *P. nigra* male parents of Ontario Canada origin were derived from pollen of trees tested and screened in Ontario by L. Zsuffa, University of Toronto. Forty-one percent of the clones had at least one parent from provenances that are more southerly and/or have more moderate climates of provenance origin than Minnesota. Eleven percent of the clones were screened in Minnesota but were either not DXN crosses or did not have parents from Minnesota provenances in their parentage. Two commercial standards, DN5 and NM6, were embedded in the trials. Nineteen NRRI clones were common to a previous clonal trial in Minnesota (Nelson et al., 2018).

Table 1

Description of clones in this test

Clone ID	Species Type	Female Parent	Origin	Male Parent	Origin	Original Source
DxN (D from MN provenances, hybrids or female parent screened in MN, 27 clones)						
20111062	DxN	D109	MN	N964-1	ONT	NRRI
20111063	DxN	D109	MN	N964-1	ONT	NRRI
20111315	DxN	D109	MN	N964-1	ONT	NRRI
2017341	DxN	288-5	MN	N944-4	ONT	NRRI
22057011	DxN	D121	MN	N40	ONT	NRRI
99001111	DxN	D121	MN	N947-5	ONT	NRRI
99007071	DxN	D121	MN	N949-2	ONT	NRRI
99007115	DxN	D121	MN	N949-2	ONT	NRRI
99007116	DxN	D121	MN	N949-2	ONT	NRRI
99037046	DxN	D200	MN	N964-6	ONT	NRRI
99037049	DxN	D200	MN	N964-6	ONT	NRRI
99038022	DxN	D200	MN	N944-4	ONT	NRRI
99038013	DxN	D200	MN	N944-4	ONT	NRRI
99059016	DxN	D123	MN	N949-2	ONT	NRRI
99098008	DxN	14CRK	MN	N964-6	ONT	NRRI
99105008	DxN	14CRK	MN	N944-4	ONT	NRRI
9732-06	DxN	D125	MN	946-2	ONT	NRRI
9732-07	DxN	D125	MN	946-2	ONT	NRRI
9732-11	DxN	D125	MN	946-2	ONT	NRRI
9732-24	DxN	D125	MN	946-2	ONT	NRRI
9732-40	DxN	D125	MN	946-2	ONT	NRRI
11786	DxN	D121	MN	Nigra1	Serbia	GWR
11793	DxN	D121	MN	Nigra1	Serbia	GWR
11794	DxN	D121	MN	Nigra1	Serbia	GWR
11798	DxN	D121	MN	Nigra1	Serbia	GWR
11799	DxN	D121	MN	Nigra1	Serbia	GWR
11807	DxN	D121	MN	Nigra3	Serbia	GWR
More southerly or moderate climate provenances than MN, not MN-screened (23 clones)						
433(NE367)	DxN	na	na	Caudina	Mediterranean	Scott Paper
10269	TxD	na	na	na	na	Potlatch
10270	TxD	na	na	na	na	Potlatch
10642	DxN	93-18	IA	NG58-3	Italy	BoiseCascad
10643	DxN	94-87	IA	NG58-3	Italy	BoiseCascad
10644	DxM	WWD6	WA	M10	Japan	BoiseCascad
10645	DxM	WWD6	WA	M10	Japan	BoiseCascad
10646	DxM	WWD6	WA	M10	Japan	BoiseCascad
10647	DxM	WWD6	WA	NG46	Italy	BoiseCascad
10648	DxN	WWD6	WA	NG51	Italy	BoiseCascad
10649	DxN	WWD6	WA	NG51	Italy	BoiseCascad
10651	DxN	WWD6	WA	NG51	Italy	BoiseCascad
10652	DxN	WWD6	WA	NG51	Italy	BoiseCascad
11765	DxT	91034	IA	ECHO-1	OR	GWR
11822	DxN	91.79.00	IA	Nigra1	Serbia	GWR
11824	DxN	91.79.00	IA	Nigra2	Serbia	GWR
11825	DxN	91.79.00	IA	Nigra2	Serbia	GWR
12820	DxT	730020	IL	ECHO-1	OR	GWR
12821	DxT	730020	IL	ECHO-1	OR	GWR
12823	DxT	730020	IL	ECHO-1	OR	GWR
22091021	TDx(D)	52-225	WAxIL	D105	WI	NRRI
22091023	TDx(D)	52-225	WAxIL	D105	WI	NRRI
22091051	TDx(D)	52-225	WAxIL	D105	WI	NRRI
Screened in Minnesota (6 clones)						
99010034	Dx(DM)	D121	MN	NC14103	MNxJapan	NRRI
NC14106	DxM	na	MN	na	Japan	UM&USFS
M/R 502.37	DxM	112(OHxOH)	OH	M11861	Japan	UM&USFS
D113	D	OP400	MN	na	MN	UM
DN5	DxN	na	na	na	na	na
NM6	NxM	na	na	na	na	Germany

