Poplar breeding and testing strategies in the north-central U.S.: Demonstration of potential yield and consideration of future research needs[1]

by Don E. Riemenschneider[2], William E. Berguson[3], Donald I. Dickmann[4], Richard B. Hall[5], J.G. Isebrands[2], Carl A. Mohn[6], Glen R. Stanosz[7] and Gerald A. Tuskan[8]

We present results from a *Populus* Regional Testing Program that has been conducted in Minnesota, Iowa, Wisconsin, and Michigan over the past six years. Our objectives have been to: 1) identify highly productive, disease resistant intra- and inter-specific clonal selections and 2) understand patterns of genotype × environment interactions within the region that would, logically, govern commercial deployment of new clones. Clones were developed by breeding and selection programs at the University of Illinois, Iowa State University, University of Minnesota, and the USDA Forest Service for experiments established in 1995. We report results of analyses of variance and principal component analyses of tree diameters and estimated above-ground biomass that demonstrate significant genotype main effects and significant genotype × environment interactions. Maximum mean annual above-ground biomass increments have surpassed 16 Mg ha $^{-1}$ y^{-1}, exceeding previously reported yields of poplars grown under similar conditions in the north-central U.S. We also discuss the breeding and selection of poplars in general with specific attention to regional research needs.

Key words: *Populus*, biomass, multi-trait selection, genotype, genotype × environment interaction

Nous présentons les résultats provenant du Programme régional d'essai de *Populus* qui a été entrepris au Minnesota, en Iowa, au Wisconsin et au Michigan au cours des six dernières années. Nos objectifs étaient de : 1) identifier les sélections clonales intra et interspécifiques hautement productives, résistantes aux maladies et 2) comprendre les patrons des interactions entre les génotypes et l'environnement au sein de la région qui, logiquement, verrait au développement commercial de nouveaux clones. Les clones ont été obtenus des programmes de reproduction et de sélection de l'Université de l'Illinois, de l'Université de l'État de l'Iowa, de l'Université du Minnesota, et du Service forestier du ministère de l'agriculture des États-Unis dont les essais ont débuté en 1995. Nous présentons les résultats des analyses de variance et les principales analyses des composantes des diamètres des arbres et des interactions significatives des génotypes avec l'environnement. Les accroissements maximaux moyens de la biomasse au-dessus du sol ont atteint plus de 16 Mg ha^{-1} an^{-1}, dépassant les rendements rapportés précédemment pour des peupliers croissant sous des conditions semblables dans le centre-nord des É.-U. Nous discutons également de la reproduction et de la sélection des peupliers en général tout en portant une attention spécifique aux besoins régionaux de recherche.

Mots-clés : *Populus*, biomasse, sélection selon plusieurs facteurs, interaction entre le génotype et l'environnement

Introduction

Cottonwoods and their inter-specific hybrids (hereinafter referred to collectively as "poplars") could augment traditional supplies of fibre and biofuels in the north-central and north-eastern United States (Ranney *et al.* 1987, Tuskan 1998). However, yields of intensively managed poplar plantations in the east have always been lower than yields demonstrated in the Pacific Northwest (Wright and Tuskan 1997). For example, mean annual above-ground biomass increments of 15.6 to 27.8 dry Mg ha^{-1} y^{-1} (stem and branches) by hybrids between *Populus deltoides* Marshall and *P. trichocarpa* Torr. & Gray were reported in the State of Washington over 15 years ago (Heilman and Stettler 1985, reviewed by Heilman *et al.* 1996). In comparison, yields of poplars derived from various inter-specific hybridizations did not exceed about 3.8 Mg ha^{-1} y^{-1} in a multi-location testing experiment in the north-central U.S. (Hansen 1992).

Unfortunately, demonstrated yields are inversely related to the amount of land suited to short rotation woody crops within the two regions. Land base has been estimated at 5.6×10^4 ha and 6.5×10^6 ha in the Pacific Northwest and the north-central U.S. respectively (Wright and Tuskan 1997). Even though expanded use of irrigation can increase the acreage suited to short rotation crops in the Pacific Northwest, it is in the national economic interest (with regard to the supply of feedstock commodities) and in the global environmental interest (with regard to potential sequestration of atmospheric CO_2) to eliminate the disproportionate relationship between potential yield and available land base. Yields of intensively managed poplar plantations in the north-central region must be increased to accomplish this goal.

Genetic selection is one method by which crop yields may be increased and a more diverse array of clones may be deployed commercially. Early success of poplar culture in the Pacific Northwest (Heilman and Stettler 1985) is a classic example of the role of breeding and selection in short rotation intensive culture of poplars. Even though poplar breeding programs were underway in the north-central region, full integration of those programs with short rotation silvicultural research was not achieved as rapidly as was the case in the Northwest. Thus, we find yields reported by Hansen *et al.* (1992) based on geno-

[1]Presented to the 21st Session of the International Poplar Commission, September 24–28, 2000, Vancouver, Washington, USA.

[2]USDA Forest Service North Central Research Station, Forestry Sciences Laboratory, 5985 Highway K, Rhinelander, WI 54501.
[3]Natural Resources Research Institute, Duluth, MN USA.
[4]Michigan State University, East Lansing, MI USA.
[5]Iowa State University, Ames, IA USA
[6]University of Minnesota, St. Paul, MN USA (retired).
[7]University of Wisconsin, Madison, WI USA.
[8]Oak Ridge National Laboratory, Oak Ridge, TN USA.

types that were originally bred and selected in the 1930s at the Oxford Paper Company (the NE-"xxxx" identification prefix), then transferred from the northeast to the north-central U.S. Unfortunately, results of clonal trials in North and South Dakota, Minnesota, and Wisconsin suggested that few NE hybrids combine all the characteristics needed in a modern commercial cultivar. In fact, all but one of the hybrids have been rejected from further trials because of poor growth, disease susceptibility or instability and are no longer recommended for planting in the north-central U.S. (Hansen *et al.* 1994).

