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Tradeoffs between yield, disease incidence and conversion efficiency for selection of hybrid poplar genotypes as bioenergy feedstocks

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ABSTRACT

Lignocellulosic biomass is an alternative source of energy that can reduce our dependency on fossil fuels and limit greenhouse gas emissions. Several techno-economic analyses have consistently shown that all the steps in biomass-to-bioproduct processes needs improvement. Simultaneous assessment of genotypes for multiple productivity characteristics and integrating information across production stages has seldom been the focus of research efforts. To address this gap, we first determined the agronomic performance of 10 poplar genotypes. Differences between genotypes in height, diameter at breast height (DBH), tree mass and yield were consistently observed. Correlation analyses revealed that height and DBH are positively correlated with tree mass and yield, whereas bark content is negatively correlated with tree mass, yield and disease incidence. Four highest-yielding genotypes were subjected to proximate, ultimate, targeted chemical analyses, along with assessment of sugar production by acid hydrolysis and enzymatic saccharification. Despite having only marginal changes in overall chemistry, the genotypes showed differential conversion efficiencies of enzymatic saccharification. Interestingly, the genotype that showed highest cellulose conversion efficiency had the lowest estimated sugar yields due to its low biomass yield, whereas the genotype with lowest conversion efficiency had the highest estimated sugar yields. These results show the importance of integrating information across the stages of biomass production and bioconversion. These results also demonstrate the complexity of biomass feedstock production and the need for future studies to assess whether these tradeoffs can be genetically separated to guide the selection of genotypes that can maximize the overall biomass feedstock production efficiency.

1. Introduction

Lignocellulosic biomass is an important renewable energy source that offers the prospect of reduced dependency on fossil fuels and a concomitant reduction in greenhouse gases [1–4]. In the recently updated Billion Ton-Report, the U.S. Department of Energy projected that the U.S. can produce 1.2 billion tons of lignocellulosic biomass by 2040, of which 411 million tons would be from dedicated energy crops [5]. Lignocellulosic biomass from trees is an abundant source of fermentable sugars to produce renewable fuels (i.e., ethanol, butanol, aviation fuels), value-added chemicals (flavor-enhancers, nutraceuticals, cosmetics) and products (animal feed, biopesticides, plastics, textiles) to name a few [6–9]. However, wide-scale production of fermentable sugars from lignocellulosic feedstocks has been hampered by the lack of cost-effective methods to overcome the natural recalcitrance of biomass prior to conversion [10–13]. Several techno-economic analyses predicting the cost of fuel ethanol derived from lignocellulosic biomass have consistently shown that every step in the biomass-to-bioproduct process needs to be improved, including the development of lignocellulosic feedstocks to make them more amenable to acid hydrolysis and enzymatic saccharification of the resultant polysaccharides [14–17].

Selecting non-food crops to supply raw material to multiple industries is key for establishing sustainable and profitable biorefinery [1,4]. With its fast-growing habit and short-rotation, hybrid poplar is a prime candidate as a sustainable biomass resource [18,19], providing

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lignocellulosic biomass for renewable fuels in as little as 1 year with coppice harvesting [20]. Traditional wood products such as pulp and paper, veneer/flake products (e.g., laminated veneer lumber, oriented strandboard) and even lumber [21–23] can be produced when hybrid poplar is managed with longer rotation ages (*ca.* 10–25 years). Hybrid poplar also has a diverse and extensively annotated gene pool [24–27] that offers opportunity for substantial near-term improvements in both environmental performance (e.g., pest/disease resistance, nutrient and water-use efficiency) and industrial performance (e.g., biomass yield and sugar production through enzymatic digestibility). However, optimal growth is typically only achieved on the most favorable sites [18,19,28,29], hence recent research has focused on the identification of poplar genotypes for superior agronomic performance in diverse environments [24,26,30–32]. Nevertheless, in the case of biofuels, where biomass conversion is an important step in fuel production, the composition of the biomass feedstock should be taken into account when assessing genotype productivity [33–35]. Although it is important to integrate information across different stages of biomass production, characterization, pretreatment and enzymatic conversion, simultaneous assessment of genotypes for multiple productivity characteristics has seldom been the focus of research efforts [4,36,37].