D is *P. deltoides*, M is *P. maximowiczii*, N is *P. nigra*, T is *P. trichocarpa*; origin is provenance or landrace; na is unknown or not applicable; *IA* from Iowa State University; *IL* from University of Illinois; WA origin for GWR D female parent clones is Walla Walla River landrace at junction of Columbia River; all D parents for NRRI clones are from Dr. Carl Mohn's program, University of Minnesota; *ONT P. nigra* male parent origin are from varied European provenances screened in Maple, Ontario Canada in Dr. Louis Zsuffa's program. USA state abbreviations: IL = Illinois, IA = Iowa, MN = Minnesota, OR = Oregon, WA = Washington, and WI = Wisconsin.

Plant propagation

Planting stock was 20-cm unrooted dormant cuttings harvested from current annual shoots of stools (cutting orchard) and kept at -2.8 °C (< 4 months) until planting. The stool beds were established and maintained near West Lafayette, Indiana, by Purdue University (R. Meilan) and ArborAmerica, LLC (G. Pardillo), using cuttings supplied by NRRI and GreenWood Resources (GWR; Portland, Oregon, USA). Clones were screened and selected for the tests based on performance in the stool beds and stock availability as a *de facto* vigor pre-screening.

Study locations

The two test sites, both of which are in Indiana, USA, were: the Southwest Purdue Agricultural Center (SWPAC; https://ag.purdue.edu/arge/pac/Pages/swpac-home.aspx), 38.7 degrees N latitude, -87.5 degrees longitude, and Pinney-Purdue Agricultural Center (PPAC; https://ag.purdue.edu/arge/pac/Pages/ppac-home.aspx),41.4 degrees N latitude, -86.9 degrees longitude. The geography and information on soils and climate for the test locations are in Table 2. Test plots on both sites were on normal agricultural soils

Table 2
Location, soil, and climate information for the Indiana test sites

	Site	
	SWPAC	PPAC
County	Knox	LaPorte
Nearest town	Vincennes	Wanatah
Latitude	38.737894	41.441828
Longitude	-87.484995	-86.923137
Slope%	10 to 18	0 to 2
Soil texture	silt clay loam	sandy loam
Soil pH	5.3	5.0
Soil particulate organic matter (%)	0.67	1.33
Soil bulk density (g/cm3)	1.46	1.56
Crop Productivity Index (CPI)	107	not available
Average high/low temperature (C)	31.1/-6.1	28.3/-10.6
Growing degree days (GDD, base 10C/30C)	3900	3050
Average precipitation (mm)	1170	1015
Average annual precipitation for study years 2011 – 2013 (mm)	1303	827

1) Soil data from https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm

CPI from Olson *et al.* 2000. 2) Climate data from https://www.usclimatedata.com/ U.S. Climate Data, three-decade (1981–2010) averages (National Centers for Environmental Information, NOAA). 3) Crop Productivity Index explanation reference (Dobos *et al.* 2012). 4) GDD https://mrcc.illinois.edu/gismaps/gddinfo.htm

Plantation establishment and maintenance

The sites were planted in spring 2011. Approximately one month prior to planting at PPAC, the site was prepared by using conventional tillage with a disk and a field cultivator. Three days before planting, Roundup® WeatherMax was applied over the plant-rows at a rate of 1.61 l/ha, along with a follow-up application of Prowl® H_2O at a rate of 7 l/ha. About one month before planting at SWPAC, the site was prepared by making a banded application of Roundup® Power Max herbicide at a rate of 2.34 l/ha; a repeat application was made approximately two weeks later. Two days prior to planting, Framework® 3.3EC herbicide was applied over the rows at a rate of 2.34 l/ha. At both locations, a trickle-irrigation line (T tape) was buried at the center of each plant-row at a depth of 8-10 cm.

Cuttings were planted at a spacing of 2.4 m within rows and 1.8 m between rows. A 2.3-m high plastic mesh deer fence (Deer Busters fencing, 650-lb breaking strength) was erected around the perimeter of each trial.

Weed control between rows after the trees were established consisted of mowing every 3-4 weeks, as needed, throughout the first three years. Weed control within rows was performed by applying undiluted Roundup® (glyphosate) with an ultralow-volume Mankar sprayer twice in year 1.