The goal of our research is to extend previous poplar breeding and selection in the north-central U.S. We have implemented a regional testing system with four test locations in Minnesota, Iowa, Wisconsin and Michigan in order to estimate clonal differences in growth and yield and the magnitude of genotype × environment interactions. Our testing has focused on eastern cottonwood (*P. deltoides*) because the species has potential as a fibre and biofuels crop *per se* and also has potential value as a parent in interspecific hybridizations. Eastern cottonwood has been selected as the primary species for woody biofuels development in the north-central U.S. because of its relative resistance to Septoria canker that limits deployment of many poplar hybrids in the region. This report describes growth and yield at age 6 years of 58 experimental clones and two commercial standards at three of our four test locations. We report, for the first time, mean annual increments of poplars grown under intensive cultivation that approach those demonstrated in the Pacific Northwest when grown under similar conditions.

Methods

Clone Selection

Experimental clones originated from breeding programs conducted by the University of Illinois, Iowa State University, University of Minnesota and USDA-Forest Service North Central Research Station. Clones were selected based on available growth and disease resistance data obtained from nursery and field experiments. Cuttings were taken from stool beds in Iowa and Minnesota and planted in nurseries at Ames, IA and Grand Rapids, MN in June 1994. Overall, we selected 43 clones of *P. deltoides*, 10 clones of *P. deltoides* × *P. maximowiczii* F_1 hybrids, two clones of *P. deltoides* × *P. nigra* F_1 hybrids (including 'DN-34' [a.k.a. 'NC-5326', cv. 'Eugenii'] as a control), one clone of *P. nigra* × *P. maximowiczii* ('NM-6' as a control), and four clones of aspen hybrids were included in the test (Table 1). Rooted one-year-old plants were lifted at both Grand Rapids and Ames in late April or early May. The current terminal shoot was pruned to approximately 10 cm and plants were held in cold storage until delivery to the planting sites during the week of May 15, 1995. All trees were delivered between May 15 and May 18 and planted within one week of delivery. Tests were established at Westport, MN; Ames, IA; Lansing, MI (results not reported herein) and Arlington, WI. In each case, the planting design was 10 randomized incomplete blocks with two-tree plots and a spacing of 3.0 m between rows and trees within rows. The planting at Westport had a single border row because of spatial constraints. All other tests had double border rows. Clones that were available in sufficient numbers were established in all 10 replications. Clones with limited ramet numbers were established in five replications. Clones were assigned to replications at random with the restriction that all replications were of identical size.

Data Analysis

We measured several growth and disease resistance traits from 1995 to 2000. Stem diameters at breast height (DBH) were measured to the nearest mm at the end of each growing season, beginning with end of the second year except for the Wisconsin site where DBH measurements began at the end of the third year. Data from the Michigan site had to be excluded from the analysis due to inconsistencies and the unbalanced nature of the data. Data from the remaining three sites were analyzed by analysis of variance (Proc GLM and VARCOMP; SAS Institute 1990) on both single and multiple location bases assuming all random effects. F-tests were by Satterthwaite approximation because of imbalance in numbers of ramets per clone (Table 2). Strong genotype × environment interactions were further explored by application of principal components analysis (Proc PRINCOMP; SAS Institute 1990). We also estimated mean clone above-ground biomass production (i.e., stem and branch dry weight) for all clones based on the equation:

$$dw = 6.16 - 2.23\ (DBH) + 0.3353\ (DBH)^2 \qquad [1]$$

(dw in kg/tree, DBH in cm). Individual biomass estimates of living trees were then converted to a per unit area basis based on plot size (9.3 m^2/tree) and assuming 100% survival. Mean annual increments (MAI) were estimated for all clones at the Minnesota, Iowa, and Wisconsin test sites. Equation (1) was developed by periodic destructive sampling of several clones per location within the regional experiment initially described in Hansen (1992). Our survival assumptions mean that mass per unit area estimates may be biased upward if current results are used to predict the yields of the same clones when planted in large plots. But survival of all clones at all sites in the current study exceeded 95%. And in any case, we know of no generally accepted, unbiased method of inferring large-plot yields from small-plot data. Thus, we state our methods and advise the reader to exercise due caution in interpretation.

Results

Genotypic Effects

We completed analyses of variance of diameters (Table 3) for all trees at all locations for age 6. Clones differed significantly in DBH at the three test locations analyzed in the current report (Table 3). Clonal differences were strongest at the Iowa test where heritability, the percent of variation among clones due to genetic effects, was 55.5% for DBH (Table 4). Elsewhere, heritability for tree DBH ranged from about 37% at Westport, MN to 43% at Arlington, WI (Table 4). The percent of variation attributable to clone effects has changed over the life of the experiments. For example, in year 1, variance in stem diameter due to clone constituted 66.3% of total variation at the Minnesota site and 20.9% at the Iowa site. Six years of growth and development have resulted in the sites becoming more uniform as regards distribution of variance (Table 4). The "best" growing clone-site combination was genotype number 80X00601, a pure *P. deltoides* that averaged 19.3 cm in DBH at age 6 at the Wisconsin test site.

Significant variation was also attributable to clone effects across locations (Table 5). However, heritability across sites was only 19% due to strong genotype × environment interactions that contribute to a higher phenotypic variance (i.e., the denominator of the heritability formula) (Table 6). Variation

Table 1. Parentage, institutional origin, and responsible breeder of clones deployed in the 1995 plantings of the North-central Regional Field Test Program.

