Vectors of Efficiency in Hybrid Poplar Genotype Testing

Neil D. Nelson*, William E. Berguson, Bernard G. McMahon, Meijun Cai, Daniel J. Buchman

Natural Resources Research Institute, University of Minnesota Duluth, 5013 Miller Trunk Highway, Duluth, MN 55811-1442, USA

* Corresponding author: Neil D. Nelson, Email: nnelson2@d.umn.edu

Abstract

The Natural Resources Research Institute Hybrid Poplar Program breeds and tests genetically improved clones for biomass production and environmental services. The testing process progresses from Nursery Progeny Tests (NPT) to Family Field Trials (FFT) to Clone Trials (CT) to Yield Blocks (YB), with limited replication of many clones in FFT and CT and a limited number of highly selected clones set out in monoclonal blocks (YB) to approximate the conditions of commercial plantations. We used correlation vectors, R^2 (coefficient of determination) and r_s (Spearman's Coefficient) for growth (DBH²) and McFadden's Pseudo R^2 for canker severity score, to determine where testing times could be altered (age – age correlations) and whole testing steps eliminated. FFT can be shortened from 5 years to 4 years. In CT, rank correlations between age 5 (half-rotation) and age 9/10 (full rotation) were significant ($R^2 = 0.39 - 0.72$), but age 5 selection missed 44 % of the top ten clones at age 9/10. Clone rank in CT at full, but not half, rotation was correlated with rank at full rotation in YB. Choosing clones at 9 years in CT adds 4 years but allows possible elimination of YB for clone selection. Both FFT and CT are necessary. Canker abundance and severity in CT at full rotation cannot be determined at earlier ages. An aggressive strategy saves 6 years of testing.

Common abbreviations in this paper are as follows: CP = controlled pollination; NPT = nursery progeny test; FFT = family field trial; CT = clone trial; YB = yield block; *P.* = *Populus*; D = *Populus deltoides* Bartr.ex Marsh (eastern cottonwood); N = *Populus nigra* L (European black poplar); M = *Populus maximowiczii* A. Henry (Japanese poplar); T = *Populus trichocarpa* Torr. & A. Gray (black cottonwood)

Keywords: hybrid poplars, *Populus*, age-age correlations, early selection, poplar breeding, clone selection

Introduction

Hybrid poplars (*Populus* spp.) are one of the most promising and tested dedicated crop sources for biomass feedstock in temperate climates. Poplar plantations have produced significant amounts of pulpwood for the paper industry in Minnesota and the Pacific Northwest, USA (Berguson et al., 2010), and are a focus of the nascent biofuel and bioproducts industry (US DOE, 2016). Achieving the national target of one billion dry tons of cellulosic biomass feedstock annually in the United States by 2030 will require producing more than 239 million dry tons each year from energy crops including hybrid poplars on agricultural soils (US DOE, 2016). The profitability of poplar plantations depends on many factors, but yield is a key determinant (Lazarus et al., 2015). Site selection and agronomic inputs can increase yield, and one of the most important inputs is the genetic composition of the planting material. The use of the most productive poplar clones has also been shown to reduce the environmental impact of biomass production (Bacenetti et al., 2016). The need for adapted, fast-growing, and disease-resistant poplar trees led us to establish the NRRI hybrid poplar genetics and breeding program. We have bred poplar families and clones and planted field tests annually in Minnesota and other areas of the USA for all but two years since 1996. Clones have been selected from these field tests. Our first generation improved clones exhibit up to a 70 percent increase in volume growth over commercially available genotypes (Nelson et al., 2018; Nelson, Berguson et al., 2019; Nelson, Meilan et al., 2019).

Even though the selection, breeding, and testing process for hybrid poplars is shorter than for most other tree species due to poplars' fast growth and amenability to cloning, up to 20 years are still required for one generation of genetic improvement in northern temperate climates, excluding parental testing and selection. The multi-step NRRI hybrid poplar improvement system, a sequential test process (Libby, 1987), is diagrammed in Figure 1. Any shortening and simplification of the

genotype testing within the process would increase genetic gain per year and save time and money, providing additional efficiency. Alternatively, it may be necessary to increase the length of testing in some steps of the breeding process to achieve accurate selection for superior performance at full rotation.

Figure 1
The NRRI (University of Minnesota Duluth - Natural Resources Research Institute) hybrid poplar breeding and testing process.

There are four potential avenues for increased efficiency with our genotype testing: 1) early selection; 2) elimination of one or more nursery-based progeny trial measurements; 3) providing clone performance estimates through FFTs, allowing the possible elimination of CTs; and 4) possible elimination of the final step, YB (monoclonal blocks of single clones meant to approximate commercial conditions), except for deriving biomass yield estimates. Vectors (quantities that have magnitude and direction) can provide this information in the form of: 1) Coefficient of Determination (R^2) combined with information on the slope of the regression line (+ or -) from which R^2 is derived; 2) Spearman's Coefficient of Rank Correlation (r_s); and 3) McFadden's Pseudo R^2 for canker scoring correlations. We can calculate these vectors for age-age correlations of clone and family growth performance and for correlations of clone and family growth performance in different steps of the testing process. Our extensive databases of field sites in Minnesota for measuring survival, growth, and canker incidence and severity provide an opportunity to determine the earliest ages at which genotype performance can be reliably estimated and whether the testing steps can be simplified or compressed. Sites applicable to this study include nursery progeny trials (1 site), FFTs (6 sites), CTs (5 sites), and YBs (2 sites).

Age-age correlations are a common approach in genetic tree improvement programs for evaluating selection at less than full rotation age (early selection, indirect selection, juvenile-mature correlations) in many tree species (see Wu, 1999 and citations in White et al., 2007), including hybrid poplars. The cumulative nature of tree growth traits, under the assumption that annual increments are normally and independently distributed, results in tree size correlations between ages increasing monotonically with age even without any genetic correlation (Bisoffi, 1993). This mathematical fact contributes to the common observation that rotation performance of trees can be

predicted at as early as one fourth of rotation age (Bisoffi and Gullberg, 1996).

Several studies document age-age correlations for poplars at traditional pulpwood production spacings. Wilkinson (1973), in a study of 350 older hybrid poplar clones in the northeastern USA, reported that selection at 1 to 4 years was inefficient for identifying the fastest-growing clones at 15 years. However, height and diameter at 9 years was highly correlated with the same variables at 15 years. Tsarev (1977) found that selection for poplar was effective at age 4 – 5 years for an implied 12-year rotation. Foster (1986) reported a high genetic correlation between ages 4 and 7 for tree bole volume of *Populus deltoides* Bartr. ex Marsh (eastern cottonwood) in the lower Mississippi Valley USA, similar to age-age genetic and/or phenotypic correlations in Mohn and Randall (1971), Randall and Cooper (1973), and Ying and Bagley (1976) for *P. deltoides*, and Mohrdiek (1979) for Aigeiros x Tacamahaca section poplar hybrids. For *Populus deltoides* and *P. x euramericana* (*Populus deltoides* x *Populus nigra*; D x N) in northern India, selection at age 3 or 4 was effective at identifying the best clones for a six-year rotation (Kumar and Singh, 2001). Chiba and Nagata (1972) found high phenotypic correlations within *Populus maximowiczii* Henry for height and diameter between years 3 and 7 for and for *Melampsora* rust susceptibility for year 1 versus year 7. Kaczmarek et al. (2013) reported that for *Populus trichocarpa* Torr. & A. Gray (black cottonwood) x *Populus deltoides* hybrids and pure *Populus deltoides* exhibited a high phenotypic correlation between survival at ages 1 and 10. Their results also indicated that selection for growth could be made at age 3 for a 10-year rotation, with only moderate to poorly performing clones changing rank between these ages. In a study of primarily *Populus deltoides* in Argentina, height and diameter growth at age 3 were significantly correlated with age 9 or 10 values (Ares, 2002), but there were large changes in individual clone rankings between these stand ages. First-year clone rankings for growth in poplars were not a good predictor of rankings at years 4 and 5 (Brown et al., 1996; Ghezehei et al., 2019). Age-age correlations have also been studied in poplar coppice rotations, e.g., Yaneza et al. (2019).

In summary, the existing literature for poplars supports effective selection between about 30 and 60 percent of rotation age for growth characters, with variation in age-age correlations likely due to site, clonal composition, region, and disease resistance. The effect of region on age-age correlations probably reflects climatic differences, e.g., the cumulative effects of recurrent killing frosts in more extreme northern continental climates such as that of Minnesota. This variability implies that it is informative and necessary to calculate age-age correlations for individual breeding programs with defined genotypes in specific regions.

Precise estimates of genetic correlations require extremely large experiments with offspring of many parents (White et al., 2007) and many ramets per genotype in a clonal experiment (e.g., Zhang et al., 2013), a condition that may not be met by the study reported here. Therefore, we employed phenotypic age-age correlations that are typically similar in size and magnitude to genetic correlations but tend to produce a more

conservative estimate of optimum selection age (Gwaze et al., 2000).