Study design

The experiment was a completely randomized design of multiple single-tree plots nested within four cultural treatment plots (blocks) at two sites. There was no replication of cultural treatments within each site. A different treatment was applied to each of the four cultural treatment blocks at each site for the first two growing seasons: Fertilization only, Irrigation only, Fertilization + Irrigation, and no fertilization or irrigation (No Treatment). For the first two years, 60 g of Osmocote® fertilizer (Scotts Miracle-Gro, 15-9-12) was applied in early spring to the Fertilizer and Fertilizer + Irrigation blocks to a small hole dug at the base of the planted cuttings. Irrigation was applied on an as-needed basis, at the discretion of the agronomist in charge of each experimental farm. Irrigation and fertilization were discontinued after the second growing season.

Clones were randomly assigned within each block, but in a way that ensured equal numbers of ramets of each clone in odd- and even-numbered rows within each block. A border row was established around the outer perimeter of each block, but not between adjoining blocks, using cuttings from a randomized assortment of genotypes. Fertilizer and Fertilizer + Irrigation treatment blocks were not adjacent. Fertilizer + Irrigation and Irrigation blocks were adjacent, as were No Treatment and Fertilizer blocks. There was a 4.9 m alley between the Irrigation and No Treatment blocks. Diffusion of fertilizer into adjoining Irrigation and No Treatment blocks was minimized by the use of slow-release fertilizer, which was applied to the soil at the base of each cutting as described below. The positioning of the Fertilizer + Irrigation and Irrigation blocks adjacent to each other minimized any "spillover effect" resulting from water diffusing into adjacent blocks. The clones from GWR were replicated six times within each block. Clones from the NRRI were replicated 10 times within each block.

Measurements

Growth data (height and diameter) and survival for all except border trees were taken at the end of each growing season (2011, 2012, and 2013) after the trees had gone dormant. Diameter at breast height (DBH, 1.4 m above ground level) was measured with 45.7-cm Haglöf Mantax aluminum calipers. A Tel-O-Pole® measuring stick (Hastings, model E35) was used to

obtain tree heights. Tree bole volume outside bark after three growing seasons was calculated by the equation developed for plantation cottonwood (*P. deltoides*) by Krinard (1988) = 0.06 + 0.002221 D^2H (r^2 = 0.987), where D is DBH and H is total tree height. This regression uses diameter in inches and height in feet to derive cubic feet. Cubic feet volume was then converted to cm^3. The emphasis of this study was on biomass production. Consequently, stem straightness and other log-quality parameters were not evaluated.

Statistical analyses

Means and ranks for total tree volume were calculated for each clone on each site at age 3 (end of third growing season). Frequency plots of tree volume by site and type of treatment exhibited a right-skewed distribution. A $\log_{10}$ transformation was applied to the volumetric data, resulting in a near-normal data distribution. Therefore, $\log_{10}$ tree volume was used as the response variable throughout the analyses.

Data from the two sites were combined and analyzed by a sequence of two ANOVA procedures. The initial ANOVA was performed in SAS 9.4 according to a linear mixed model treating site, treatment (irrigation, fertilization), and site x treatment interaction as fixed variables and clone and any interaction with clone (site x clone, treatment x clone, and site x treatment x clone) as random variables.

The model had a significant R^2, and all effects were significant in the initial ANOVA except treatment x clone and site x treatment x clone (see Results). Thus, the latter two interactions were excluded from the predictor list, and a new ANOVA final model was developed using the remaining five significant variables: site, treatment, clone, site x treatment, and site x clone. The final ANOVA was a mixed-model analysis performed in R 3.5.1 using the software package 1me4. Random variables were clone and clone x site. Variance components for fixed and random effects were calculated as percentages of total variance in the final ANOVA model.

Heritabilities for the clonal population pooled across both sites were calculated as follows:

Broad-sense heritability, $H^2 = \frac{\sigma_A^2+\sigma_I^2}{\sigma_A^2+\sigma_I^2+\sigma_E^2}$,

Narrow-sense heritability, $h^2 = \frac{\sigma_A^2}{\sigma_A^2+\sigma_I^2+\sigma_E^2}$,

where σ_A^2 is the additive genetic variance, σ_I^2 is the non-additive genetic variance (variance of the interaction of clone and site), and σ_E^2 is the error variance (variance among trees caused by micro-environmental effects) (White et al., 2007).