Clone	Parentage
113.64	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
117.53	*deltoides* × *nigra* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
119.16	*deltoides* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
11AAG9012	Aspen hybrid by P. McGovern, Grand Rapids, MI
12XAA9005	Aspen hybrid by P. McGovern, Grand Rapids, MI
171–1	*deltoides* selection from NC-51 regional provenance tests
180-1	*deltoides* selection from NC-51 regional provenance tests
192-2	*deltoides* selection from NC-51 regional provenance tests
193-5	*deltoides* selection from NC-51 regional provenance tests
220-5	*deltoides* selection from NC-51 regional provenance tests
25	*deltoides* × *maximowiczii* selection by V. Steenacker (Belgium)
252-4	*deltoides* selection from NC-51 regional provenance tests
313.23	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
313.55	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
42-7	*deltoides* selection from NC-51 regional provenance tests
51-2	*deltoides* selection from NC-51 regional provenance tests
7300501	*deltoides* selection, University of Illinois
8000105	*deltoides* selection, University of Illinois
80X00601	*deltoides* selection, University of Illinois
80X00603	*deltoides* selection, University of Illinois
80X01132	*deltoides* selection, University of Illinois
8XAA9004	Aspen hybrid by P. McGovern, Grand Rapids, MI
91.01-07	*deltoides* selection by R. Hall (ISU)
91.05-02	*deltoides* selection by R. Hall (ISU)
91.05-06	*deltoides* selection by R. Hall (ISU)
91.05-08	*deltoides* selection by R. Hall (ISU)
91.05-10	*deltoides* selection by R. Hall (ISU)
91.06-13	*deltoides* selection by R. Hall (ISU)
91.07-06	*deltoides* selection by R. Hall (ISU)
91.08-09	*deltoides* selection by R. Hall (ISU)
91.65-00	*deltoides* selection by R. Hall (ISU)
91.79-00	*deltoides* selection by R. Hall (ISU)
CRANDON	putative *alba* × *grandidentata* hybrid, a commercial clone to serve as experimental control
D1	*deltoides* selection from NC-51 regional provenance tests (a.k.a. 41-3)
D10	*deltoides* selection from NC-51 regional provenance tests (a.k.a. 180-6)
D101	*deltoides* selection by C. Mohn (U of Minn.)
D102	*deltoides* selection by C. Mohn (U of Minn.)
D104	*deltoides* selection by C. Mohn (U of Minn.)
D105	*deltoides* selection by C. Mohn (U of Minn.)
D106	*deltoides* selection by C. Mohn (U of Minn.)
D108	*deltoides* selection by C. Mohn (U of Minn.)
D109	*deltoides* selection by C. Mohn (U of Minn.)
D110	*deltoides* selection by C. Mohn (U of Minn.)
D112	*deltoides* selection by C. Mohn (U of Minn.)
D114	*deltoides* selection by C. Mohn (U of Minn.)
D120	*deltoides* selection by C. Mohn (U of Minn.)
D121	*deltoides* selection by C. Mohn (U of Minn.)
D2	*deltoides* selection from NC-51 regional provenance tests (a.k.a. 41-2)
D3	*deltoides* selection from NC-51 regional provenance tests (a.k.a. 44-4)
D5	*deltoides* selection from NC-51 regional provenance tests (a.k.a. 269-4)
D7	*deltoides* selection from NC-51 regional provenance tests (a.k.a. 170-3)
MWH12	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
MWH14	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
MWH17	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
MWH2	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
MWH20	*deltoides* × *maximowiczii* hybrid by C. Mohn (U of Minn.) and D. Riemenschneider (USFS)
NC5326	Commercial hybrid clone for experimental control
NM6	Commercial hybrid clone for experimental control
OHIO RED	*deltoides* selection from NC-51 regional provenance tests

attributable to clone effects across locations has also changed over the course of the experiment (Fig. 1), ranging from about 3% in year 3 to over 10% in years 5 and 6. Clone effects in our multi-location analyses were greatest in year 1 (tree height and basal stem calliper – data not shown) but declined after the first winter. Winter damage at the Minnesota test site to several clones originating from the University of Illinois and Iowa State University programs resulted in a transient decline in clone effects with a concomitant increase in genotype × environment interactions.

Location Effects

Variance attributable to location was a strong source of variation in DBH after six years of growth in our multi-location analysis of variance (Tables 5 and 6), accounting for 28.6% of variation in tree DBH (Table 6). Variance due to locations has been significant throughout the life of the experiment, accounting for more variability than any other source until the current measurement year (Fig. 1). Average location DBH at Arlington, WI was exceptionally high with a mean of 14.4 cm. The Wisconsin test site has exceeded all other sites in mean tree height and diameter growth since the end of the first growing season, most likely because of its high quality agricultural soil. Growth was lowest at the Minnesota test site (Table 2), a result we attribute to the lower overall edaphic quality of the site, rather than to any large-scale environmental factor. In quantitative support of this attribution we have seen commercial plantings of our control clones on higher quality soils within 50 km of our Minnesota test site with mean annual increments in excess of 9 Mg ha^{-1} y^{-1} at age 4. The effect of blocks within locations was small relative to other sources of variation. Variation in tree DBH attributable to blocks in individual location analyses ranged from 6.7% at Westport, MN to 1.2% at Arlington, WI. Across all locations, variation due to blocks was also small (Table 6).

Genotype × Environment Interactions

Results of the combined location analysis of year 6 DBH demonstrated that variance attributable to clone × location interaction accounted for 20.6% of total variation, nearly double that attributable to the main effect of clone (Table 6). Much of the genotype × environment interaction in year 6 continued to be associated with the relative performance of the aspen clones, *P. deltoides* of Iowa and Illinois origin, and *P. deltoides* of Minnesota origin. For example, the four aspen clones continue to perform well at Ames, IA with clones 11AAG9102 and 12XAA9005 ranking among the best at Ames but ranking less well relative to the commercial standards at Minnesota and Wisconsin (Table 2). Further, the best pure *P. deltoides* clones at the Iowa test site were of southern (mostly Iowa and Illinois) origin while the most promising selections for Minnesota were derived from pure *P. deltoides* that were subject to nursery and field selection within Minnesota (i.e., 8000105 and

Table 2. Mean diameter (DBH, cm) after the sixth growing season and number of trees per location (n) for each clone tested in the 1995 Regional Field Test.