To address this gap between selection based on biomass productivity and bioconversion efficiency, this paper aims to 1) characterize the agronomic performance of 10 poplar genotypes planted in a short rotation coppice system, 2) evaluate the biomass of the poplar genotypes in terms of physical and chemical properties, 3) assess the sugar production of poplar genotypes after acid hydrolysis and enzymatic saccharification, and 4) integrate the information across the stages of biomass production and bioconversion to identify genotypic characteristics for optimal biomass productivity.

2. Materials and methods

2.1. Study site description and planting

The study site, previously used as a pasture, was located at the University of Tennessee's East Tennessee Research and Education Center (ETREC, 35° 50.5486′N, 83° 57.6293′W). Ten poplar genotypes (8 hybrids and 2 first generation of cottonwood genotypes selected from natural populations representing four different taxonomic designations (*P. trichocarpa* × *P. deltoides* (TD), *P. deltoides* × *P. maximowiczii* (DM), *P. deltoides* × *P. deltoides* (DD; different *P. deltoides* genotypes were crossed), first generation of *P. deltoides* genotypes (D)) were planted in blocks at the end of March 2012. Unrooted cuttings (46 cm long) were manually planted at three different spacings: 3 m × 0.9 m, 3 m × 1.2 m and 3 m × 2.1 m of inter- and intra-row spacing, respectively. These spacing levels were chosen based on the commonly recommended spacings for hybrid poplar in short rotation [19,20,24] with the main goal of using available standard forestry operation/equipment as much as possible to control weeds, apply fertilized and harvest the biomass. Each block included five rows that were 25.6 m in length with total block areas of 384 m^2. Based on the availability of cuttings and survival of saplings, there were one to three replicated blocks per poplar genotype. Weed control at the time of planting was provided by spraying pre-emergence herbicides (pendimethalin [Pendulum 3.3 EC] at 4.7 L/ha, and Imazaquin [Scepter 70 DG] at 0.07 L/ha) and glyphosate ([Cornerstone] at 2.3 L/ha) and post-planting by directed spray of glyphosate and mowing between rows in the first year.

2.2. Field sampling and specimen processing

In each block, the height and diameter at breast height (DBH) of 2-year old trees were measured, this ranged from 14 to 24 trees, depending on tree survival. After two growing seasons, the main stem and woody branches were harvested and weighed to obtain woody aboveground biomass for each tree. Two stem disks (each 5.1 cm long) were collected at breast height (1.37 m) from a given tree among the 10 poplar genotypes sampled; the number of trees subjected to destructive sampling for each variety varied between 4 to 6 trees in order to provide sufficient material for analyses. The diameter and green weight of each disk were measured at the time of harvest. The first set of disks was dried at 105 °C until constant weight to determine moisture content (%), total woody tree mass (main stem and woody branches) and wood/bark ratio. The four best performing hybrid genotypes at the 1.2 m spacing, based on yield (t/ha), were selected for the proximate and wood chemistry analyses. The samples from four blocks of each variety were combined to obtain enough material for the analyses. All further analyses were conducted with disks from the second set that were dried at 40 °C before carefully being debarked. The separate wood and bark samples were each milled to a <40 mesh particle size with a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). Only the wood samples were used for chemical composition and sugar release analyses, as the compounds in the bark are known to inhibit quantification assays and enzyme activities. Since it is unlikely that the feedstock would be debarked for commercial thermochemical operations, elemental, proximate and ultimate analyses were carried out on the "whole" sample which contained the bark and wood. Since the ground bark and wood samples had already been separated, we mixed them back together according to the bark percentage of each sample for the proximate and ultimate analyses. Particle size estimates for the four best performing hybrids were determined by image analysis (ImageJ) of pictures of the particles widely dispersed over a light box customized to attenuate backlighting.

Finally, to assess the incidence of canker on the trees, a qualitative disease rating was developed. Trees were rated for incidence of canker on the branches and stem as follows: rating of 0 = no cankers visible; rating of 1 = small cankers (diameter <7.6 cm) visible on stem but appearing to heal over; rating of 2 = large unhealed cankers (diameter >7.6 mm) visible on either stem or branches; rating of 3 = many large cankers on both stem and branches, appearing to impact tree health/growth. The incidence of canker was rated in year 2 and 3. After the disease rating in year 3, trees were coppiced and the regrowth was rated for canker incidence in year 4.