Overall clone rank was calculated by averaging $\log_{10}$ tree volume for each site x treatment combination for each clone, followed by averaging the former means to derive a mean for each clone across the two sites. Rank 1 is the largest (fastest growing) clone; Rank 56 is the smallest clone. Spearman's Coefficient of Rank Correlation was used to determine whether clone rank changes contributed to clone x site interactions. A separate Spearman's test was done for rank (basal area basis) in a previous set of clone trials in Minnesota versus rank ($\log_{10}$ tree volume) in the current study for the 19 NRRI clones common to both studies.

Histogram plots of tree volume indicated that variances may not be equal between the two sites or among the four treatment types. Three variance comparisons were conducted using $\log_{10}$ tree volume to determine whether variances differed by treatment for: all data, PPAC site-only treatment, and SWPAC site-only treatment. Four tests were used for these variance comparisons: O'Brien test, Brown-Forsythe test, Levene's test, and Bartlett's test. Variances were not significantly different among treatments for each site (see Results), so Tukey's HSD test was used to compare the difference among treatments within each site. Due to unequal variances between sites, the comparison of sites by treatment was tested with Wilcoxon's non-parametric test using the JMP software package.

Due to the unequal replication of NRRI clones (6) and GWR clones (10) and some relatedness between clones (only 23 % of clones do not share a male and/or female parent with at least one other clone), we questioned whether to use observed clone growth values or Best Linear Unbiased Predictor (BLUP)-derived clone values in the analyses. A plot of clone rank based on BLUP and observed values indicated there was no significant difference between the methods (R^2 =0.999). Therefore, we based all analyses on observed values.

Results

Means and analysis of variance

Survival measured after the third growing season was excellent, 99.7 % for PPAC, 94.6 % for SWPAC.

Table 3 shows the average tree height, DBH, and total volume for each site. Height growth averaged 1.78 m per year over the first three years of the planting for all clones over all treatments and 2.02 m per year for the fastest-growing six clones (top 10th percentile). Tree bole volume for the fastest-growing 10 % of the clones was 55 % larger than tree volume average for all clones over all treatments and 70 % larger than the average of the two commercial check clones.

Table 3
Mean of height, diameter, and tree volume for each site after 3 years' growth. $\log_{10}$ is of tree bole volume in cm^3.

Parameter		PPAC	SWPAC	Top 10% clones across both sites	Two commercial check clones
Height, m	Mean	5.51	5.14	6.07	5.16
	Standard deviation	0.82	1.41	0.94	
	Median	5.6	5.3	6.16	
DBH, cm	Mean	6.5	7.0	8.3	6.6
	Standard deviation	1.4	2.0	1.8	
	Median	6.5	6.9	8.0	
Volume, cm^3	Mean	9,700	11,400	16,400	9,600
	Standard deviation	3,900	7,400	8,200	
	Median	9,300	9,800	14,600	
$\log_{10}$ cm^3 Volume	Mean	3.95	3.97	4.17	3.94
	Standard deviation	0.19	0.28	0.20	
	Median	3.97	3.99	4.16	

Except where noted, data are for all clones over all treatments. The commercial check clones are DN5 and NM6.

The initial ANOVA is shown in Table 4. All effects were significant except treatment x clone and site x treatment x clone.

Table 4
Initial ANOVA. Dependent Variable: $\log_{10}$ Volume. Log is of tree bole volume in cm^3.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	1	0.20513605	0.20513605	6.53	0.0107
Treatment	3	5.48694017	1.82898006	58.20	<.0001
Clone	55	74.71707519	1.35849228	43.23	<.0001
Site*Treatment	3	2.26267549	0.75422516	24.00	<.0001
Site*Clone	55	11.07431514	0.20135118	6.41	<.0001
Treatment*Clone	165	5.20517045	0.03154649	1.00	0.4729
Site*Treatment*Clone	165	5.22768376	0.03168293	1.01	0.4577

Variance components for growth based on the final ANOVA are exhibited in Table 5. The clone effect was 36 % of total variance, almost 10 times the effect of site, treatment, and site x treatment combined, and over four times the effect of site x clone interaction. Experimental error accounts for over half of the variance.

Based on the variance component data in Table 5, broad-sense heritability, H^2,and narrow-sense heritability,h^2, are:

$$H^2 = \frac{\sigma_A^2 + \sigma_I^2}{\sigma_A^2 + \sigma_I^2 + \sigma_E^2} = \frac{0.0219 + 0.0054}{0.0219 + 0.0054 + 0.0314} = 0.46$$

$$h^2, = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_I^2 + \sigma_E^2} = \frac{0.0219}{0.0219 + 0.0054 + 0.0314} = 0.37$$

Table 5
Variance components for $\log_{10}$ volume, final ANOVA. Clone, site x clone, and model error are random effects.