	Minnesota		Iowa		Wisconsin		Experiment-wide	
Clone	DBH (cm)	n	DBH (cm)	n	DBH (cm)	n	DBH (cm)	n
113.64	11.8	10	11.0	6	16.2	5	12.6	21
117.53	12.4	10	12.1	10	12.1	10	12.2	30
119.16	11.0	8	14.6	8	9.2	6	11.8	22
11AAG9102	11.9	10	15.9	6	10.7	5	12.7	21
12XAA9005	12.0	18	14.1	16	13.6	10	13.1	44
171-1	11.1	8	11.9	10	13.4	9	12.2	27
180-1	10.9	19	11.1	18	12.4	18	11.5	55
192-2	10.6	20	9.1	18	11.6	18	10.4	56
193-5	10.8	19	9.7	19	11.7	17	10.7	55
220-5	10.5	18	17.2	10	17.3	9	14.0	37
25	12.5	8	13.4	6	13.8	8	13.2	22
252-4	12.9	10	15.5	10	17.0	10	15.1	30
313.23	11.6	19	9.6	15	16.6	19	12.8	53
313.55	12.1	20	13.6	20	16.6	11	13.7	51
42-7	10.4	10	17.1	10	14.5	9	14.0	29
51-2	12.3	10	13.0	10	17.0	9	14.0	29
7300501	9.7	10	18.7	10	18.0	9	15.4	29
8000105	7.5	10	16.6	10	14.5	9	12.8	29
80X00601	10.2	10	13.7	10	19.3	10	14.4	30
80X00603	8.8	10	16.6	10	16.2	6	13.5	26
80X01132	8.3	10	16.7	12	15.3	9	13.6	31
8XAA9004	12.4	10	13.5	8	16.0	8	13.8	26
91.01-07	—	—	8.4	10	—	—	8.4	10
91.05-02	10.2	15	10.4	10	17.4	10	12.3	35
91.05-06	10.8	19	10.7	20	13.4	15	11.5	54
91.05-08	10.4	10	9.8	9	15.3	10	11.9	29
91.05-10	9.8	18	9.1	20	13.0	20	10.7	58
91.06-13	10.5	11	8.7	6	14.6	7	11.2	24
91.07-06	10.4	22	9.0	10	12.2	10	10.5	42
91.08-09	10.4	20	9.9	10	15.1	10	11.4	40
91.65-00	10.4	10	11.4	10	14.2	10	12.0	30
91.79-00	9.1	10	10.2	8	13.9	8	10.9	26
CRANDON	12.0	19	15.5	18	18.1	20	15.2	57
D1	8.7	10	10.8	9	12.7	8	10.6	27
D10	8.5	10	7.0	9	10.1	9	8.5	28
D101	10.6	10	11.2	10	12.0	9	11.2	29
D102	10.8	10	9.1	6	10.5	8	10.3	24
D104	10.8	20	10.8	20	12.4	19	11.3	59
D105	12.2	20	13.1	17	17.1	20	14.2	57
D106	11.3	9	11.0	20	9.5	4	10.9	33
D108	11.1	20	12.5	19	16.0	20	13.2	59
D109	12.3	18	13.0	19	17.0	21	14.2	58
D110	11.4	10	12.8	9	13.8	12	12.7	31
D112	12.1	10	12.2	10	16.4	9	13.5	29
D114	12.3	15	11.8	17	14.4	6	12.4	38
D120	10.6	10	10.2	10	16.0	9	12.1	29
D121	13.1	10	10.9	10	13.5	10	12.5	30
D2	—	—	11.4	9	—	—	11.4	9
D3	9.8	10	11.4	9	12.5	9	11.2	28
D5	9.0	19	10.0	15	14.4	19	11.2	53
D7	10.8	10	12.0	10	12.7	10	11.8	30
MWH12	9.9	19	8.1	18	13.7	10	10.0	47
MWH14	12.1	8	10.9	8	12.0	10	11.7	26
MWH17	10.2	10	8.8	6	12.5	11	10.8	27
MWH2	10.4	19	9.6	8	12.0	11	10.7	38
MWH20	9.8	12	7.7	8	10.3	3	9.1	23
NC5326	10.7	20	13.1	16	13.9	20	12.5	26
NM6	12.4	10	13.4	10	17.3	8	14.2	28
OHIORED	11.0	10	14.5	9	17.4	8	14.1	27
All clones	10.8	760	11.9	694	14.4	627	12.3	2081

80X01132 vs. D109and D121) (Table 2). Several hybrids, including clone 25, rank high for DBH across the region, but we are concerned about the incidence of Septoria canker in these clones that may impact performance over time.

Clone × location interactions were small on an experiment-wide basis for year 1 height and basal calliper (data not shown). For example, variation attributable to clone × location interaction for tree height over three locations account-

Table 3. Analyses of variance of age 6-year tree DBH (cm) at Westport, MN; Ames, IA and Arlington, WI in the 1995 Regional Field Test. Analyses assumed an all random effects model computed using Type III mean squares and expected mean squares. F-tests were done using the Satterthwaite approximation (Snedecor and Cochran 1967).

Location	Source of variation	df	Mean square	F	p	Expected mean square
Minnesota						
	Block	9	12.90	7.29	<0.0001	$\sigma_e^2 + 1.90\sigma_{cb}^2 + 69.89\sigma_b^2$
	Clone	56	17.22	9.68	<0.0001	$\sigma_e^2 + 1.93\sigma_{cb}^2 + 12.94\sigma_c^2$
	Clone*Block	322	1.79	1.49	<0.0001	$\sigma_e^2 + 1.95\sigma_{cb}^2$
	Within Plot	372	1.20			σ_e^2
Iowa						
	Block	9	8.05	2.22	0.0204	$\sigma_e^2 + 1.84\sigma_{cb}^2 + 61.85\sigma_b^2$
	Clone	58	75.81	20.94	<0.0001	$\sigma_e^2 + 1.88\sigma_{cb}^2 + 11.27\sigma_c^2$
	Clone*Block	293	3.62	0.98	0.5829	$\sigma_e^2 + 1.91\sigma_{cb}^2$
	Within Plot	333	3.71			σ_e^2
Wisconsin						
	Block	9	8.05	2.67	0.0054	$\sigma_e^2 + 1.73\sigma_{cb}^2 + 54.55\sigma_b^2$
	Clone	58	75.81	8.87	<0.0001	$\sigma_e^2 + 1.74\sigma_{cb}^2 + 10.36\sigma_c^2$
	Clone*Block	293	3.62	1.33	0.0089	$\sigma_e^2 + 1.83\sigma_{cb}^2$
	Within Plot	333	3.71			σ_e^2

ed for only 1.3% of total variation or about one-fifth of variation attributable to the clonal main effect. Clone × location interaction for stem calliper was larger as a percent of total variation (4.1%), but still smaller than the clonal main effect. Genotype × environment interactions increased significantly by year 3 and have remained high relative to the clone main effect through the life of the experiments (Fig. 1).