All of the data analyzed here are from Minnesota USA, which has a continental northern temperate climate. Rotation length for hybrid poplars at pulpwood production spacings in Minnesota is approximately 10 – 12 years, which frames our breeding process steps and age-age correlations. Crown closure occurs at about ages 3 – 5 at the spacings in the FFTs, CTs, and YBs here, depending on the specific spacing. Variables evaluated in this study are survival, diameter breast height squared (DBH²), and canker incidence and severity.

Materials and Methods

The NRRI hybrid poplar breeding and testing process

The NRRI Hybrid Poplar Breeding Process is a sequential breeding and testing system depicted in Figure 1. Phenotypic selection includes testing and recurrent selection of full-sib intra-specific families followed by inter-specific hybridization in a greenhouse to produce F₁ clones for testing and deployment. The rationale for the process is justified in Berguson et al. (2017). Our focus has evolved to emphasize *Populus deltoides* x *Populus nigra* hybrids (aka *P. x canadensis* known by the shorthand designations D x N and DN) after prior field experimentation with a range of parental *Populus* species and hybrid combinations in the Midwest USA. The *P. deltoides* parents are primarily of Minnesota USA provenance. The system is based on a full rotation length of 10 – 12 years in Minnesota and the other far northern tier of U.S. states, and 5 – 7 years in the southern portion of the Midwest. In this paper, we refer to ages 9 and 10 as “full rotation” age. Details of greenhouse growing conditions and establishment and maintenance for the field plots providing data for this study are in the Materials and methods — Greenhouse breeding and Plantation establishment and maintenance sections, respectively.

Phenotypic selection and collection of test families

We select male and female parents for breeding from our field tests of pure species in Minnesota and from pure species genotypes, including non-native species, selected by cooperators outside Minnesota in Canada and the United States. Our internal program's family and clone selections of offspring of selected parents are based on growth and canker incidence and severity, typically at 3 – 5 years stand age. Cankering is not a significant concern in *P. deltoides* in Minnesota.

Parent selections were based upon previous performance data from existing mature FFTs, from trees that had adequate floral branches of both seed and pollen candidate clones (on separate trees due to the dioecious nature of *Populus* spp.). Parents were also selected in older University of Minnesota existing collections and FFTs to fill breeding targets or matrix workplans. Vigorous one- and two-year-old floral-bearing branches, located in the upper crown of targeted parent trees, are collected and are preferred over older, gnarled branches of the lower crown. Flowering in existing genetic trials begins

between the ages of six and eight years in eastern cottonwood (*P. deltoides*) and European black poplar (*P. nigra*). Floral branches were collected from sexually mature parent trees in January, when they were in a fully dormant state and floral buds were intact.

Greenhouse breeding

Roots were initiated on female branches while keeping the buds dormant and intact. These female cuttings with roots were then planted in pots in our breeding greenhouse, where flowers emerged. Male branches were forced to produce pollen in a separate greenhouse over two months in January and February. Female flowers were pollinated in isolation breeding hoods in the NRRI breeding greenhouse. Pollen and female flower forcing were both at a minimum temperature of 20 degrees C. Seed was collected when capsules dehisced and planted in germination flats. This seed was germinated in the greenhouse using an intermittent mist system. The germinated seedlings were transplanted to Spencer Lemaire ConeTainer tubes and grown over the summer and fall, moved outside for growth and development through the onset of dormancy, then stored in dark and cold greenhouse bays over the winter. The following spring, the seedlings were planted in the nursery for progeny trials. Details of the greenhouse breeding are in the online Supplementary Data.

Nursery progeny trials (NPT)

Between 25 and 75 seedlings (genotypes) from each full-sib family produced in the breeding were established at 0.3 m within row and 1.0 m between rows at the University of Minnesota North Central Research and Outreach Center (NCROC) site near Grand Rapids, MN. Individual clones were unreplicated. The goal was 75 seedlings per family, but we used the number available, with 25 seedlings as the minimum. These NPT plantings served as the cutting supply (stoolbeds, cutting orchard) for the FFTs, as well as an early-stage screening for climatic adaptability and disease resistance. Four measures of shoot growth were used to determine growth performance in the NPT as follows: All seedlings (genotypes) were measured for total height after one year of growth (**Yr 1 seedling ht**), and some were measured after two years of growth (**Yr 2 seedling ht**). Most seedlings (those slated for stump sprouting) were cut to within 5 cm above ground in the winter after the first year of growth. These trees subsequently sprouted from the stump in year 2, and the terminal shoot was measured at the end of year 2 (**Coppice 1**). These same trees were then cut again to within 5 cm above ground in the winter after the second year of growth, subsequently sprouted from the stump in year 3, and the terminal shoot was measured at the end of year 3 (**Coppice 2**). As the NPT trials are unreplicated tests at the genotype level, the family mean of all four NPT growth measurements were analyzed for their relationship to family mean growth in FFTs, with the primary purpose being to choose the fastest-growing full-sib families for the ensuing FFTs. However, plant material availability in the coppice stems (stools) was the primary underlying reason for inclusion in FFT establishment. Coppice 1 or 2 shoot growth are measured to gauge cutting

supply for the FFTs, regardless of potential predictive value. Typically this procedure results in approximately the top 50 % of families (based on family mean shoot growth rate) using the Coppice 1 and 2 results being selected for propagation in the greenhouse. Rooting success in the greenhouse is the ultimate criteria determining opportunity for planting in the field with balanced representation of families, clones, and ramets. A common target in FFTs is 30 full-sib families, with 20 individual clones per family and 5 ramets (replicates) of each clone. As reported in Berguson et al. (2017), this design allows us to evaluate sources of variance among families, within families, and within clones, all important values to better understand the potential of the overall breeding program to improve yield.

Shoots (whips) of clones within each selected family were harvested for cutting material. The selection of clones within family for harvesting was random, except that only clones that could produce the required number of cuttings for the field plot design were included, so that there was some bias toward faster-growing clones within each family, although the magnitude and significance of this effect is practically not quantifiable. The cutting production requirement, in addition to eliminating slower-growing families and clones, also eliminated clones that became diseased or were otherwise maladapted.

Family field trials (FFT)

Family Field Trials test full-sib families with seedling selections clonally replicated within each family. Dormant shoots (whips) harvested from the NPT in winter were stored below freezing and processed into 'mini' cuttings 7.6 – 10.1 cm in length, each cutting having at least two viable lateral vegetative buds and no terminal buds. These 'mini' cuttings were rooted in 107 ml cells containing a coarse peat blend soil mix (Premier Tech Horticulture Pro-Mix HP Bio + MYCO) and grown for eight weeks in the greenhouse starting in late March, then moved outside for three weeks of environmental acclimation starting in early May. The six FFTs were then planted with these rooted cuttings in May/June at 3.0 x 3.0 spacing, with the exceptions being the Woelfel FFT at 2.4 x 3.0 m and the MN Power FFT at 1.6 x 1.6 m.

Clone trials (CT)

The purpose of clone trials is to further evaluate the growth and disease performance of selected individual clones that performed well in the FFTs. Dormant shoots were harvested from the FFTs in winter. The remainder of the propagation and planting was the same as the FFT procedures. All spacing was 3.0 x 3.0 m. Growth parameter measured was DBH² (tree diameter outside bark at 1.4 m squared). All clone trials included one or more commercial check clones ('NM6', 'DN5', 'DN2', 'DN34' ('Eugenei')).

Yield blocks (YB)

Yield Blocks are considered the final stage of field testing and consist of clones set out in monoclonal blocks meant to approximate commercial conditions and to estimate biomass yield. Most of the clones in the YBs are those that have performed well in the FFTs and CTs. FFT and CT generally do not provide sound estimates of biomass yield per unit land because of

wide variation in growth rate of adjoining trees in the plots. The number of selected clones included in a single YB in the trials used in this study varied from five clones for the LEA Grand Rapids YB to 22 for the Hansen YB. The LEA Grand Rapids YB included the commercial check clone, 'NM6' (*Populus nigra* x *Populus maximowiczii*). Propagules for the YBs have varied in our process—some trials used unrooted cuttings, some rooted cuttings. In this study, the LEA Grand Rapids YB was established with 23 cm length unrooted cuttings, Hansen YB with 8 cm length containerized mini rooted cuttings. YB spacing was 3.0 x 3.0 m at Hansen and 2.4 x 2.4 m at LEA Grand Rapids.

Clone selection for FFT, CT, YB

Clone selection for FFTs is described above.

Clones for CTs were selected from our Program's FFTs in Minnesota and from other sources within the region.

Selection of most clones for the CTs was based on diameters measured in our FFTs from ages 3 – 5 years, along with canker scores and other categorical observations related to tree form. The FFTs have a family genetic structure, including clonally replicated full-sib individuals, and typically contain up to 900 genotypes. In addition to new clones selected from the FFTs, a small subset of those clones that performed well in earlier tests was included as a comparison to the new set of clones. Also, two commercial standards, 'DN2' (*Populus deltoides* x *Populus nigra*) and 'NM6', were embedded within the clone trials.