2.3. Wood chemistry analyses

Milled wood samples were sequentially extracted with water and ethanol using an automated extraction system (ASE 350, Dionex Corp. CA, USA) following the National Renewable Energy Laboratory (NREL) protocol for quantifying extractives content in biomass [38]. The extracted samples were then dried in an oven (40 °C, > 3 days) to a constant weight and used to measure cellulose, hemicellulose, and lignin content following the corresponding NREL protocols [39]. High pressure liquid chromatography (HPLC) was employed to quantify the structural monomeric sugars after a two-step acid hydrolysis. The HPLC system was equipped with an Aminex HPX-87P column (Bio-Rad, 300 nm × 7.8 nm, 9 μm particle size) attached to a micro-guard Carbo-P guard column (Bio-Rad), and a refractive index detector (Perkin Elmer). The detector temperature was set at 50 °C and the oven temperature at 85 °C. The injection volume was 30 μl with 0.25 mL/min of flow rate. Acid insoluble lignin was measured gravimetrically while acid soluble lignin was measured by UV/VIS spectrometry at 240 nm wavelength. A pyrolysis-gas chromatography mass spectrometer (py-GCMS, Perkin Elmer) was utilized to determine the ratio of syringyl (S) and guaiacyl (G) lignin. Approximately 200 μg of sample were pyrolyzed at 450 °C for 12 s using a Frontier EGA/Py-3030 D pyrolyzer. Then, a Perkin Elmer Clarus 680 gas chromatograph coupled with a Clarus SQ 8C mass spectrometer was used for the chromatographic separation of the pyrolysis vapors and the identification of the evolved components, respectively. The GC column was 60 m × 0.25 mm ID × 0.25 μm film thickness (Agilent). Helium was used as the carrier gas at a 1 mL/min flow rate and an 80:1 split ratio was maintained. A total of 200 chromatographic peaks with S/N ≥ 2000 were extracted using the

Table 1
Yield parameters of 10 poplar genotypes after two years of growth. Estimated mean and standard errors (in parentheses) are shown. Letters indicate significant difference ($p<0.05$) between genotypes at 0.9 m, 1.2 m and 2.1 m spacing.