Principal component analysis of clone and location mean DBH resulted in two interpretable components of interest that accounted for 85% of joint variation (Table 7). Principal component 1 (PC 1) loaded positively on each of the three locations while principal component 2 loaded strongly and positively on Minnesota but negatively on Iowa and Wisconsin. We interpret PC 1 as an effect due to consistent performance of some clones across locations (i.e., "stability" or "plasticity"). Principal component 2 (PC 2) appears to be an effect due to the interaction between Minnesota and the other test sites, the source of which we have already discussed relative to the results of multiple location analysis of variance.

Table 4. Variance components and derived statistics from analyses of variance of tree diameter (cm) after six growing seasons at three 1995 Regional Field Test locations.

Source of variation	Location Minnesota	Iowa	Wisconsin
Variance Component			
Block	0.177	0.047	0.124
Clone	0.997	5.526	4.565
Block * clone	0.446	0.711	0.829
Residual	1.235	3.711	5.185
Total	2.854	9.995	10.703
Variance Component (% of Total)			
Block (%)	6.2	4.7	1.2
Clone (%)	34.9	55.3	42.7
Block * clone (%)	15.6	7.1	7.7
Residual (%)	43.3	37.1	48.4
Derived Variances			
Genotypic variance	0.997	5.526	4.565
Phenotypic variance	2.678	9.948	10.579
Heritability	0.372	0.555	0.432

Performance in Relation to Commercial Standards

Several new test clones exceeded the two control clones in height and stem calliper at the end of year 1. For example, the number of clones growing taller in year 1 compared to DN-34 was 18, 47, 46, and 22 at the Minnesota, Iowa, Wisconsin, and Michigan sites respectively (data not shown). Experiment-wide, 35 clones grew taller than DN-34. Overall, the two fastest growing clones at Minnesota were D105, a pure eastern cottonwood, and MWH2, a hybrid between eastern cottonwood and an Asian black poplar (*P. maximowiczii*). Fastest growing clones at Iowa were the various aspen hybrids.

Fewer clones exceeded the commercial standards in DBH at the end of six years. However, several clones continue to outperform DN-34 and NM-6 at all locations. The relative advantage of some clones is remarkable (Table 8). For example, mean above-ground (stem and branch) biomass for the two controls was 5.0 Mg ha $^{-1}$ y^{-1} at Minnesota (very close to that reported by Hansen [1994] for DN-34 in a regional plantation network). In comparison, the best clone in Minnesota has produced an estimated 6.79 Mg ha $^{-1}$ y^{-1}, while the mean of the best five clones was 6.23 Mg ha $^{-1}$ y^{-1} (Table 8). The relative advantage of the "best" clones is even greater at Iowa and Wisconsin, where the best clones exceed the controls by 135% and 58%, respectively. The best clone in Wisconsin has a mean annual increment (MAI) at the end of age 6 years of over 17 Mg

Table 5. Analysis of variance of tree diameter (breast height, cm) after the sixth growing season for three locations in the 1995 Regional Field Test. Assumptions and computations were done as described in Table 3.

Source of Variation	df	Mean square	F	p	Expected mean square
Location	2	1700.29	42.04	<0.0001	$\sigma_e^2 + 1.82\sigma_{cb(l)}^2 + 10.38\sigma_{cl}^2 + 59.03\sigma_{b(l)}^2 + 590.27\sigma_l^2$
Block/Location	27	12.74	3.33	<0.0001	$\sigma_e^2 + 1.83\sigma_{cb(l)}^2 + 62.10\sigma_{b(l)}^2$
Clone	58	83.64	2.46	<0.0001	$\sigma_e^2 + 1.83\sigma_{cb(l)}^2 + 11.12\sigma_{cl}^2 + 32.72\sigma_c^2$
Clone * Location	112	34.55	9.02	<0.0001	$\sigma_e^2 + 1.84\sigma_{cb(l)}^2 + 11.33\sigma_{cl}^2$
Clone * Block/Location	889	3.86	1.24	0.0006	$\sigma_e^2 + 1.90\sigma_{cb(l)}^2$
Within Plot	992	3.12			σ_e^2

$ha^{-1}\ y^{-1}$, which is greater than any previously reported MAI estimates in the North-central Region (Hansen *et al.* 1992). MAI curves show no sign of culmination, especially at the more productive locations (Fig. 2). In fact, estimation of relative growth rate in mean clone basal area ($cm\ cm^{-1}$) indicates that relative growth rate increased from age 5 years to age 6 years (data not shown).

Discussion

The current study is typical of poplar breeding and selection programs. Experimental clones were produced by crossing parents intra- and inter-specifically, sometimes with parental selection but as often with parents that were untested in the environment within which their progeny might be commercially deployed. Selection pressure was applied to the progeny populations for various characteristics including growth and resistance to those diseases that could be observed given the time span devoted to the tests. In sum, selections represented the application of "best intuition" and pure empiricism. In the following section we discuss the results of our past methodology along with our suggestions for future improvements that might lead to a better quantitative understanding of alternative breeding and testing strategies than we have achieved thus far.

Results of current study

We have demonstrated, within the limits of our methods, that poplar yields in the north-central U.S. can exceed 17 Mg $ha^{-1}\ y^{-1}$ at age 6 years. Our methods, however, require a relatively cautious interpretation and scope of inference. First, we used rooted cuttings (one-year-old nursery stock) as the propagule for field planting. This approach was used because our array of genotypes was expected to express a range of rooting abilities (i.e., aspens versus eastern cottonwood versus Tacamahaca × Aegeiros hybrids). Had we deployed unrooted cuttings, differences in rooting ability might have outweighed differences in growth potential, an outcome we did not desire. Second, our experiment utilized small, two-tree plots. Thus, inter-genotypic competition would be expected and there always exists the possibility that yield of genotypes with high growth potential is overestimated because the trees compete directly with genotypes of lower growth potential rather than with con-genotypic trees. Last, some of our experimental clones (especially those belonging to *P. deltoides* × *P. maximowiczii* pedigrees) are expressing susceptibility to Septoria canker and their individual-tree growth and per-unit-area MAI could reasonably be expected to decline over the next few years. Overall, we expect our growth and yield estimates to represent upper bounds and additional large-plot experiments of selected genotypes will be needed to obtain more realistic estimates of actual yield.

Table 6. Variance components and derived statistics from combined analyses of variance of tree diameter (cm) after 6 years at three locations in the 1995 Regional Field Test.