The fastest-growing disease-resistant clones in the CTs with adequate cutting material available in stool beds were selected for the YB tests in most cases. However, the clones selected for the 2006 Hansen YB were selected from clone tests established before 2006, not from the 2006 Hansen CT. Other clones in the YBs were chosen to satisfy an industrial cooperator's request for evaluation of specific clones.

Genotype identities

The pedigrees of the full-sib families and clones and the number of replications in the trials are summarized in Tables 1 and 2. Detailed parentage of individual clones are in Tables A1 – A3 in the online Supplementary Data. All families are full sib, produced through controlled pollinations. Taxa include: *Populus deltoides* (D); *Populus nigra* (N); and subsequent matings: D x D and/or D x N pedigrees; and additional full-sib families between *P. deltoides* (D) x *Populus maximowiczii* (M) to produce D x M pedigrees. No successful N x M pedigrees were created by NRRI. The focus of our breeding program has evolved to emphasize the D x N cross. Crosses involving *Populus maximowiczii* and *Populus trichocarpa* are generally maladapted to midwestern USA conditions (Nelson et al., 2018).

Table 1
Composition of Family Field Trials (FFT).

Site	Year planted	CP breeding population	Number of full-sib families	Number of clones	D x D Number of ramets (n)	D x M Number of ramets (n)	D x N Number of ramets (n)	D x T Number of ramets (n)	Total Number of ramets (n)
Woelfel	2001	1998	36	910	1652	673	76	0	2401
Sturges1	2002	1999	30	690	0	776	611	176	1563
Housman	2004	1997,1999, 2002	26	475	0	546	478	147	1171
Schultz	2007	2003,2004	25	219	0	0	564	51	615
Wanderscheid	2008	2005,2006	36	371	881	0	712	75	1668
MN Power	2015	2012	30	611	0	0	2898	0	2898
Totals over all sites			183	3310	2533	1995	5339	449	10316

CP = controlled breeding. n = observations (individual trees measured at 5 years).

Table 2
Composition of Clone Trials (CT) and Yield Blocks (YB).

Site	Year planted	<i>P. deltoides</i> / D x D	D x M	D x N	<i>P. nigra</i>	N x M	Total clones	Total ramets (n)
Hansen (CT)	2006	(21 / 23) 44	8	14	0	1	67	370
Schultz (CT)	2007	(16 / 33) 49	4	10	0	0	63	340
Captain (CT)	2009	(0 / 1) 1	4	36	1	0	42	203
Hansmeyer (CT)	2009	(0 / 1) 1	4	37	1	0	43	198
Smith (CT)	2009	(0 / 0) 0	4	38	1	0	43	229
Total unique clones in CT		94	16	62	1	1	174	1340
Hansen (YB)	2006	(5/3) 8	4	10	0	0	22	589
LEA GrRpdS (YB)	2007	(0/0) 0	2	2	0	1	5	129
Totals (YB)		8	6	12	0	1	27	718

n = observations (individual trees).

Site locations and descriptions

The study sites are described in Table 3. All sites are in Minnesota USA. The range in latitude and longitude of the sites is 46.0420 to 47.4452 and -93.4919 to -95.2378, respectively.

Table 3
Study site descriptions.

Site	Captain CT	MN Power FFT	LEA Grand Rapids*YB	Hansen CT&YB
County (MN)	Todd	Itasca	Itasca	Todd
Nearest town (MN)	Eagle Bend	Cohasset	Grand Rapids	Eagle Bend
Latitude	46.2096	47.2484	47.2435	46.1297
Longitude	-95.1225	-93.7009	-93.4919	-95.058
Slope (%)	2-6	0-10	0-6	2-6
Soil texture	sandy loam	loamy very fine	fine sandy loam	sandy loam
Soil pH	6.2	6.1	5.3	6
Soil particulate organic matter (%)	1.88	0.52	10.42	2.43
Soil bulk density (g/cm ³)	1.49	1.59	1.36	1.61
Average high/low temperature (C)	26.9/-19.8	26.8/19.3	26.8/19.3	24.2/-17.1
Minnesota Crop Productivity Index (range 0 to 100)	88	45	90	85
Average precipitation – rainfall (mm)	723	735	735	638
Site	Hansmeyer CT	Housman FFT	Schultz FFT&CT	Smith CT
County (MN)	Todd	Todd	Todd	Clearwater
Nearest town (MN)	Browerville	Miltona	Clarissa	Bagley
Latitude	46.0420	46.0549	46.1952	47.4452
Longitude	-95.0336	-95.1268	-94.8613	-95.2378
Slope%	2-6	2-6	2-6	1-3
Soil texture	sandy loam	sandy loam	sandy loam	very fine sandy loam
Soil pH	6	6.2	6	6.5
Soil particulate organic matter (%)	2.43	1.88	2.43	1.43
Soil bulk density (g/cm ³)	1.61	1.49	1.61	1.46
Average high/low temperature (C)	24.2/-17.1	24.2/-17.1	1.4972067	26.1/-20.5
Minnesota Crop Productivity Index (range 0 to 100)	85	88	85	90
Average precipitation – rainfall (mm)	638	638	769	648
Site	Sturges FFT	Wanderscheid FFT	Woelfel FFT	
County (MN)	Otter Tail	Todd	Todd	
Nearest town (MN)	Rose City	Clarissa	Bertha	
Latitude	46.1352	46.1129	46.2657	
Longitude	-95.1653	-95.0118	-95.1447	
Slope%	2-6	2-6	2-6	
Soil texture	sandy loam	sandy loam	sandy loam	
Soil pH	6.3	6.5	6.3	
Soil particulate organic matter (%)	2.2	3	2.2	
Soil bulk density (g/cm ³)	1.5	1.48	1.5	
Average high/low temperature (C)	24.2/-17.1	1.4972067	26.9/-19.8	
Minnesota Crop Productivity Index (range 0 to 100)	88	85	88	
Average precipitation – rainfall (mm)	638	769	723	

● *LEA Grand Rapids data also applies to the nursery progeny tests (NPT), which are located on the same experimental farm (an insignificant difference in latitude and longitude).

● Soil data from <https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>

● Climate data from <https://www.usclimatedata.com/> U.S. Climate Data, three-decade (1981–2010) averages (National Centers for Environmental Information, NOAA).

Crop Productivity Index from <https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm>

Figure 2
Map of study sites.

Field trial design

The NPT was unreplicated. FFTs, CTs, and YBs were randomized complete blocks. All FFTs were single-tree plots, with 3 replications (Housman, Schultz, Sturges 1, Woelfel) or 5 replications (Wanderscheid, MN Power) of from 20 to 36 families. CTs were all single-tree plots with 6 replications of up to 67 clones. The YB tests blocks each had 9 interior trees that were measured, surrounded by a two-row buffer of the same clone, with three replications (blocks) of each clone, resulting in a maximum of 27 tree measurements per clone in each YB. Using this design, tree growth is more representative of growth in large-scale commercial plantations than it is in FFTs and CTs. YB Hansen had 3 replications of 22 clones, and YB LEA Grand Rapids had 3 replications of 5 clones.

Plantation establishment and maintenance

The sites were prepared and planted in the spring, sprayed with glyphosate at 3.51 liters of 41.0 % active ingredient per hectare and tilled. Containerized plants were hand planted on pre-marked fields at pulpwood production spacings described above. The sites were immediately sprayed with the pre-emergent chemical Scepter 70DG (Imazaquin) at 292 ml of 70.0 % active ingredient per hectare. Further weed competition was effectively controlled through mechanical means during the first three growing seasons. Sites were not irrigated. Planting

year for the sites is given in Tables 1 and 2. Survival measured at 2 – 4 years ranged between 76 % and 99 % for FFTs, CTs, and YBs.

Growth measurement

For the NPTs, shoot length was measured in four different ways as described above. For the FFTs, CTs, and YBs, diameter at 1.4 m above ground (breast height), DBH, was measured on each tree. The growth variable analyzed for FFTs, CTs, and YBs was DBH², as this metric relates directly to cross-section area of the tree bole (basal area) and tree volume (Krinard, 1988). We also used cumulative basal area for each 9-tree plot in the YBs as a metric for YB growth, a metric that is affected by survival.

Pathogen assessments

The primary plant pathogen of concern for hybrid poplar culture in Minnesota and elsewhere in the eastern half of North America has been a stem- and branch-cankering disease caused by the fungal pathogen *Sphaerulina musiva* (Peck) *Quaedvlieg, Verkley & Crous*, formerly known as *Septoria musiva*. We see canker development on shoots as early as the first year in tightly spaced nursery stoolbeds, but more commonly by the third year of growth in the wider-spaced field trials and plantations.