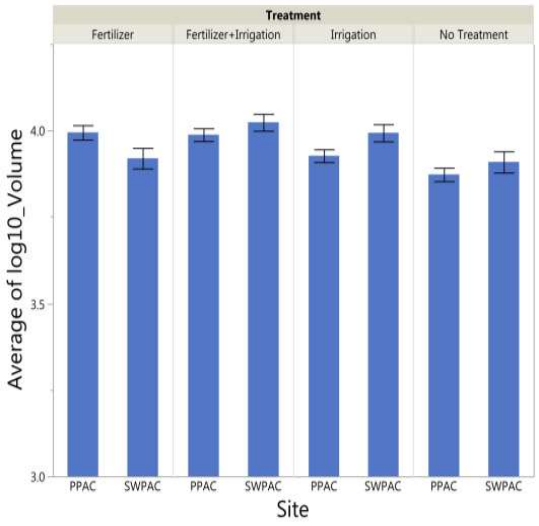
Source	Variance components	% of variance components
Fixed factors (Site, Treatment, Site * Treatment)	0.0023	3.77%
Clone	0.0219	35.90%
Site*Clone	0.0054	8.85%
Error	0.0314	51.48%

Results from four methods of testing for equality of variances are displayed in Table 6. Variances were significantly different between sites but equal for treatments within each site.

Table 6
P-values for variance comparison by four methods. Null hypothesis is that variances are equal. P-value< 0.05 is rejection of the null hypothesis.

	All data Site x Treatment	Site PPAC only Treatment	Site SWPAC only Treatment
O'Brien test	0.0002	0.8676	0.1847
Brown-Forsythe test	0.0003	0.8353	0.1692
Levene's test	0.0002	0.7294	0.1742
Bartlett's test	<0.0001	0.7990	0.1918

The mean and standard error for $\log_{10}$ tree volume within each site and treatment type are displayed in Figure 1.



Results of Tukey's HSD test for comparing the difference in log10 tree bole volume due to treatments within each site are shown in Table 7. At PPAC, bole volume (anti-log) under Fertilizer and Fertilizer + Irrigation treatments was 32 % greater and significantly different than under No Treatment. At SWPAC bole volume for Fertilization + Irrigation was 29 % greater and significantly different than for No Treatment.

Table 7
The mean values of $\log_{10}$ tree bole volume in cm^3 by four treatment types within each site at age 3. Data at same column sharing same letters have insignificant difference of the means based on Tukey's HSD test. Data are not comparable across the columns.

Treatment type	PPAC	SWPAC
Fertilizer	3.99^A	3.92^B
Fertilizer + Irrigation	3.99^A	4.02^A
Irrigation	3.93^{AB}	3.99^{AB}
No Treatment	3.87^B	3.91^B

Wilcoxon's test of the significance of tree bole volume differences of mean treatment values between the two sites is in Table 8. The only significant difference between sites for treatments was for the Irrigation treatment, wherein the SWPAC site had a larger tree volume under Irrigation than did the PPAC site.

Table 8
Wilcoxon test of site differences for mean values of log_{10} tree bole volume in cm^3 for two sites within each of four treatments at age 3. Comparison is within each row, not within columns.

Treatment	PPAC	SWPAC	Probability > Chisq
Fertilizer	3.99	3.92	0.0634
Fertilizer + Irrigation	3.99	4.02	0.2067
Irrigation	3.93	3.99	0.0212
No Treatment	3.87	3.91	0.3141

Clone ranks

Mean clone ranks are presented in Table 9. Taxa and provenance effects are summarized in Table 10. DxN clones of Minnesota provenance for the female *P. deltoides* parent with hybrids or female parent screened in Minnesota (DN-MN) dominate the top 10th and 25th percentiles for growth. DN-MN clones were 48 % of the total clonal population but were 83 % and 71 % of the 10th and 25th percentiles, respectively.

There was no significant rank change (Spearman's test, Table 11) between the two sites for No Treatment, Fertilizer, and Irrigation treatments. Fertilizer + Irrigation did result in significant rank change, but the p value was just above 0.05 (0.07).

A surprising result of this study was the superior performance and significant representation of *P. deltoides x P. nigra* (DxN) clones with a female *P. deltoides* parent of Minnesota origin within the top growth percentiles of clone rank (Table 10). We did not expect to see such broad adaptability across a north–south gradient, and it prompted a careful evaluation of the clonal performance and GxE interaction revealed by this study, relative to past results in Minnesota.