Spacing (m)	Poplar genotype and taxon										
		185*	**230***	302	**303***	312	**339***	342	356	373	412
		TD	DM	TD	TD	DD	TD	TD	DD	D	D
0.9	**Height (m)**	5.5 (1.1)^{ab}	5.6 (1.5)^{a}	4.6 (0.8)^{c}	5.2 (0.6)^{b}	3.8 (0.7)^{d}	5.4 (1.4)^{ab}	4.7 (1.0)^{c}	4.0 (0.5)^{d}	4.2 (0.4)^{cd}	2.9 (0.6)^{e}
	DBH (cm)	4.9 (1.2)^{ab}	5.2 (1.7)^{a}	3.9 (1.0)^{c}	4.5 (0.9)^{b}	3.1 (1.1)^{d}	5.0 (1.2)^{a}	3.7 (1.1)^{c}	2.8 (0.7)^{d}	2.9 (0.8)^{d}	2.0 (0.8)^{e}
	Tree mass (kg)	5.4 (3.2)^{abc}	7.7 (4.3)^{a}	3.4 (1.9)^{bc}	3.7 (0.7)^{bc}	2.0 (1.2)^{c}	5.5 (2.9)^{ab}	3.6 (1.3)^{bc}	2.4 (0.6)^{bc}	1.8 (0.3)^{bc}	2.2 (1.5^{bc}
	Yield (t/ha)	20.0 (11.9)^{abc}	28.5 (15.7)^{a}	12.4 (6.9)^{bc}	13.9 (2.7)^{bc}	7.5 (4.6)^{c}	20.5 (10.9)^{ab}	13.5 (5.0)^{bc}	9.0 (2.0)^{bc}	6.6 (1.1)^{bc}	8.2 (5.6)^{bc}
	Bark (%)	21 (3.0)^{a}	22.3 (4.9 ^{a}	23.3 (6.7)^{a}	23.9 (2.4)^{a}	22.3 (1.7)^{a}	19.3 (3.4)^{a}	22.1 (1.8)^{a}	17.8 (1.0)^{a}	21.4 (5.1)^{a}	20.7 (4.7)^{a}
	Wood yield (t/ha)	16.0 (10.0)^{abc}	22.7 (13.2)^{a}	9.5 (5.3)^{bc}	10.6 (2.2)^{bc}	5.8 (3.6)^{c}	16.9 (9.0)^{ab}	10.6 (4.2)^{bc}	7.4 (1.6)^{bc}	5.2 (0.5)^{bc}	6.6 (4.8)^{bc}
1.2	**Height (m)**	6.2 (1.0)^{ab}	6.4 (1.4)^{a}	4.7 (1.1)^{c}	6.0 (0.8)^{b}	4.1 (0.7)^{d}	6.1 (0.7)^{ab}	4.8 (0.9)^{c}	3.3 (0.6)^{e}	4.5 (0.2)^{cd}	3.0 (0.8)^{e}
	DBH (cm)	5.8 (1.4)^{b}	6.3 (1.7)^{a}	4.3 (1.1)^{d}	5.4 (0.9)^{bc}	3.7 (1.3)^{e}	5.2 (1.4)^{c}	4.1 (1.1)^{de}	2.3 (0.8)^{f}	4.1 (1.0)^{de}	2.2 (1.3)^{f}
	Tree mass (kg)	5.7 (2.7)^{ab}	8.6 (5.0)^{a}	6.0 (4.9)^{ab}	3.1 (2.0)^{b}	2.4 (1.2)^{b}	4.8 (2.6)^{ab}	3.1 (0.8)^{b}	1.2 (0.9)^{b}	2.3 (0.3)^{b}	2.3 (1.9)^{b}
	Yield (t/ha)	16.0 (7.4)^{ab}	23.8 (14.0)^{a}	16.7 (13.7)^{ab}	8.6 (5.6)^{b}	6.6 (3.2)^{b}	13.4 (7.2)^{ab}	8.5 (2.2)^{b}	3.3 (2.5)^{b}	6.5 (0.8)^{b}	6.4 (5.2)^{b}
	Bark (%)	20.2 (5.5)^{b}	21.4 (5.2)^{b}	26.3 (4.9)^{ab}	23.7 (7.0)^{ab}	28.5 (7.8)^{a}	21.6 (3.3)^{b}	22.4 (3.1)^{ab}	30.6 (5.3)^{a}	24.4 (1.0)^{ab}	26.8 (4.9)^{ab}
	Wood yield (t/ha)	13.0 (6.5)^{ab}	19.2 (12.2)^{a}	11.9 (9.1)^{ab}	6.8 (4.9)^{b}	4.6 (1.8)^{b}	10.6 (5.9)^{b}	6.6 (1.8)^{b}	2.4 (1.9)^{b}	4.9 (0.7)^{b}	4.8 (4.1)^{b}
2.1	**Height (m)**	6.3 (1.4)^{a}	5.7 (1.3)^{b}	4.6 (0.6)^{d}	5.3 (1.0)^{c}	4.0 (0.9)^{e}	5.4 (0.8)^{bc}	4.7 (0.7)^{d}	2.9 (0.6)^{f}	3.7 (0.5)^{e}	3.9 (0.7)^{e}
	DBH (cm)	6.1 (2.0)^{ab}	6.6 (1.9)^{a}	4.8 (1.1)^{c}	4.9 (0.8)^{c}	3.8 (1.7)^{e}	6.1 (1.7)^{b}	4.7 (0.9)^{cd}	1.9 (0.7)^{f}	2.6 (0.8)^{f}	4.0 (1.6)^{de}
	Tree mass (kg)	9.8 (3.8)^{a}	9.4 (4.5)^{a}	7.2 (3.4)^{abc}	9.1 (6.7)^{ab}	4.0 (3.1)^{bc}	9.2 (2.5)^{ab}	5.3 (3.3)^{abc}	1.7 (0.8)^{c}	1.9 (0.5)^{c}	7.4 (7.5)^{abc}
	Yield (t/ha)	15.6 (6.0)^{a}	14.9 (7.2)^{a}	11.4 (5.4)^{abc}	14.5 (10.6)^{ab}	6.4 (4.9)^{bc}	14.5 (4.0)^{ab}	8.4 (5.3)^{abc}	2.7 (1.2)^{c}	3.1 (0.8)^{c}	11.7 (11.8)^{abc}
	Bark (%)	19.6 (3.8)^{cd}	21.2 (3.1)^{bcd}	24.6 (3.9)^{ab}	23.4 (3.8)^{abc}	22.1 (3.1)^{abcd}	18.1 (3.1)^{d}	23.7 (4.3)^{abc}	28.2 (3.6)^{a}	23.2 (2.9)^{abcd}	25.7 (5.7)^{abc}
	Wood yield (t/ha)	12.7 (5.3)^{a}	11.9 (6.0)^{a}	8.6 (4.2)^{abc}	10.8 (7.2)^{ab}	5.1 (4.0)^{bc}	11.9 (3.3)^{a}	6.6 (4.4)^{abc}	1.9 (0.8)^{c}	2.4 (0.7)^{bc}	9.1 (9.5)^{abc}

*indicates the high yielding genotypes in the study

TurboMass GC/MS software. Among those 200 chromatographic peaks, the S-lignin and G-lignin fragments were identified by comparing each of the mass spectra to the NIST library of fragmentation patterns from the Turbomass software. From the sum of S and G lignin, the ratio of S/G ratio was calculated.