Cankers or lesions of unknown cause were observed and scored categorically in the NPTs, CTs, and FFTs in the autumn of each year. Cankers were not scored for the YBs, but obvious canker damage was noted. Following natural leaf senescence and leaf fall, incidence of cankers or lesions on the branches and boles of each tree was rated according to a three-class scoring system: 0 = cankers absent; 1 = cankers present but rare; and 2 = cankers present with multiple areas of sunken, necrotic tissue on main boles and/or branches. All canker scoring was done by one individual, an experienced poplar geneticist and breeder, to reduce variability, as the canker scoring system involves some subjectivity.

Statistical analysis

Vectors chosen for the analysis of growth are the Coefficient of Determination (R^2) for linear regression and Spearman's Coefficient of Rank Correlation (r_s). McFadden's Pseudo R^2 was calculated for the categorical (ordinal) canker data fitted to the nominal logistic model, due to the non-normality of the canker score data. All age-age correlations were based on individual clone values. The linear regression line for tree size from earlier to later ages always has a positive slope, as trees do not get smaller with age. The logistic regression of canker score for age-age correlations would be expected to have a positive slope, as trees rarely have less cankering at older ages, except in the infrequent case of cankers that heal over. McFadden's Pseudo R^2 was only used for the age-age correlations for canker score in CTs because canker scoring only was done in CTs. Statistical analysis was performed in JMP Pro 14 and R 3.5.1. Replication was identical for each clone at the beginning of each field trial but varied from clone to clone after year 1 due to differential survival. Therefore, DBH² was averaged for each clone

within each site. The data distribution of mean DBH² was plotted and was close to a normal distribution for most sites; therefore, no data transformation was applied to the growth data. Age-age correlations were FFT: ages 3, 4, and 5; CT: ages 3, 4, 5, 9, 10; and YB: ages 3, 4, 5, 9, 10. For correlation of clones between breeding and testing process steps (NPT to FFT, FFT to CT, CT to YB), DBH² at 4 or 5 years was used for FFT, and DBH² at ages 3, 4, 5, 9, and 10 for CT and YB. The primary NPT to FFT correlations were family mean to family mean, although we did test genotype-to-clone regressions for one site (MN Power), where family mean to family mean correlations were non-significant. FFT to CT correlations and CT to YB correlations were clone to clone only. Clones were ranked by DBH² for each CT. Clonal variance and repeatability and environmental variance in FFT and CT were calculated according to equations 1 and 2 in Baltunis and Brawner (2010), based on a mixed model with replication as a fixed factor and clone as a random factor.

Correlations of DBH² and clone rank with age at different stand ages are in Tables 4, 5, and 6 for FFT, CT, and YB, respectively. The pattern of individual clone ranks at 3 and 5 years versus age 9 or 10 is illustrated in Table 7. The canker scores by site, stand age, and taxon type in the clone trials are in Figure 3. Correlations (McFadden's Pseudo R²) of canker scores between stand ages in the clone trials are in Table 8, and the pattern of canker score changes in CTs with stand age for taxon types over all sites is illustrated in Figure 3.

As shown in Table 4 for FFTs, the correlations between DBH² at ages 3 and 4 versus DBH² at age 5 were highly significant on all sites, higher for age 4 versus age 5 than for age 3 versus 5. The Spearman's rank correlations (r_s) (Table 4) were also all highly significant for ages 3 and 4 versus age 5, again higher for age 4 vs age 5 than for age 3 vs age 5.

Results

Age-age correlations

The range of overall mean DBH² (cm²) at age 5 for sites was: FFT 44.62 – 108.57 (excluding the MN Power site, which was not measured at age 5); CT 53.09 – 112.21; YB 93.29 – 117.85. Detailed DBH² data for the FFTs, CTs, and YBs for all sites, species types, and ages are in Tables A5, A6, A7 of the online Supplementary Data. There was variation in spacing between different sites (see above), so the DBH² ranges cannot be directly compared in all cases.

Figure 3

The frequency percentage of canker scores for each site, species type and age in the clone trials. 0 = cankers absent; 1 = cankers present but rare; and 2 = cankers present with multiple areas of sunken, necrotic tissue on main boles and/or branches.

Table 4

Family Field Trials - The linear regression fit R^2 and Spearman's correlation coefficients for DBH² between different years.

Age	R^2			Spearman's correlation coefficients		
	3	4	5	3	4	5
Woelfel (n=910 individual trees)						
3	1	0.874	0.692	1	0.932	0.817
4	0.874	1	0.875	0.932	1	0.930
5	0.692	0.875	1	0.817	0.930	1
Sturges1 (n=690 individual trees)						
3	1	0.763	0.577	1	0.882	0.778
4	0.763	1	0.874	0.882	1	0.933
5	0.577	0.874	1	0.778	0.933	1
Housman (n=475 individual trees)						
3	1	0.858	0.635	1	0.935	0.809
4	0.858	1	0.860	0.935	1	0.920
5	0.635	0.860	1	0.809	0.920	1
Schultz (n=219 individual trees)						
3	1	0.881	0.691	1	0.937	0.840
4	0.881	1	0.876	0.937	1	0.942
5	0.691	0.876	1	0.840	0.942	1
Wanderscheid (n=371 individual trees)						
3	1	0.751	0.663	1	0.863	0.816
4	0.751	1	0.954	0.863	1	0.972
5	0.663	0.954	1	0.816	0.972	1

Table 5 enumerates the age-age correlations in CTs, with the correlation between earlier ages and age 9 or 10 being the most important. Age 10 is the approximate lower bound of rotation age in Minnesota. All age-age correlations were significant at the $p < 0.05$ level, age 5 providing a much better correlation with age 10 for DBH² and clone rank than ages 3 and 4, i.e., half-rotation seems to provide a good estimate of growth and rank results at full rotation in the CTs. Even though the significant Spearman's Coefficients indicated no rank change with age in the clone trials, there was some variability in ranking for DBH² of individual clones with age on the different sites (Table 7). Clone rank for each genotype in the CTs at different ages are in Table A4 in the online Supplementary Data. An average of 42 % of the 10 top-ranked clones at age 9 or 10 were in the top 10 clones at age 3, 56 % at age 5. This varied by site (Table 7).

Table 5

Clone Trials - The linear regression fit R^2 and Spearman's correlation coefficients for DBH² between different years for clone trials. All coefficients are from the significant linear fits or correlations. All coefficients are significant at $p < 0.05$. Blank cells are those without pairwise data.

Age	R^2					Spearman correlation coefficients				
	3	4	5	9	10	3	4	5	9	10
Hansen (n=67 clones)										
3	1	0.830	0.695	0.260		1	0.873	0.800	0.485	
4	0.830	1	0.882	0.408		0.873	1	0.921	0.606	
5	0.695	0.882	1	0.600		0.800	0.921	1	0.728	
9	0.260	0.408	0.600	1		0.485	0.606	0.728	1	
10										
Schultz n=63 clones)										
3	1	0.832	0.711		0.316	1	0.905	0.843		0.577
4	0.832	1	0.898		0.491	0.905	1	0.958		0.728
5	0.711	0.898	1		0.720	0.843	0.958	1		0.838
9										
10	0.316	0.491	0.720		1	0.577	0.728	0.838		1
Captain (n=42 clones)										
3	1	0.840	0.673		0.266	1	0.900	0.795		0.479
4	0.840	1	0.913		0.559	0.900	1	0.940		0.695
5	0.673	0.913	1		0.708	0.795	0.940	1		0.828
9										
10	0.266	0.559	0.708		1	0.479	0.695	0.828		1
Hansmeyer (n=43 clones)										
3	1	0.937	0.888		0.364	1	0.959	0.946		0.611
4	0.937	1	0.953		0.432	0.959	1	0.981		0.675
5	0.888	0.953	1		0.601	0.946	0.981	1		0.742
9										
10	0.364	0.432	0.601		1	0.611	0.675	0.742		1
Smith (n=43 clones)										
3	1	0.676	0.567		0.193	1	0.730	0.603		0.361
4	0.676	1	0.911		0.269	0.730	1	0.936		0.430
5	0.567	0.911	1		0.390	0.603	0.936	1		0.561
9										
10	0.193	0.269	0.390		1	0.361	0.430	0.561		1

Yield Block age-age correlation results (Table 6) were different and more variable than for FFTs and CTs. For absolute DBH² and DBH² rank (r_s), ages 3, 4, and 5 were generally poorly correlated with absolute DBH² at age 9 or 10. We also analyzed age-age correlations in the Hansen YB using clone average cumulative basal area, which gave the same pattern as the DBH² metric, with slightly lower R^2 and Spearman's Coefficients (data not shown).

Table 6

Yield Blocks - The linear regression fit R^2 and Spearman's correlation coefficients for clone mean DBH² between different years. All coefficients are from the significant ($p < 0.05$) linear fits or correlations. Insignificant fits are labelled *ns*. Blank cells are those without pairwise data.