A rank comparison of NRRI clones common to the current study and a previously reported trial in Minnesota (Nelson et al., 2018) is presented in Table 12. A Spearman's test comparing these two datasets gave a Spearman's correlation coefficient of 0.8053 ($p<0.0001$), indicating no significant rank change for the two studies. One NRRI DxN clone, 99038022, was in the top 10th percentile in both studies. Three NRRI DxN clones (99038022, 20111315, and 99007116) were in the top 25th growth percentile in both studies.

Table 9
Mean clone rank across and within both sites based on the log_{10} mean tree bole volume in cm^3 of mean site x treatment values at age 3 for each clone. Rank is ordered from highest value to lowest value. Across-site values for tree bole volume are average of average site values.

Clone	Across both sites log_{10} volume	Across both sites Rank	PPAC Rank	SWPAC Rank
11807[MN]	4.25	1	1	1
99007071[MN]	4.18	2	8	2
11793[MN]	4.18	3	2	3
10642[S]	4.15	4	6	5
99038022[MN]	4.13	5	5	9
11798[MN]	4.13	6	10	7
11794[MN]	4.12	7	16	4
433[S]	4.12	8	19	6
20111315[MN]	4.11	9	12	8
11824[S]	4.11	10	3	13
10648[S]	4.10	11	13	10
99038013[MN]	4.10	12	9	14
11799[MN]	4.09	13	7	16
99007116[MN]	4.08	14	11	15
99059016[MN]	4.07	15	20	11
10270[S]	4.06	16	4	24
11786[MN]	4.05	17	22	17
99007115[MN]	4.03	18	21	19
10649[A]	4.02	19	38	12
20173417[MN]	4.01	20	27	18
10643[S]	4.01	21	26	21
11825[S]	4.01	22	18	29
22057011[MN]	4.00	23	17	31
9732-24[MN]	3.99	24	31	22
99098008[MN]	3.99	25	30	25
99037046[MN]	3.99	26	23	32
10651[S]	3.97	27	34	26
20111062[MN]	3.97	28	32	27
11822[S]	3.97	29	40	20
10644[S]	3.96	30	14	43
9732-11[MN]	3.96	31	37	28
M/R 502.37[O]	3.95	32	15	45
99105008[MN]	3.95	33	24	40
DN5[O]	3.95	34	36	30
9732-40[MN]	3.95	35	44	23
10645[S]	3.94	36	25	42
NM6[O]	3.94	37	35	33
20111063[MN]	3.93	38	33	36
10646[S]	3.93	39	29	44
99037049[MN]	3.92	40	39	34
D113[O]	3.89	41	42	39
9732-06[MN]	3.89	42	46	35
9732-07[MN]	3.88	43	45	37
22091021[MN]	3.87	44	28	48
10652[S]	3.84	45	48	41
99001111[MN]	3.83	46	50	38
10647[S]	3.80	47	47	46
22091051[MN]	3.78	48	41	51
22091023[MN]	3.78	49	43	50
NC14106[O]	3.73	50	51	49
10269[S]	3.70	51	52	52
11765[S]	3.70	52	53	47
99010034[O]	3.69	53	49	53
12821[S]	3.60	54	54	54
12820[S]	3.53	55	56	55
12823[S]	3.52	56	55	56

[MN] are *Populus deltoides x P. nigra* clones with *P. deltoides* parents of Minnesota (MN)provenances, hybrids or female *P. deltoides* parent screened in MN. [S] are clones of more southerly or moderate climatic origins than MN, not screened in MN. [O] are other clones (non-DxN or DxN of unknown provenances, but screened in MN).

Table 10
Geographic/climatic origins of top-ranking clones. Rank percentiles are average for both sites.

Clone growth rank percentile	DxN[1] clones, MN provenance for *P. deltoides* parents	Clones [2]southern/moderate climatic origin	[3]Other clones screened in MN
Top 10% (6 clones)	5 out of 6 clones	1 out of 6 clones	zero
Top 25% (14 clones)	10 out of 14 clones	4 out of 14 clones	zero

[1]DxN clones are *P. deltoides x P. nigra* hybrids with MN (Minnesota) provenance for the *P. deltoides* female parent, hybrids or female *P. deltoides* parent screened for survival, growth, and disease resistance in MN. [2] are clones of more southerly or moderate climatic origins than MN and not screened in MN. [3](non-DxN or DxN of unknown provenance, but screened in MN).

Table 11
Spearman's correlation coefficients of clone ranks based on log_{10} tree bole volume between the two Indiana sites within four treatment types.