2.4. Elemental analysis

Inorganic composition of the mixed bark and wood from the four selected poplar genotypes was determined by inductively coupled plasma optical emission spectrometry with an Optima 7300DV spectrometer (ICP-OES, Perkin Elmer, USA) after microwave digestion. Approximately 0.5 g of sample was digested using a Multiwave 3000 microwave (Anton Paar, VA, USA) in 4 mL of nitric acid (67–70%), 3 mL of hydrogen peroxide (30%), and 0.2 mL of hydrofluoric acid (HF, 51%) at 180–210 °C for 60 min. After digestion, 1 mL of 4.0% boric acid solution was added to the digested sample to neutralize the hydrofluoric acid and to facilitate dissolution of precipitated fluorides. The solutions were transferred to 50 mL polypropylene centrifuge tubes and diluted to 50 mL with deionized water. The samples were filtered with 0.45 μm PTFE filter before ICP-OES analysis.

Ash content of the mixed bark and wood was determined by combusting 1 g of material in a furnace at 575 °C for 24 h (Fisher Scientific Isotemp Programmable Muffle Furnace 750). Volatile matter was determined using the same amount of sample (1 g) in covered crucibles placed in a muffle furnace (Themolyne, Thermo Scientific, USA) for 10 min at 910 °C; the amount of fixed carbon was obtained by subtracting the percentage of moisture, volatile matter, and ash. For ultimate analysis, 20 μg samples were analyzed with a 2400 Series II CHNS/O analyzer coupled with AD6 autobalance controller (Perkin Elmer, USA). The higher heating value (HHV) was calculated using ultimate analysis data as described by Yin [40].

2.5. Dilute acid pretreatment and saccharification

Since compounds in bark can cause inhibition of enzymatic saccharification, only debarked wood samples were used for biofuel chemical property analyses and three dilute-acid pretreatment conditions were tested. Ground wood samples were mixed with prepared sulfuric acid solution (low 0.08%, moderate 0.2% and high 1.0%) in an Erlenmeyer flask and autoclaved at 121 °C for 60 min. The biomass loading was 20%. The acid-pretreated samples were diluted with 15 g of sodium citrate buffer solution (0.05 M, pH 5.5). The samples were mixed with cellulase enzymes (Cellic® CTec2, Novozymes Corp) to assure 60 FPU/g of substrate in a total reaction volume of 20 mL. The content was incubated for 72 h in a shaker incubator at 50 °C with 150 rpm agitation. Saccharified aliquots (0.5 mL) were taken at 0, 1, 3, 6, 12, 24, 48, 72 h of incubation. Each aliquot was boiled to inactivate the enzymes and then centrifuged at 10000 rpm for 5 min. The supernatant was filtered with 0.2 μm Teflon filter into a HPLC vial for soluble carbohydrates content measurement.

2.6. Statistical analysis

To compare the differences among genotypes and taxa in yield and disease parameters, chemical composition and thermo/bio-chemical conversion properties, analysis of variance (ANOVA) was conducted in SAS 9.4 (SAS Institute, Cary, NC, USA) at a significance level of $p < 0.05$ and means when different were separated using Tukey test. The correlation of determination between measured traits were assessed in Excel (Microsoft Corp., Redmond, WA, USA).