Age	R^2					Spearman correlation coefficients				
	3	4	5	9	10	3	4	5	9	10
Hansen (n=22 clones)										
3	1	0.889	0.584	ns		1	0.939	0.785	ns	
4	0.889	1	0.816	ns		0.939	1	0.905	ns	
5	0.584	0.816	1	0.218		0.785	0.905	1	0.481	
9	ns	ns	0.218			ns	ns	0.481	1	
10										
LEA Grand Rapids (n=5 clones)										
3										
4		1	0.990		ns		1	1		ns
5		0.990	1		ns		1	1		ns
9										
10		ns	ns		1		ns	ns		1

Table 7

Overlap in ranking for DBH² of individual clones at different ages in Clone Trials. All sites except Hansen are versus age 10; Hansen is versus age 9. Top ten clones equal 81st percentile for all sites combined.

Site	% of top-ranked 10 clones in year 9 or 10 at year 3	% of top-ranked 10 clones in year 9 or 10 at year 5
Hansen	20 %	40 %
Schultz	40 %	80 %
Captain	60 %	60 %
Hansmeyer	50 %	50 %
Smith	40 %	50 %
Average for 5 sites	42 %	56 %

Age-age correlations for canker score in CTs (Table 8) were significant at the $p < 0.5$ level for all age pairings from age 3 to age 7, but the McFadden's pseudo R^2 values were all low, i.e., cankering at older ages was not well predicted by canker scores earlier in the rotation. The square root of all McFadden's pseudo R^2 values were positive as expected, i.e., cankering proceeds from lighter to heavier with age. The canker score pattern varied with taxon (species type) and site in the CTs (Fig. 3). See also Figure A1 in online Supplementary Data. The D x M and N x M crosses were ultimately most cankered; N, D, and D x D the least; and D x N intermediate.

Table 8

Clone Trials - The McFadden's pseudo R^2 from the nominal logistic model fit for Canker Scores between different years. All values are from significant ($p < 0.05$) fits. Blank cells are those without pairwise data.

Age	3Yr	4Yr	5Yr	6Yr	7Yr
Hansen (n=370 individual trees)					
3Yr					0.061
7Yr	0.061				1
Schultz (n=340 individual trees)					
4Yr		1	0.179	0.097	0.044
5Yr		0.179	1	0.146	0.175
6Yr		0.097	0.146	1	0.184
7Yr		0.044	0.175	0.184	1
Captain (n=203 individual trees)					
3Yr	1	0.280	0.231		
4Yr	0.280	1	0.284		
5Yr	0.231	0.284	1		
Hansmeyer (n=198 individual trees)					
3Yr	1	0.116	0.074		
4Yr	0.116	1	0.089		
5Yr	0.074	0.089	1		

Correlations between breeding and testing process steps

Correlations between NPT growth measures and FFT family mean growth and rank are in Table 9. All are family mean to family mean correlations, as the focus of the NPT is to select families for the FFT. All correlations for the MN Power site were non-significant. All NPT growth measures were significantly

correlated with family mean DBH² and rank at 4 and 5 years stand age in the Woelfel FFT. The highest correlation at the Woelfel site, (Spearman's coefficient = r_s , NPT Year 1 Seedling Ht. vs FFT family rank) was 0.589, a moderate correlation explaining 34 % of the variation in family mean rank. The highest correlation between NPT growth measures and FFT DBH² (Woelfel, R^2 , NPT Year 1 Seedling Ht. vs FFT DBH²) explained

32.5 % of total variation in family mean DBH². No other R^2 or r_s for either site came close to this correlation (Table 9). The non-significant fits for the MN Power site may be partially due to the smaller population size at that site than at Woelfel ($N = 62$ vs $N = 30$). Using the sum of the NPT growth measures as a predictor did not improve the correlations over using the single growth measure that returned the highest correlation (Table 9). When raw (genotype to clone) data, instead of family mean to family mean, were used for the regressions for NPT growth measures vs the MN Power site growth data, all regressions were significant at the $p < 0.05$ level (data not shown). This indicates that the NPT growth measures are effective selection criteria for clone growth in the FFTs but are not as effective for selecting families for the FFTs at the population sizes in this study. As explained under Methods, the main purpose of NPT growth measures is to select the best families to proceed into FFTs.

Table 9

The linear regression fit R^2 and Spearman's correlation coefficients between NPT growth measures and FFT Dbh², family mean to family mean. *NPT sum* is the sum of shoot lengths (height growth) for all four NPT growth measures. All fits for the MN power site (*italics*) are statistically non-significant, but all fits for the Woelfel site (**bold**) are statistically significant ($p < 0.5$) but relatively weak, explaining less than 35 % of the variation. Blank cells are those without pairwise data.

NPT	MN Power site, vs yr4Dbh ² (n=30 observations)		Woelfel site, vs Yr5Dbh ² (n=62 observations)	
	R^2	Spearman's correlation coef	R^2	Spearman's correlation coef
NPT Year 1 seedling ht.	<i>0.002</i>	<i>0.066</i>	0.325	0.589
NPT Year 2 seedling ht.			0.096	0.320
NPT Coppice 1	<i>0.007</i>	<i>0.049</i>	0.144	0.361
NPT Coppice 2	<i>0.042</i>	<i>0.126</i>		
NPT sum	<i>0.022</i>	<i>0.091</i>	0.153	0.411

Scatterplots of FFT DBH² versus NPT shoot length growth measures (Figs. A2, A3 in online Supplementary Data) indicated a positive slope for the regression line for significant R^2 ; viz, faster growth in the NPT tends to indicate faster growth in the FFTs. Scatterplot matrices between NPT growth measures (Figs. A2, A3 in online Supplementary Data) showed a positive relationship, e.g., seedling heights were positively and closely related to coppice shoot lengths in the NPTs. The correlation vectors for growth and rank were positive as expected.

Regressing clone values in the CTs on clone values in the FFTs at ages 4 and 5 years for the 6 FFTs in Table 1 and the 5 CTs in Table 2 for growth rate (DBH²) (actual growth rate = R^2 , clone rank for growth rate = r_s) were made to test whether clone performance in FFTs could be used to predict clone performance in CTs. Almost all R^2 and r_s were non significant at the $p < 0.05$ level (data not shown). Out of 36 such linear regressions for actual growth, only 3 had a significant R^2 , the largest explaining 30 % of the variation in CT clone means. Only two r_s out of 36 for rank correlations were significant ($r_s = 0.543$,

0.829). Genetic variances and clonal repeatabilities for FFTs and CTs are in Table 10.

Table 10

Genetic variance among clones and clonal repeatabilities in FFT and CT. All repeatabilities are significant at the $p < 0.0001$ level, computed from standard errors of repeatability (data not shown).

Test Type	Site	Number of observations	Number of clones	Genetic variance among clones	Residual variance	Repeatability
CTs	Captain	203	42	531.27	551.85	0.490
	Hansen	370	67	464.41	720.29	0.392
	Hansmeyer	198	43	558.85	673.84	0.453
	Schultz	340	63	583.90	1131.25	0.340
	Smith	229	43	78.25	351.89	0.182
Ave per CT site				443.34	685.82	0.371
FFTs	Housman	1144	475	211.62	728.68	0.225
	Schultz	615	219	1442.28	1012.02	0.588
	Sturges1	1433	690	504.40	441.98	0.533
	Wanderscheid	1666	371	591.00	592.61	0.499
	Woelfel	2371	910	469.76	318.49	0.596
Ave per FFT site				643.81	618.76	0.488

Regressions for clone means in CTs versus YBs for the Hansen CT and YBs are in Table 11. R^2 and Spearman's coefficient were significant at the $p < 0.05$ for age 3, 4, and 5 comparisons. However, the correlations between age 5 in the CTs and age 9 in the YB were non-significant. DBH² and rank correlations for CT age 9 versus YB age 9 were significant.

Table 11

The linear regression fit R^2 and Spearman's correlation coefficients for clone average DBH² between Clone Trials and the Hansen Yield Block at different years for Hansen (n=22 clones, 3 blocks per clone). Coefficients in italics are non-significant ($p > 0.05$).

Age		Clone Trial (Hansen)							
		R^2				Spearman's correlation coefficients (r_s)			
		3	4	5	9	3	4	5	9
Yield Block (Hansen)	3	0.68	0.61	0.34	<i>0.03</i>	0.75	0.74	0.55	<i>-0.14</i>
	4	0.47	0.53	0.34	<i>0.01</i>	0.64	0.68	0.59	<i>-0.08</i>
	5	0.23	0.34	0.26	<i>0.01</i>	0.47	0.56	0.57	<i>0.15</i>
	9	<i>0.10</i>	<i>0.03</i>	<i>0.01</i>	0.29	<i>-0.23</i>	<i>-0.14</i>	<i>0.14</i>	0.46

Clone growth performance in the Hansen CT at ages 3, 4, and 5 predicted the performance of the same clone in the Hansen YB at the same age, but not growth at age 9 in that YB. However, both R^2 and r_s were significant for growth and rank correlations for CTs at age 9 versus Hansen YB at age 9. Coefficients of variation for DBH² were similar for CTs and the YB at equal ages and decreased from age 3 to age 9 (data not shown), indicating that the selection of the best performing clones in the CTs for inclusion in the Hansen YB did not reduce growth variation in the YB and was not a factor in the correlation patterns. We also analyzed correlations between clone average DBH² in CTs versus clone average cumulative basal area (cm²) in YBs, which gave the same pattern as DBH² metrics, with slightly lower R^2 and Spearman's Coefficients (data not shown).