Variance component	Estimate
Location	3.07
Block (location)	0.13
Clone	1.46
Clone * location	2.21
Clone * block (location)	0.64
Within plot	3.23
Total	10.74
Variance component (% of total)	Estimate
Location	28.6
Block (location)	1.2
Clone	13.6
Clone * location	20.6
Clone * block (location)	6.0
Within plot	30.1
Derived variances	Estimate
Genotypic variance	1.46
Phenotypic variance	7.54
Heritability	0.19

Several of the best hybrids and pure *P. deltoides* have been established in scale-up stool beds at Grand Rapids, MN and Rhinelander, WI. These clones exceeded the control clones in height and diameter at the end of year 6 at all locations or grew exceptionally well at two or more locations. Additionally, those clones had either a low incidence of Septoria canker, or no canker, after year 4. We established the stool beds to support future large-plot growth and yield tests. As a cautionary note, we realize that some of the selections could fall out after two or three more years of observation in the Regional Field Test, while other clones may advance.

Short-term research needs

We need a better understanding of the origin of genotype × environment interactions, especially between Minnesota and Iowa. Both states maintain poplar breeding programs housed

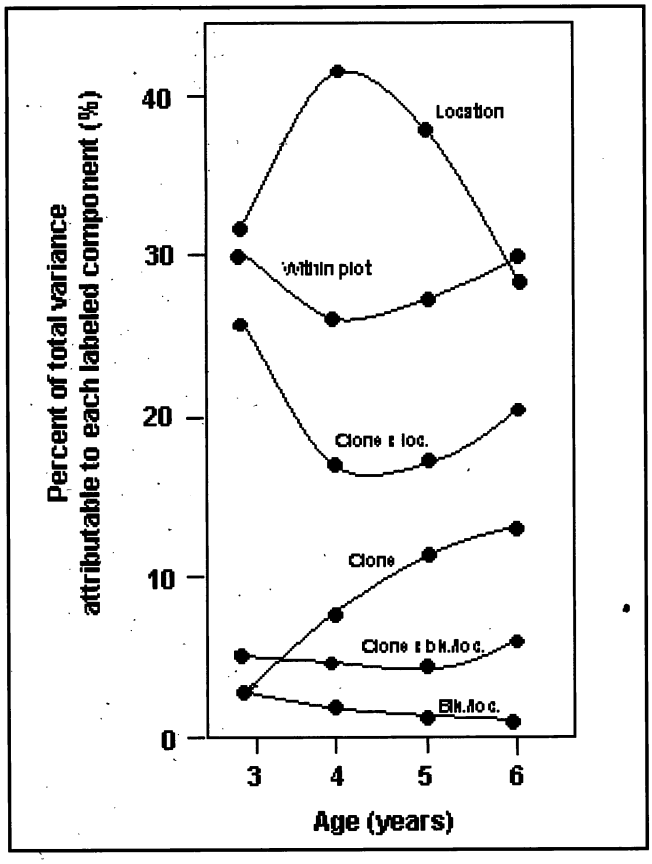

Fig. 1. Components of variance as a percent of total variation over time at three locations in our North-central Regional Clone Test Program.

at the Minnesota Hybrid Poplar Research Cooperative, NRRI, Duluth, Minnesota and Iowa State University, Ames, Iowa, respectively. Reduction or, at least, prediction of genotype × environment interactions is needed if these two programs are to conduct collaborative breeding research and crop development throughout the north-central U.S. To accomplish this we are implementing two modifications of our Regional Field Testing Program. First, we are adding an additional test site in Minnesota that is approximately equidistant between our existing test sites in Minnesota, Iowa, and Wisconsin. This modification should allow more precise delineation of geographic boundaries within which clone stability would be expected. Second, we are implementing a breeding effort designed to test whether genotype × environment interactions are arising due to: 1) parental selection, 2) juvenile selection at local nursery test sites, or 3) both of the above. F_1 hybrids between select *P. deltoides* of Minnesota and Iowa origin will be crossed with the same set of inter-specific parents, either *P. maximowiczii* or *P. nigra*. Unselected seedling populations will be reared in short-term tests in both states, where selections will be made. This breeding and selection experiment will produce populations with parental origin and juvenile test location in factorial combination and facilitate separation of the two effects noted above by subsequent field testing.

Our second short-term research need is to develop methods to identify clones that express long-term field resistance to Septoria canker based on tests of young plants. Selection for

Table 7. Results of principal component analyses applied to clone mean DBH (cm) measured at three test locations. Location loadings suggest principal component 1 reflects mean clone performance across all sites whereas principal component 2 reflects strong genotype × environment interactions between the Iowa and Wisconsin sites, taken together, and the Minnesota site.

	Eigenvectors	
Location	Component 1	Component 2
Minnesota	0.345	0.931
Iowa	0.651	–0.329
Wisconsin	0.676	–0.158
% Total variance (eigenvalues)	51%	31%

resistance to Septoria canker is mandatory in the north-central and northeastern U.S. Yet, the only method for accomplishing this selection is through long-term field testing of clones that have been selected for other criteria. The disadvantages of this strategy are many. First, long-term field testing requires a substantial investment in time and funding. Much of this investment is spent on field evaluation of clones (especially hybrids between *P. deltoides* and species of the section Tacamahaca) that become heavily cankered during their third or fourth growing season. Second, delayed identification of canker susceptibility can dictate the selection intensity allocated to other traits in a multiple trait selection strategy. For example, if only a few clones are canker-resistant it is impossible to achieve high selection intensities for growth, rooting ability, or resistance to other pests. An attempt to predict long-term canker resistance in the field based on quantitative responses of young trees to artificial inoculation with *Septoria musiva* has shown some promise (Weiland *et al.* 2000). A full evaluation of the efficiency of the method, however, requires additional testing and analysis of the relationship between juvenile test and long-term field result.