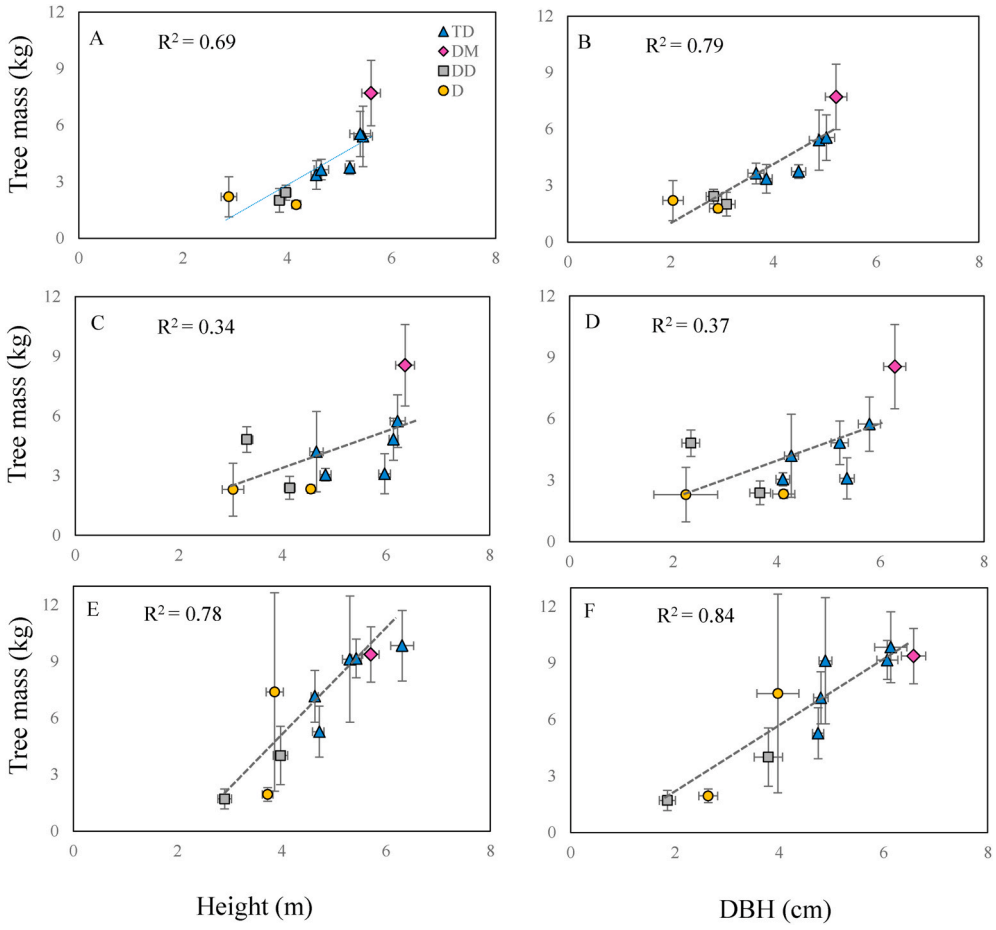

Fig. 1. Correlation between different yield parameters among poplar genotypes. The correlation between height (m) vs tree mass (kg) (A, C, E) and diameter at breast height (DBH (cm)) vs tree mass (kg) (B, D, F) when planted at 0.9 m (A, B), 1.2 m (C, D) and 2.1 m (E, F) spacing respectively. Standard error bars denote variability in height, DBH and tree mass within each genotype.

3. Results

3.1. Variation in agronomic characteristics of poplar hybrid genotypes

The growth, tree mass and biomass yield data for ten poplar genotypes planted at three different spacings are shown in Table 1. The best performing genotype was the hybrid 230, having highest (at 0.9 and 1.2 m spacings) or second highest (at 2.1 m spacing) average tree mass, with values ranging from 7.7 to 9.4 kg. The lowest performing genotypes were 356 (DD) and 373 (D), with average tree mass ranging from 1.7 to 2.6 kg for all three spacings. Differences between the genotypes in height, diameter at breast height (DBH), tree mass and yield were consistently observed at all three spacing levels. Correlation analyses showed moderate relationships between height and tree mass (R^2 = 0.34) and DBH and tree mass (R^2 = 0.37) at the 1.2 m spacing (Fig. 1 C, D). Strong correlations for height vs. tree mass (R^2 = 0.69–0.78) and DBH vs. tree mass (R^2 = 0.79–0.84) were obtained for the lower and higher (0.9 and 2.1 m) spacings (Fig. 1 A, B and E, F). Similarly, the correlations between height and yield (Supplemental Fig. 1 A, C, E) as well as DBH and yield (Supplemental Fig. 1 B, D, F) were moderate to strong. Within the same genotype, spacing impacted the height and DBH, with larger trees growing at wider spacings, as expected (Supplemental Table 1). However, these changes in height or DBH did not significantly impact tree mass or yield (except for genotype 339). Taken together, the results indicate that DBH and height are accurate predictors of tree mass and yield of poplar genotypes.