In addition, we calculated the average genetic gain at age 9 (full rotation) in the Hansen YB (cumulative basal area of 9-tree plots, cm²) by comparing clone performance for the top 5 clones (77th percentile) (DBH²) selected in the Hansen CT at age 5 and 9, using only the 22 clones that appeared in both YB and CT. We had to base genetic gain on the population mean of the Hansen YB, as no commercial check clones were included in the YB. This calculation gave a genetic gain of 5 % (1 % per year) for selection at 5 years in the CT, and 11.3 % genetic gain (1.3 % per year) for selection at 9 years in the CT. Genetic gains in CTs versus commercial check clones in other of our populations are 27 % – 83 % higher than those based on population means (Nelson et al., 2018; Nelson, Meilan et al., 2019; Nelson, Berguson et al., 2019), depending on site and

population composition. Thus, the genetic gain comparison here is only relative and is not an estimate of meaningful genetic improvement against current commercial practice. Furthermore, these gain estimates are for only one YB and appear to be much lower than expected from previous genetic gain estimates based on many other CTs.

Discussion

As expected, age-age correlations for growth and rank are similar across the FFTs, CTs, and YBs (Table 4, 5, 6; r_s must be squared to compare directly with R^2). Rank is the most important selection criteria in the breeding process and ultimately should translate to growth and yield.

The age-age correlations for DBH², actual and clone rank, had a similar pattern within the five FFTs—age 4 was a better predictor than age 3 of age 5 results, and the values for each age-age correlation were similar between sites (Table 4). The clone rank results are the most critical, and the results in Table 4 indicate that we can effectively select clones from the FFTs for further testing in the CTs based on growth rank at age 4 in the FFTs (r_s of 0.920 – 0.972 for age 4 ranks versus age 5 ranks) instead of waiting to age 5, saving one year in the testing process.

The age-age correlations for DBH² in the CTs, actual and clone rank, had a similar pattern on all five sites; viz., correlations with age 9 or 10 results increased from age 3 to age 4 to age 5 (Table 5). Absolute values R^2 and r_s for these same age-age correlations were much lower on one site, Smith (Table 5). Two of the five sites, Schultz and Captain, had r_s values exceeding 0.82 for the age 5 to age 10 correlation—the other three sites r_s ranged from 0.56 to 0.74 for the age 5 to age 9 or 10 correlation. The age 5 to age 10 rank correlation explained an average of 55 % of the variation (r_s^2), indicating that age 5 (half-rotation) is a moderately good age at which to select clones within the CTs for additional testing in the YBs. An average of 56 % (range 40 % to 80 % for the five sites) of the clones ranked in the top ten ranking at age 10 in the CTs were in the top ten ranked clones at age 5. This result for individual clones is better for clone selectivity than the report by Ghezehei et al. (2020), wherein only 3 top-ten clones out of the 66 clones tested for biomass at age 4 were still in the top ten at age 8. It is highly likely that climate, site, and genetic composition can produce different age performance patterns in field trials, again illustrating the need for early selection studies under specific growing conditions, disease prevalence, and genetic composition.

These CT results in totality are consistent with those in most of the other age-age studies on *Populus* cited in the Introduction which indicate that clones can be selected at approximately one-half rotation. By selecting at age 5, we miss about 44 % of the clones that would have been selected in the 81st percentile (top 10 clones) if selected at age 9 or 10 (Table 7); however, saving four or five years of testing with this procedure may be worth the possible reduction in genetic gain from the clone mis-selection at age 5. Selection at age 5 in CTs is our

standard operating procedure in the current testing process in Minnesota, so these results confirm our methods in a broad sense but do not allow us to reduce the age at which we select the best clones. However, growth performance of clones selected at age 5 in the Hansen CT are not correlated with growth of the same clones at age 9 in the YB, whereas age 9 selection in the Hansen CT identifies clones that perform well at age 9 in the Hansen YB (Table 11), although the significant R^2 and r_s^2 only explain 29 % and 21 % of the variation for this correlation. This suggests selecting clones in CTs at age 9 or 10 (near-full rotation in Minnesota) will identify clones that will have superior growth performance at full rotation in the YBs, albeit with significant but low correlation. The Hansen CT and YB were on the same site in close proximity, suggesting that genotype x environment interactions could reduce correlations between CT and YB even further when the CT and YB were on different sites. The effectiveness of full-rotation selection in CT indicates one aggressive strategy would be to choose superior clones at age 9 or 10 in the CTs and eliminate YB tests as a prerequisite for clone selection for commercial deployment. YB tests will still be necessary for precise estimates of biomass yield where required by a grower. This scenario would add 4 or 5 years onto the CT length (9 or 10 years instead of 5 years) but save 9 years by eliminating YB tests, for a net savings of at least 4 years. However, eliminating the YB step for clone selection would transfer some identification of clones susceptible to cankering during the nine years of YB to the commercial deployment stage. Another codicil is that the correlations of age 9 CT and age 9 YB growth and rank are based on data from only one site (Hansen).

Yield Blocks (Table 6) manifested only poor age-age correlations for age 5 versus age 10, with an r_s of 0.481 for the Hansen site and non-significant r_s for the LEA Grand Rapids site. This indicates that we must continue YBs to age 9 or 10 (full rotation) to obtain a reliable estimate of clone performance, as found by Stanton (2001). Similarly, Niemczyk and Thomas (2020) found that in northern climates of North America, 8 years is a reliable age at which to select fast-growing and disease-resistant poplar clones for commercial deployment. We already adopt this procedure in YBs to derive accurate biomass yield estimates for our clones. The low age-age correlations in YBs may reflect the possibility that clones perform differently from their growth in CTs when tested in the mono-clonal blocks of YBs, due to the more uniform competition in YBs, although the significant correlation between growth in the Hansen CT and YB at age 9 (Table 11) argues against this explanation. Another factor is that in the Hansen YB cankering developed between ages 5 and 9. By age 9 in the Hansen YB, 6 out of 22 total clones, and 3 out of 10 DN clones, had significant or heavy cankering (data not shown), which could have affected growth and reduced the age-age correlations. The poor age-age correlations in the YBs are an example of the phenomenon described by Ghezehei et al. (2020), wherein some clones become less suitable, and others more suitable, with stand age. The development of significant cankering in the Hansen YB in 30 percent of the DN clones that had not shown cankering in the FFTs and CTs preceding the YB also

demonstrate the value of testing clones for nearly full rotation length in the YBs to eliminate disease problems that were not manifest in earlier tests.

Canker incidence and severity in the CTs varied by taxon type and age and were generally much more variable by age than growth (DBH²) (Table 8 and Fig. 3). Severe cankering (Canker Score 2) was almost non-existent in D and D x D genotypes, prevalent in D x M, and intermediate (but still low) in D x N (Fig. 2). These taxon cankering results were as expected, from our experience. Age-age correlations for canker score were low for all sites. The patterns of canker score with age did not consistently follow the expected pattern of increasing incidence and severity with age. In some instances, canker score estimates were higher at earlier than later ages (Fig. 3). This variability is undoubtedly a function of the subjectivity of the visual ordinal canker scoring system, including the possibility of not seeing cankers on some trees during some scoring sessions, as well as some cankers healing over by a later age. Therefore, apart from eliminating clones with heavy cankering at any age, our results indicate that we cannot judge a reliable age for predicting cankers before full rotation age. Our aim is the elimination of clones that show severe cankering at any age in the breeding and testing process, whether in NPTs, FFTs, CTs, or YBs. We can effectively identify clones prone to cankering by just scoring cankers at the endpoint of the CT process, whether this is half rotation (5 years) or full rotation (9 or 10 years), instead of our current practice, which has been to score cankering after each growing season.

For achieving disease resistance in poplars, a holistic plan is needed that tracks clones over time in regions where they are deployed. Disease resistance of poplars can change with geographic regions and climate and with evolution of the disease organism through mutations. Disease evolution can be expected to be faster with greater selection pressure on the disease organism, such as when large areas of poplar plantations are planted in a relatively small region. Our field testing for disease in poplars has some similarity to clinical testing of new pharmaceuticals. Not all side effects or efficacy failures are discovered in the clinical tests (field tests analogy)—some are not identified until the approved drug (poplar genotype analogy) is in use by the patient population over years or decades (commercial deployment of poplar genotype analogy). Fortunately for our focus on the D x N hybrids, there are some examples of durable *Sphaerulina musiva* (*Septoria*) canker resistance holding up over decades in some existing D x N hybrids, including *Populus x euramericana* (DN34, *Eugenei*), *Raverdeau*, *Robusta*, *I-45/51*, and *Wisconsin 5* (unpublished long term field observations), which shows that resilient canker resistance is possible with other D x N hybrids. After many years, even some durable clones are likely to be susceptible to fungal diseases due to the evolution of the pathogen, making genetic diversity, deployment strategies, and periodic introduction of new cultivars essential (McDonald, 2014). This fact is one of the strongest justifications for continuing poplar breeding programs. Recent papers on *Sphaerulina musiva* in hybrid poplars provide background on canker resistance research (Dunnell et

al., 2016; Niemczyk and Thomas, 2020; Tabima et al., 2020), including the importance of deploying resistant clones.