Long-Term Research Needs

One of our long-term goals is to be more quantitatively rigorous in the application of multiple-trait selection strategies. Various methods exist whereby multiple traits may be considered during the design of testing and selection strategies in order to maximize the aggregate value of selected genotypes. Strategies have been discussed and reviewed as they apply to tree breeding in general (Cotterill and Dean 1990) and to poplars specifically (Riemenschneider *et al.* 1996). The most rigorous, and theoretically most efficient, selection strategy is the discriminant function selection index where selection is based on an index (I) comprised of the sum of various phenotypic traits (X_i), each of which are given an appropriate weight (b_i) as follows:

$$I = b_1X_1 + b_2X_2 + \ldots + b_nX_n \qquad [2]$$

Estimation of the various weights (b_i) requires knowledge of the economic value of a "unit" improvement in each trait comprising the index. Such estimation assumes that the relationship between a unit of phenotypic improvement is linear with economic value throughout the range of expression observed in the population under selection, an assumption that may or may not be true (Namkoong 1979). We hypothesize that the assumption is rarely true for most poplar traits, excepting wood volume or mass, and that an understanding

Table 8. Biomass yields of control (DN34 and NM6) and newly tested clones at age 6 years were estimated by an individual tree biomass equation (see Materials and Methods). Then, yields per hectare were estimated by multiplying individual tree yield by field test stocking (1075.6 trees ha^{-1} at 3.05 m × 3.05 m spacing) and calculating the clone mean. The percent advantage of the best clones was determined.

	Minnesota		Iowa		Wisconsin	
	DBH (cm)	Mg ha^{-1} y^{-1}	DBH (cm)	Mg ha^{-1} y^{-1}	DBH (cm)	Mg ha^{-1} y^{-1}
Control mean	11.52	5.02	13.25	7.17	15.61	10.84
Best clone	13.13	6.79	18.69	16.84	19.28	17.16
Mean of 5 best clones	12.64	6.23	17.28	13.53	17.81	14.94
Advantage of best clone (%)	14.0	35.3	41.1	135.0	23.5	58.3
Advantage of best 5 clones (%)	9.7	24.1	30.4	88.8	14.1	37.8

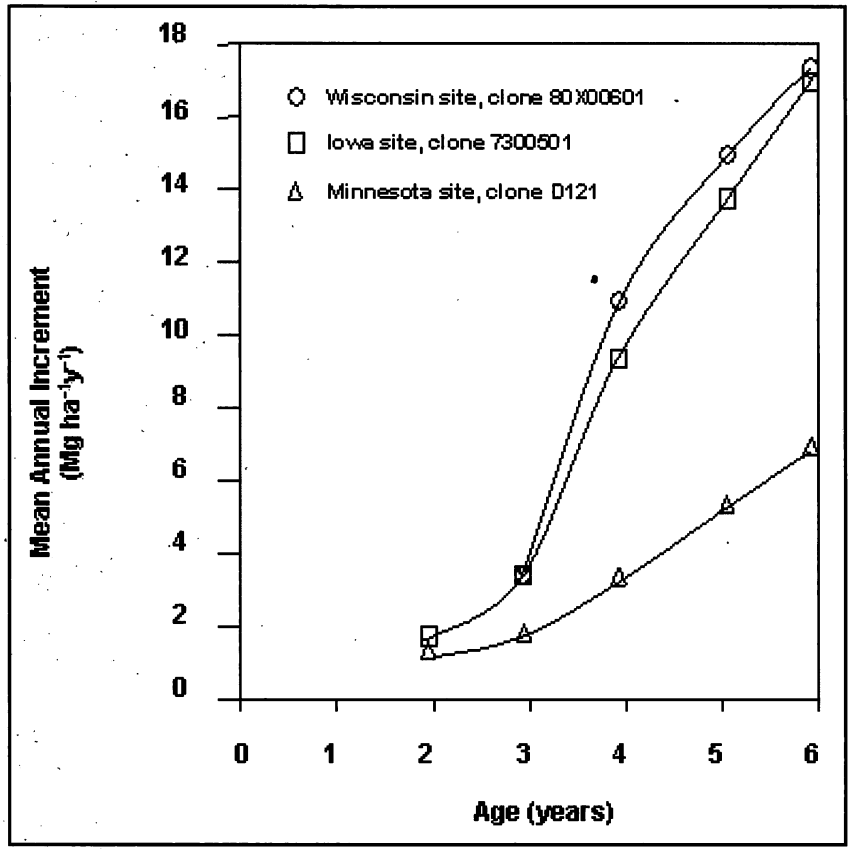

Fig. 2. Mean annual increments (Mg ha^{-1} y^{-1}) for the "best" growing clones at each of three test locations in the North-central Regional Field Test.

of the relationship between expression and economic value is needed to achieve efficient selection strategies.

Consider rooting ability as an example. The production of adventitious roots by poplar cuttings is an important characteristic because the commercial propagule of choice is a dormant unrooted hardwood cutting. Rooting is known to vary genetically within poplar populations (Wilcox and Farmer 1968, Ying and Bagley 1977, Riemenschneider *et al.* 1996) and is an historically important selection criterion in breeding programs. Thus, incorporation of rooting ability into multiple-trait selection strategies is reasonable. The need to place some value on the expression of rooting ability follows, as do the assumptions regarding linearity between value and expression. Unfortunately, rooting is probably not that simple. For example, poor rooting ability, over some quantitative range, may be inadequate to ensure survival, regardless of genetic differences within that range. Value over such a range is 0.0. Alternatively, aggressive rooting, again over some quantitative range, may not confer genotype-based differences in value within that range and may cause value reduction if preferential partitioning of photosynthate reduces very early stem and leaf growth. Our reasoning leads to the hypothesis that the relationship between aggregate genotypic value and the expression of rooting ability is an "S" shaped curve, not a linear relationship as is required by selection theory. Expression of other characteristics such as resistance to some diseases and various measures of wood quality could be similarly related to genotypic value based on similar reasoning.

We see the need to understand relationships between expression and value as a prerequisite to refining our application of selection theory to poplar breeding and testing. We speculate further that such an understanding might be achieved through a redirection of physiological genetics research. It has been noted that the application of physiological genetics to the definition and implementation of a crop ideotype for poplars has been difficult (Dickmann and Keathley 1996) and that a more focused approach could be useful. We suggest that understanding the relations between traits such as rooting, dis-

ease incidence and disease severity and their associated value (effect on stem growth) would provide such focus and a new *modus operandi* (Dickmann and Keathley 1996) for the application of physiological research to the needs of poplar breeding.