Treatment type	Spearman's correlation coefficient	p-value
Fertilizer	0.5722	<0.0001
Fertilizer + Irrigation	0.2430	0.0711
Irrigation	0.5542	<0.0001
No Treatment	0.4682	0.0003

Table 12
Ranks of NRRI clones common to current study in Indiana and previous clone trial in Minnesota (Nelson *et* al. 2018). Rank is ordered from highest value to lowest value. Bole volume values are across-site average of average site values.

Clone	Across both sites log_{10} Volume in current study	Rank in current study	Rank in previous MN clone trial
99007071	4.18	2	24
99038022	4.13	5	5
20111315	4.11	9	4
99038013	4.1	12	29
99007116	4.08	14	9
99059016	4.07	15	1
99007115	4.03	18	20
9732 -24	3.99	24	6
99098008	3.99	25	35
9732 -11	3.96	31	12
M/R 502 .37	3.95	32	51
99105008	3.95	33	41
NM6	3.94	37	59
20111063	3.93	38	34
99037049	3.92	40	36
22091021	3.87	44	63
99001111	3.83	46	47
22091051	3.78	48	60
NC14106	3.73	50	54

Rank in current study based on log_{10} tree bole volume at age 3 in cm^3(Table 9). Rank in MN study based on basal area at age 5.

Discussion

The data clearly indicate that the fastest-growing 10 % of the clones in this study are highly improved in comparison with commercial clones commonly used in the Midwestern United States (e.g., DN5 and NM6) and the rest of this collection of clones (Table 3). Site, treatment, clone, and site x treatment and site x clone interactions all had significant effects on tree bole volume (Tables 4, 5). However, the clone effect was dominant, constituting 36 % of the total variance. Site explained much less of the variance, and site x clone somewhat less of the variance, than in a study on six sites in Minnesota that employed 19 of the same clones used in the present study (Nelson et al., 2018). There were significant treatment effects in the present study, but they were a relatively minor component of the overall variance. The broad-sense heritability estimate for the clonal population (0.46) is within the $0.21 < H^2 < 0.50$ range reported for *Populus* stem growth in several studies (reviewed in Riemenschneider et al., 1996).

We estimate a rotation of 5-7 years for the two sites in this study, based on mean annual increment per hectare versus that for 8 to 12-year rotations in Minnesota (D. Buchman, unpublished data, 2018). Thus, the three-year growth exhibited in this study represents approximately one-half of the rotation. In our experience, clone performance at half rotation is indicative of ultimate clonal rank at full rotation (Nelson et al., 2018). Kaczmarek et al. (2013) found that rank (based on tree-volume measures) for faster-growing *Populus* clones at age 3 did not significantly change for the same clones at age 10 but did change for moderate to poorly performing clones. Thus, the results described here likely represent relative performance at full rotation, at least for the better-performing clones.

Fertilizer and Irrigation and the Fertilizer x Irrigation interaction affected growth at one or both sites (Table 7). The response to Fertilization and Fertilization + Irrigation (29-31 %) was within the 20-60 % range reported in other studies of fertilization of *Populus*. Irrigation differentially increased growth on the two sites (Table 8), with trees at SWPAC benefitting more from irrigation than those at PPAC (Table 7). This is somewhat surprising, given that SWPAC has a finer texture soil and had 476 mm more natural precipitation per year over the three years of the study than did PPAC (Table 2). The test site at SWPAC had a slope of 10-18 % versus only 0-2 % at PPAC. SWPAC is also hotter than PPAC, and SWPAC soil has only half as much organic matter as PPAC (Table 2), all factors that may help explain the difference in irrigation response. The frequency and amount of irrigation was based on the judgment of a different expert agronomist at each site which also may explain some of the difference in response to irrigation. Even though there were significant treatment effects within and between sites, they may not be important in the overall interpretation of the results, as they explained a very small portion of the variance (Table 5).

The working paradigm for our breeding program, which is focused on the Midwestern United States, is that the scale of breeding investment that the bioenergy and bio-products industry is likely to support in the foreseeable future will