The overall correlation patterns for growth and rank for the unreplicated NPTs versus FFTs indicate that we can continue to measure coppice shoot lengths (Coppice 1 and/or Coppice 2) in the NPTs, because we need those measurements to choose families that have enough cuttings for the FFTs. There seems to be no advantage in using the other NPT growth measures for family selection. The fact that clone-to-clone regressions for all NPT growth measures versus FFT growth were significant, and that the NPT growth measures were correlated, is further evidence for simplifying the NPT measurement protocol by solely measuring coppice growth. This study is, to our knowledge, the first to correlate growth rate in NPTs with growth of families and clones in ensuing FFTs for hybrid poplars.

The almost complete lack of correlation of clone performance in FFTs versus CTs means that there is no evidence that clone means in the FFTs can be used to estimate clone means in the CTs with the clone replication levels that we commonly use in our FFTs. The data indicate that we should retain CTs in our breeding and testing process. Additive genetic variation is captured in the FFT, making family selection an essential step (Berguson et al., 2017). Indeed, multiple clones from single full sib families commonly are represented in the fastest growing 80th – 90th percentile ranks of clones in our CTs (Nelson et al., 2018; Nelson, Berguson et al., 2019; Nelson, Meilan et al., 2019).

This lack of correlation of clone performance in FFTs versus CTs may be due to the clone replication levels (3–5 replicates per clone) in the FFTs. It may also be true that selection in the FFTs is effective, but the effectiveness is not revealed in the correlation analyses because only the selected portion of the FFTs is carried forward to the CTs. The unselected portion of the FFTs may have underperformed in the CTs had they been tested there. If selection in the FFTs was indeed effective, then this should be reflected in lower clone variances and clonal repeatabilities in the CTs than in the FFTs, assuming comparable environmental variance. Table 10 indicates that this is the case, i.e., similar residual (environmental) variance in the FFTs and CTs and lower genetic variance and clonal repeatability in the CTs than in the FTs.

The lack of correlation for growth and clone rank between half-rotation in the Hansen CT and near-full rotation in the Hansen YB (age 9), but significant correlation at near-full rotation in both CT and YB (Table 11), suggests some value in using near-full rotation, rather than half rotation, for clone selection in the CTs. This would add 4 or 5 more years to the length of the CTs compared to our current procedure, but it may be worth the extra genetic gain that could ensue from better selection in both the CTs and YBs. As explained above, by selecting clones at age 5 in the CTs we miss 44 percent of the clones that are in the top ten (81st percentile) for growth at full rotation in the CTs. The decision of whether to select clones at age 5 or age 9 or 10 in the CTs should be an economic determination based on added genetic gain at the end of the pipeline (full rotation in the YB) from selection at near-full instead of half-rotation in the CTs versus the cost of adding four or five more years onto

the CT segment of testing, an analysis that is beyond the scope of the current study. The most cautious approach would be to select clones at near-full rotation in the CTs, which would have the added advantage of providing four or five extra years to identify clones with canker susceptibility and remove such clones from further consideration for deployment. As explained above, one strategy would be to select superior clones for growth in CTs at near-full rotation (age 9), but eliminate YBs for clone selection, employing YBs only for estimating biomass yields. However, commercial deployment will likely only use a maximum of 10 clones at any given time, and maybe one can afford to lose some high performers if the scale (number of clones tested) of the breeding and testing program is sufficiently large (e.g., >100 clones) or there are only small differences between the growth rate of clones in the top 75th percentile.

Summarizing the data, the most efficient and aggressive strategy for clone selection with acceptable accuracy and precision is as follows: 1) Measure only coppice shoot lengths in NPTs, eliminating measurement of seedling heights at ages 1 and 2; 2) Select superior clones and families at age 4 in the FFTs, instead of at age 5, reducing FFTs by 1 year; 3) Select clones superior in growth rate in the CTs at age 9, instead of age 5, adding 4 years to the CT tests; 4) Eliminate YBs, except for providing estimates of biomass yield where needed, saving 9 years. The net savings in time with this strategy for FFTs, CTs, and YBs are $(-1 + 4 - 9) = 6$ years saved, for a total testing time for clone selection of 13 years instead of 19 years, excluding the 3 or 4 years required for NPT and cutting production in stoolbeds; 5) Score canker presence and severity in the CTs only at the end point of the CT (half or full rotation), instead of after every growing season.

Although this study is highly valuable for our breeding program, the results are specific to the climate, soils, and genetic populations of the experiments. As such, the greatest values for applications to other breeding programs are the analytic approach to determine the most efficient clone testing schemes and the considerations that need to be included in such evaluations.

Conclusions

Our goal was to use correlation vectors to better define efficiency in the genotype testing process for our hybrid poplar program and other poplar programs that use a sequential procedure to derive superior clones. The results indicate that in FFTs we can select the fastest-growing families and clones within those families at 4 years instead of the current 5 years. In CTs, the age 5 to age 10 rank correlation explained an average of 74 % of the variation, indicating that age 5 (half rotation) is a moderately good age at which to select clones within the CTs for additional testing in the YBs. However, selecting at age 5 in the CTs missed 44 % of the clones that were in the top ten clones (81st percentile) at age 9 or 10. In addition, clone growth and rank at age 5 in the CTs was not correlated with those

growth parameters in the YBs at near-full rotation (age 9), but growth and rank at age 9 in the CTs was correlated with growth and rank at age 9 in the YB. These patterns indicate that a more accurate selection of clones will be obtained by selecting clones at age 9 in the CTs, instead of the current 5 years. Whether this extra time is worthwhile can be determined by a cost-benefit analysis beyond the scope of the current study. The genetic gain per year at full rotation for selection at 9 years in a CT was one third higher than selection at 5 years in the same CT, which gives a relative idea of the tradeoffs for the early and late selection options. Nursery Progeny Tests can be simplified to only measuring the shoot lengths of coppice in stoolbeds, eliminating 2 – 3 other measurements that we currently make. The clone replication that we currently use in FFTs is not sufficient for us to select superior clones in FFTs without the CTs that follow FFTs in our current process, i.e., we cannot eliminate CTs. Except for eliminating clones with heavy cankering at any age and differentiating canker prevalence and severity for different taxon groups (D, D x D, D x M, D x N, N, N x M), from the data presented here we cannot judge a reliable age for predicting cankers at full rotation, probably due to the accuracy and precision of the canker scoring method. The correct testing perspective to increase disease resistance is to eliminate clones that show severe cankering at any age in the total breeding protocol, whether in NPTs, FFTs, CTs, YBs, or after commercial deployment.

Acknowledgements

At the University of Minnesota's NRRI in Duluth, development and testing of NRRI clones was funded by State of Minnesota appropriations to the Minnesota Hybrid Poplar Research Cooperative (MHPRC), State Special appropriations to the University of Minnesota Duluth Natural Resources Research Institute (NRRI), Minnesota Agricultural Utilization Research Institute (AURI) and U.S. DOE BETO Sun Grant Initiative Poplar Woody Crops Program (contract # DEFC36-05GO85041). In addition, the following companies contributed through their membership in the MHPRC: Verso Corporation, Champion International, International Paper, Boise Cascade, Potlatch Corporation (now PotlatchDeltic), UPM-Blandin and Minnesota Power (MN Power). Verso Corporation generously allowed us to embed most of the field plots in this study within their commercial hybrid poplar plantations and provided site preparation and maintenance. Analysis and writing were funded by the USDA-NIFA Agriculture and Food Research Initiative Competitive Grants Program Sustainable Bioenergy and Bioproducts Challenge Area (grant no. 2018-68005-27635/project accession no. 1015244). Some clones in the tests were provided by the U.S. Forest Service Rhinelander Forestry Sciences Laboratory (Dr. Don Riemenschneider). Parents from outside the NRRI program used in producing inter-specific hybrids at NRRI were provided by the University of Minnesota (the late Dr. Carl Mohn's program), University of Toronto (the late Dr. Louis Zsuffa), and Iowa State University (the late Dr. Rick Hall). Anda

Bellamy is gratefully acknowledged for editing the paper for journal submission. An anonymous reviewer is acknowledged for materially improving the manuscript.

Appendix A. Supplementary Electronic Materials

Supplementary data to this article can be found online.