Testing and selection strategies are further dependent on breeding strategies that, for poplars, exist in vast array. Many intra- and inter-specific hybridization strategies have proven possible (Stettler *et al.* 1996). And, within each of the possible species combinations, any number of subordinate breeding and selection strategies might be imposed (Bisoffi and Gullberg 1996). The prevalence and impact of Septoria canker in the north-central U.S. has led us to favour intra-specific recurrent breeding of *P. deltoides* as the preferred strategy because the species is highly resistant to the disease. Most of the clones tested in the experiments described herein were pure *P. deltoides* (Table 1) and current results demonstrate that yields can be high (Table 2). However, hardwood cuttings of *P. deltoides* root erratically or not at all in our region, which has led us to adopt F_1 inter-specific breeding (Table 1) and backcross breeding (with *P. deltoides* as the recurrent parent) as simultaneous pursuits. This is problematic because any introduction of parentage from the section Tacamahaca has proven to increase susceptibility to Septoria canker, necessitating strong selection pressure for canker resistance. Multiple breeding strategies increase genetic diversity and thus the probability that achieved yields will be sustainable. Multiple strategies also cause division of effort and complicate testing to the extent that the distribution of variances and covariances, that define optimum testing designs, are population-dependent. We see no way to identify the "best" breeding strategy for the north-central U.S. other than by the continued application of empirical research and the experimental comparison of alternative strategies, as inefficient and time-consuming as that might seem.

Conclusions

Results of the current study have demonstrated to our satisfaction that poplars grown under short-rotation intensive culture in the north-central U.S. can produce mean annual increment above-ground biomass yields that exceed 17 Mg ha^{-1} y^{-1} at age 6 years. We fully recognize that such yields need to be verified through large-plot field testing as does continued resistance of selected experimental clones to important diseases, especially Septoria canker. We suggest that though our breeding programs have succeeded, as evidenced by the results reported herein, our breeding strategies need to be refined. Refinement will rely on the fulfilment of short-term research needs such as a better understanding of the origins of genotype × environment interactions and the development of early screening methods for canker resistance. Refinement also relies on the fulfilment of long-term research needs such as an improved understanding of the relationship between trait expression and the value each trait confers to the aggregate genotypic worth. Refinement of our breeding strategies will likely rely on continued empirical experimentation.

Acknowledgements

The authors wish to thank the agencies that are financially supporting this research, including the Oak Ridge National Laboratory, managed by UT-Battelle, LLC for the U.S. Department of Energy under contract DE-AC05-00OR22725, and the U.S. Department of Agriculture, Forest Service.

References

Bisoffi, S., and U. Gullberg. 1996. Poplar breeding and selection strategies. *In* R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman and T.M. Hinckley (eds.). Biology of *Populus* and its implications for management and conservation. pp. 139–158. NRC Research Press, National Research Council of Canada, Ottawa, ON

Cotterill, P.P. and C.A. Dean. 1990. Successful tree breeding with index selection. Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia. 79 p.

Dickmann, D.I. and D.E. Keathley. 1996. Linking physiology, molecular genetics, and the *Populus* ideotype. *In* R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman and T.M. Hinckley (eds.). Biology of *Populus* and its implications for management and conservation. pp. 491–514. NRC Research Press, National Research Council of Canada, Ottawa, ON

Hansen, E. 1992. Mid-rotation yields of biomass plantations in the north central U.S. Res. Pap. NC-309. St. Paul, MN. U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 8 p.

Hansen, E.A., Ostry, M.E., W.D. Johnson, D.N. Tolsted, D.A. Netzer, W.E. Berguson and R.B. Hall. 1994. Field performance of *Populus* in short-rotation intensive culture plantations in the north-central U.S. Res. Pap. NC-320. St. Paul, MN. U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. 13 p.

Heilman, P.E., T.M. Hinckley, D.A. Roberts and R. Ceulemans. 1996. Production physiology. *In* R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman and T.M. Hinckley (eds.). Biology of *Populus* and its implications for management and conservation. pp. 459–489. NRC Research Press, National Research Council of Canada, Ottawa, ON

Heilman, P.E. and R.F. Stettler. 1985. Genetic variation and productivity of *Populus trichocarpa*. II. Biomass production in a 4-year plantation. Can. J. For. Res. 15: 384–388.

Namkoong, G. 1979. Introduction to quantitative genetics in forestry. U.S. Department of Agriculture, Technical Bulletin No. 1588. 342 p.

Ranney, J.W., L.L. Wright and P.A. Layton. 1987. Hardwood energy crops: the technology of intensive culture. J. For. 85: 17–28.

Riemenschneider, D.E., H.E. Stelzer, and G.S. Foster. 1996. Quantitative genetics of poplars and poplar hybrids. *In* R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman and T.M. Hinckley (eds.). Biology of *Populus* and its implications for management and conservation. pp. 159–181. NRC Research Press, National Research Council of Canada, Ottawa, ON.

SAS Institute. 1990. SAS/STAT Users Guide. Version 6, Third Edition. SAS Institute, Cary, NC.

Stettler, R.F., L. Zsuffa and R. Wu. 1996. The role of hybridization in the genetic manipulation of *Populus*. *In* R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman and T.M. Hinckley (eds.). pp. 87–112. Biology of *Populus* and its implications for management and conservation. NRC Research Press, National Research Council of Canada, Ottawa, ON

Tuskan, G.A. 1998. Short-rotation woody crop supply systems in the United States: What do we know and what do we need. Biomass and Bioenergy 14: 307–315.

Weiland, G.E., J.C. Stanosz and G.R. Stanosz. 2000. Short-term assays of resistance to Septoria canker predict long-term field performance. Phytopath. 90: S83.

Wilcox, J.R. and R.E. Farmer Jr. 1968. Variation and inheritance of juvenile characters of eastern cottonwood. Silv. Genet. 16: 162–165.

Wright, L.L. and G.A. Tuskan. 1997. Strategy, results and directions for woody crop research funded by the US Department of Energy. *In* Proceedings of the 1997 Pulping Conference. pp 791–799. TAPPI Press, Atlanta, GA.

Ying, C.C. and W.T. Bagley. 1977. Variation in rooting capability of *Populus deltoides*. Silv. Genet. 26: 204–207.