preclude numerous breeding zones with narrowly adapted genotypes and small latitudinal bounds, i.e., specialists (Zalesny et al., 2009). Rather, the industry will require a collection of clones that exhibit a high degree of site adaptability and high yields over a range of approximately 38 to 48 degrees north latitude; i.e., *generalists* (Zalesny et al., 2009). This is different than the usual provenance-focused approach in traditional tree improvement programs with seed reproduction in pure species. The present study and Nelson et al. (2018) provide evidence that it may be possible to breed and select geographically robust inter-specific hybrid poplar clones, at least for DxN hybrids. Our hypothesis is that the *P. nigra* male component of DxN hybrids imparts broad adaptability to these genotypes. Regardless of the exact role of *P. nigra* in adaptability, genetic theory and empirical evidence point to increased heterozygosity and heterosis in inter-specific genotypes, in comparison to pure species (Li and Wu 1996). Heterozygosity and heterosis are associated with broader adaptability at the individual genotype level (Lerner 1954; Mitton and Grant 1984; Gillespie and Turelli 1989; Wu 1998). Unlike DxN hybrids, we would expect pure *P. deltoides*, the female component, to behave more like other pure species, wherein northern provenances would likely underperform southern provenances when moved southward to the latitude of the southern provenances (Eldridge et al., 1972; Ying and Bagley, 1976; Loehle, 1998). We will test the latter expectation with clonal trials in Minnesota, Iowa, and Indiana that will be established in 2019 and include DxD and DxN hybrids.

DxN clones linked to Minnesota parentage and/or screening outperformed most clones of more southerly or otherwise more moderate climatic origins in the present study, which took place far south of Minnesota. Most of the clones derived from more moderate climates were in the lower 50th percentile in rank. Only one was in the top 10th percentile. The GWR DxN clones with Minnesota origin of the *P. deltoides* female parent performed well, even though, unlike the NRRI DxN clones, they were not screened in Minnesota. The NRRI DxN clones, with both Minnesota provenance origin and screening, also performed well on these Indiana sites, which were 5-9 degrees latitude south of where the screening took place in Minnesota.

Strong genetic control in DxN hybrids is also exhibited by the absence of rank change between the two Indiana sites (Table 11). Nelson et al. (2018) also found an absence of rank change and a low GxE interaction in relation to the clone effect in a study of 69 clones, 77 % of which were DxN hybrids, on six sites in Minnesota. Furthermore, there was no rank change for the 19 clones common to this study and the Nelson et al. (2018) experiment (Table 12). In addition, three NRRI DxN clones were in the top 25th rank percentile in both studies, in spite of the large latitudinal difference.

Neither Fertilization nor Irrigation had a significant effect on clone rank (Table 11). The combined Fertilizer + Irrigation treatment did have an effect on clone rank (Table 11), but p for the Spearman coefficient was just above the 0.05 level, so this result is equivocal. The lack of any clear treatment effect on clone rank is again evidence of strong genetic control in this collection of clones, which is dominated by DxN hybrids, providing further corroboration of the broad adaptability of DxN clones.

An important synthesis of our previous study of clones on six sites in Minnesota (Nelson et al., 2018) and the current study is that broadly adapted hybrid poplar genotypes can be identified, particularly for DxN hybrids. Zalesny et al. (2009) found certain older clones that had stable biomass production across portions of Minnesota, Wisconsin, and Iowa. European experience with *Populus* has been that natural hybrid DxN members of the *P. x canadensis* taxon contain the most broadly adapted hybrid poplars for that temperate climate (Clifton-Brown et al., 2019).

In addition to Nelson et al. (2018), the present study, and ongoing field tests in Minnesota, Iowa, and Indiana, and further wide-range geographic experiments will be valuable in bolstering or refuting our hypothesis of broad adaptability in DxN hybrids. Data are available for coincident DxN clones from past field tests with cooperators in New York and Michigan and will be analyzed along with our other study results and reported in a future scientific paper that will address the discovery of geographically robust DxN clones over very broad latitudinal and longitudinal ranges.

A useful approach in further studies of clones adapted to wide geographic ranges would be to monitor phenology (shoot initiation and growth cessation). Such studies could lead to a better understanding of the physiological mechanisms behind the geographic robustness of these clones and refinements in clonal selection.

General conclusions can be drawn from this study. Clones in the top tenth percentile in volume growth were highly improved. The clonal effect was dominant in comparison to site, treatment, and all interaction effects. Clonal rank was the same on both sites regardless of treatment, except for the Fertilizer x Irrigation treatment. There was no rank change for clones common to this study in Indiana and a clone trial on six sites in Minnesota (Nelson et al., 2018), which are widely separated in latitude, providing further evidence of broad adaptability of DxN hybrids with female *P. deltoides* parents of Minnesota provenance. It may be possible to locate a breeding and screening center for such hybrids in Minnesota for deployment over a wider area, possibly eliminating the need for narrow breeding zones and allowing reduced intensity of testing at specific latitudes, thus saving time and money. Further field tests and analyses of other completed tests are planned to refine this hypothesis.

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