References

- Ares A (2002) Changes through time in traits of poplar clones in selection trials. *New Forests* 23:105–119. <https://doi.org/10.1023/A:1015687708505>
- Bacenetti J, Bergante S, Facciotto G, Fiala M (2016) Woody biofuel production from short rotation coppice in Italy: Environmental-impact assessment of different species and crop management. *Biomass & Bioenergy* 94:209–219. <http://dx.doi.org/10.1016/j.biombioe.2016.09.002>
- Baltunis B, Brawner JT (2010) Clonal stability in *Pinus radiata* across New Zealand and Australia. I. Growth and form traits. *New Forests* 40: 305–322. <https://doi.org/10.1007/s11056-010-9201-4>
- Berguson W, Eaton J, Stanton B (2010) Development of hybrid poplar for commercial production in the United States: The Pacific Northwest and Minnesota experience. In: Braun R, D Karlen and D Johnson (eds) Sustainable Feedstocks for Advanced Biofuels, Sustainable Alternative Fuel Feedstock Opportunities, Challenges and Roadmaps for Six U.S. Regions. Proceedings, Sustainable Feedstock for Advance Biofuels Workshop. Ankeny, IA: Soil and Water Conservation Society, pp 282–299.
- Berguson WE, McMahon BG, Riemenschneider DE (2017) Additive and non-additive genetic variances for tree growth in several hybrid poplar populations and implications regarding breeding strategy. *Silvae Genetica* 66:33–39. <https://doi.org/10.1515/sg-2017-0005>
- Bisoffi S (1993) Age-age correlations for the evaluation of forest reproductive material. In: Terrasson D (ed) Proceedings, Quality of Forest Reproductive Material in the Field of Application of European Community Rules, European Commission, Paris, 9–10 December, 1993. CEMAGREF Editions, pp 75–91.
- Bisoffi S, Gullberg U (1996) Poplar breeding and selection strategies. In: Stettler RF, HD Bradshaw Jr, PE Heilman and TM Hinckley (eds) Biology of *Populus* and its Implications for Management and Conservation – Part I. Ottawa, ON: NRC Research Press, National Research Council of Canada, pp 139–158.
- Brown KR, Beall FD, Hogan GD (1996) Establishment-year height growth in hybrid poplars; relations with longer-term survival. *New Forests* 12(2):175–186.
- Chiba S, Nagata Y (1972) Rust resistance and growth of *Populus maximowiczii* clones selected from the progenies of intraspecific hybridization. Tokyo, Japan: Proceedings, IUFRO Genetics – SABRAO Joint Symposia, C-6(V), pp 1–7.
- Dunnell KL, Berguson B, McMahon B, LeBoldus JM (2016) Variation in resistance of *Populus nigra* to *Sphaerulina musiva* in the North-Central United States. *Plant Disease* 100:287–291. <https://doi.org/10.1094/pdis-05-15-0516-re>
- Foster GS (1986) Provenance variation of eastern cottonwood in the lower Mississippi Valley. *Silvae Genetica* 35(1):32–38.
- Ghezehei SB, Nichols EG, Maier CA, Hazel DW (2019) Adaptability of *Populus* to physiography and growing conditions in the Southeastern USA. *Forests* 10:118. <https://doi.org/10.3390/f10020118>
- Ghezehei SB, Wright J, Zalesny RS, Guthrie Nichols E, Hazel DW (2020) Matching site-suitable poplars to rotation length for optimized productivity. *Forest Ecology and Management* 457:117670. <https://doi.org/10.1016/j.foreco.2019.117670>
- Gwaze DP, Bridgewater FE, Byram TD, Woolliams JA, Williams CG (2000) Predicting age-age genetic correlations in tree-breeding programs; a case study of *Pinus taeda* L. Theoretical Applications of Genetics 100:199–206. <https://doi.org/10.1007/s001220050027>
- Kaczmarek DJ, Coyle DR, Coleman MD (2013) Survival and growth of a range of *Populus* clones in central South Carolina USA through age ten: Do early assessments reflect longer-term survival and growth trends? *Biomass & Bioenergy* 49:260–272. <https://doi.org/10.1016/j.biombioe.2012.12.005>
- Krinard RM (1988) Volume equations for plantation cottonwood trees (*Populus deltoides*). USDA Forest Service, Southern Forest Experiment Station, SO-347, 4 p. <https://doi.org/10.2737/SO-RN-347>
- Kumar D, Singh NB (2001) Age-age correlations for early selection of clones of *Populus* in India. *Silvae Genetica* 50(3):103–108.
- Lazarus W, Headley WL, Zalesny RS (2015) Impacts of supply-level differences in productivity and land costs on the economics of hybrid poplar production in Minnesota, USA. *Bioenergy Research* 8(1):231–248. <https://doi.org/10.1007/s12155-014-9520-y>
- Libby WJ (1987) Testing for clonal forestry. *Annales Forestales* 13(1–2):69–75.
- McDonald BA (2014) Using dynamic diversity to achieve durable disease resistance in agricultural ecosystems. *Tropical Plant Pathology* 39(3):191–196. <https://doi.org/10.1590/S1982-56762014000300001>
- Mohn CA, Randall WK (1971) Inheritance and correlation of growth characters in *Populus deltoides*. *Silvae Genetica* 20:182–184.
- Mohrdiek O (1979) Juvenile-mature and trait correlations in some aspen and poplar trials. *Silvae Genetica* 28:107–111.
- Nelson ND, Berguson WE, McMahon BG, Cai M, Buchman DJ (2018) Growth performance and stability of hybrid poplar clones in simultaneous tests on six sites. *Biomass & Bioenergy* 118:115–125. <https://doi.org/10.1016/j.biombioe.2018.08.007>
- Nelson ND, Berguson WE, McMahon BG, Meilan R, Smart LB, Gouker FE, Bloese P, Miller R, Volk TA, Cai M, Buchman D (2019) Discovery of geographically robust hybrid poplar clones. *Silvae Genetica* 68(1):101–110. <https://doi.org/10.2478/sg-2019-0018>
- Nelson ND, Meilan R, Berguson WE, McMahon BG, Cai M, Buchman D (2019) Growth performance of hybrid poplar clones on two agricultural sites with and without early irrigation and fertilization. *Silvae Genetica* 68(1):58–66. <https://doi.org/10.2478/sg-2019-0011>
- Niemczyk M, Thomas BR (2020) Growth parameters and resistance to *Sphaerulina musiva*-induced canker are more important than wood density for increasing genetic gain from selection of *Populus* spp. Hybrids for northern climates. *Annals of Forest Science* 77:26. <https://doi.org/10.1007/s13595-020-0931-y>
- Randall WK, Cooper DT (1973) Predicted genotypic gain from cottonwood clonal tests. *Silvae Genetica* 22:165–167.
- Stanton BJ (2001) Clonal variation in basal area growth patterns during stand development in hybrid poplar. *Can. J. For. Res.* 31 (12):2059–2066. <https://doi.org/10.1139/x01-150>
- Tabima JF, Sondrelli KL, Kerio S, Feau N, Sakalidis ML, Hamelin RC, LeBoldus JM (2020) Population genomic analyses reveal connectivity via human-mediated transport across *Populus* plantations in North America and an undescribed subpopulation of *Sphaerulina musiva*. *MPMI* 33(2):189–199. <https://doi.org/10.1094/MPMI-05-19-0131-R>
- Tsarev AP (1977) Minimum age for evaluating growth rate of *Populus* in variety trials. *Lesovendienie* 3:67–71.
- US Department of Energy (US DOE) (2016) 2016 Billion-Ton Report: Advancing Domestic Resources for a Thriving Bioeconomy. In: Langholtz MH, BJ Stokes and LM Eaton (Leads) Volume 1: Economic Availability of Feedstocks. Oak Ridge, TN: Oak Ridge National Laboratory, ORNL/TM-2016/160. <http://doi.org/10.2172/1271651>
- White CC, Adams WT, Neale DB (2007) Forest Genetics. Wallingford Oxfordshire, United Kingdom: CAB International. <https://doi.org/10.1079/9781845932855.0000>
- Wilkinson RC (1973) Inheritance and correlation of growth characters in hybrid poplar clones. In: Proceedings, Northeastern Forest Tree Improvement Conference. Durham, NH: University of New Hampshire, pp 121–130.
- Wu HX (1999) Study of early selection in tree breeding. 2. Advantage of early selection through shortening the breeding cycle. *Silvae Genetica* 48(2):78–83.
- Yaneza, MA, Zamudio F, Espinoza S, Ivkovic M, Guerra F, Espinoza C, Baettig RM (2019) Genetic variation and growth stability of hybrid poplars in high-density short-rotation coppice stands in central Chile. *Biomass and Bioenergy* 120:84–90. <https://doi.org/10.1016/j.biombioe.2018.11.011>
- Ying CC, Bagley WT (1976) Genetic variation in eastern cottonwood in an eastern Nebraska provenance study. *Silvae Genetica* 25:67–73.

Zhang P, Wu F, Kang X (2013) Genetic control of fiber properties and growth in triploid hybrid clones of *Populus tomentosa*. Scandinavian Journal of Forest Research 28(7):621–630. <http://dx.doi.org/10.1080/02827581.2013.829868>