Since the wood used for the biochemical production of biofuels is usually debarked, we separated the wood and bark for the tree disk samples and assessed the variability of these components in the genotypes. Interestingly, bark amount (%) was not significantly different between genotypes at the 0.9 m spacing whereas bark amount varied between 20.2–30.6% at the 1.2 m spacing and 18.1–28.2% at the 2.1 m spacing. Within the same genotype, there was no significant impact of spacing on bark except for genotype 356 (Supplemental Table 1). The amount of bark did not significantly alter the wood yield compared to total yield except in genotype 339 (TD) that had the lowest bark amount at two of the three spacing levels. To examine whether bark amount has an impact on yield, we performed correlation analysis between bark and growth characteristics, DBH (Fig. 2 A, C, E), tree mass (Fig. 2 B, D, F) and height (Supplemental Fig. 2). We found that bark amount was negatively correlated with DBH (R^2 = 0.6–0.71), height (R^2 = 0.62–0.74) and with tree mass (R^2 = 0.37–0.42) at 1.2 and 2.1 m spacing but was not correlated with any parameters at 0.9 m spacing (R^2 = 0.00).

One of the objectives of the study was to identify poplar genotypes that are high yielding and tolerant to canker disease. Since bark tissue is considered as a barrier that protects the tree from insects and diseases, we assessed whether the variability in bark amount in the genotypes impacts their canker disease incidence (Table 2). Disease rating conducted on two-year-old trees found that there was very low incidence of canker except in genotype 339 (TD) in at least two of three spacing levels. In year three, disease severity increased and genotypes 339 and 342 showed severe canker incidence, genotypes 185, 230 and 302 showed moderate levels of canker and genotypes 312, 373 and 412 showed lowest canker incidence. After coppicing in year 3, the disease incidence was assessed on the regrowth in year 4 and it was found that overall genotypes 185, 230, 302, 339 and 342 had more severe disease

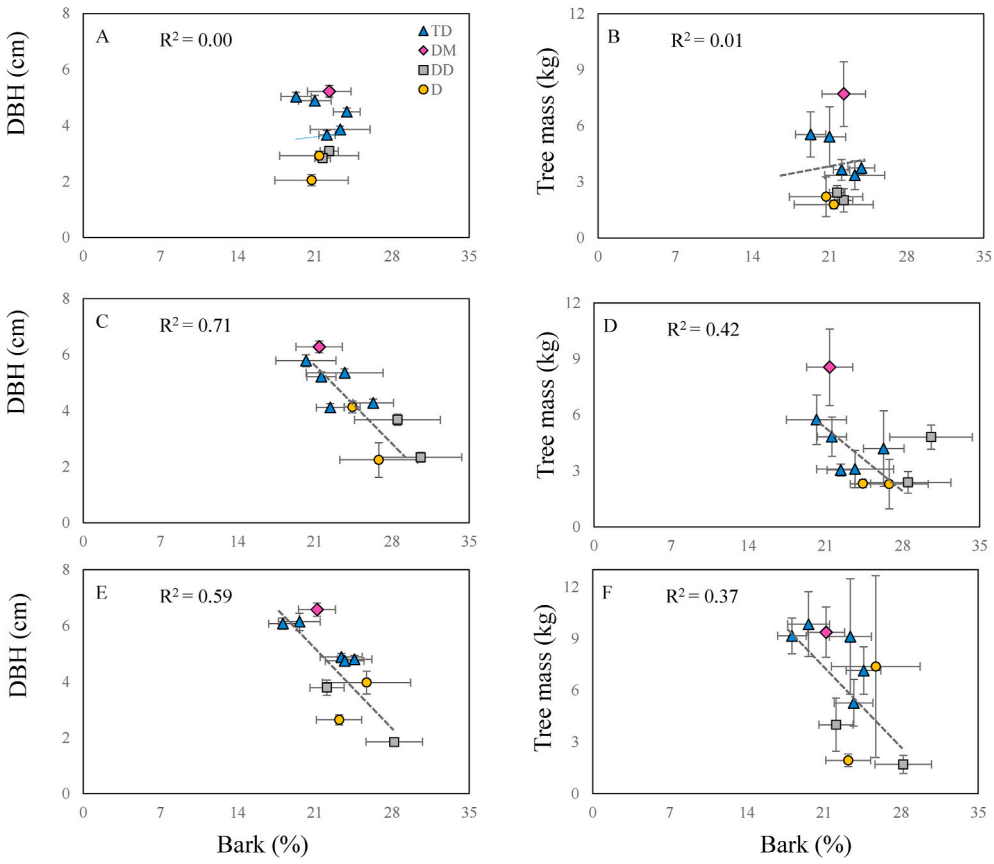

Fig. 2. Correlation between DBH and biomass with bark among poplar genotypes. The correlation between DBH (cm) vs bark (%) (A, C, E) and biomass (kg) vs bark (%) (B, D, F) when planted at 0.9 m (A, B), 1.2 m (C, D) and 2.1 m (E, F) spacing respectively. Standard error bars denote variability in DBH, biomass and bark within each genotype.

incidence and genotypes 312, 356, 373 and 412 with low (to no) disease incidence. Given that the *P. deltoides* populations are known to be more disease tolerant [23], perhaps it is not unexpected that the genotypes 312, 356, 373 and 412 had low (to no) disease incidence. Although there were no uniform trends within genotypes, trees grown in wider spacing level had more incidence of canker than trees grown with narrower spacing (Supplemental Table 2). Correlation analysis of bark amount and disease rating in year 3 (before coppicing; Fig. 3 A, C, E) and year 4 (after coppicing; Fig. 3 B, D, F) found that there was no correlation between bark amount and disease at 0.9 m spacing; however, the genotypes with higher bark amount showed less canker incidence at 1.2 m spacing (R^2 = 0.49–0.71) and 2.1 m spacing (R^2 = 0.21–0.35).

Overall, the analysis of agronomic traits revealed that height and DBH are strong indicators of tree mass and yield of genotypes. Although spacing did not significantly impact agronomic parameters within genotypes, the differences between genotypes were more pronounced at wider spacing. Therefore, future studies of genotypic selection should consider planting at 1.2 m spacing. The correlation analyses between bark amount with yield parameters and disease revealed that the selection of genotypes for optimal growth will involve tradeoffs between disease tolerance and yield potential. Growers should consider these aspects and cost of planting at higher densities to select appropriate spacing levels for commercial cultivation.

3.2. Conversion properties of four hybrid poplar genotypes

Based on the agronomic performance of relatively high yield and variable disease tolerance, we selected four genotypes 185 (TD), 230 (DM), 303 (TD) and 339 (TD) grown at 1.2 m spacing to conduct more detailed compositional and (bio)chemical analyses.

3.2.1. Elemental composition analyses

The inorganic composition of biomass plays an important role in thermochemical conversion processes. The presence of a high concentration of inorganics, particularly in the bark, can cause problems such as decreased reactor efficiency by slagging and fouling of reactors [41]. We analyzed the inorganic composition of the whole (wood and bark) samples of the four best performance genotypes by proximate and ultimate analysis and the data are shown in Table 3. The ash content ranged from 1.5 to 2.1% (on a dry basis) and there was a statistical difference within and between taxa. Genotype 185 had a lower ash content (1.5%) than variety 339 (2.0%), both belonging to the same taxa (TD), whereas genotype 230 (DM) has the highest ash content (2.1%). It is worth noting that the ash content of all four genotypes were higher than the recommended ash content (less than 1%) for thermochemical conversion by the Department of Energy [42]. The higher bark-wood ash contents (Table 3) compared to the wood-alone ash contents (Table 5) are consistent with the fact that ash content is higher in bark than it is in wood, therefore increasing ash contents would coincide with increasing bark contents of the samples. There was no significant difference in volatile matter, fixed carbon, overall carbon and hydrogen contents between the taxon or between genotypes (Table 3). Similarly, no significant differences in the higher heating values were found among the four best performing genotypes. The inorganic composition of the whole samples is provided in Table 4. Subtle differences between the genotypes were observed. For example, genotype 185 (TD) had the lowest Ca (3, 763 mg/kg), Mg (762 mg/kg), P (588 mg/kg), and Si (129 mg/kg); however, it has the highest Na (31.7 mg/kg) and the genotype 230 (DM) had the highest Ca (5803 mg/kg). While these data identify no single poplar variety standing apart as being unique, it should be acknowledged that specific elements, such as alkali and alkaline earth metals,

Table 2
Disease incidence rating of 10 poplar genotypes after two (Y2), three (Y3) and four (Y4) years of growth. Estimated mean and standard errors (in parentheses) are shown. Letters indicate significant difference ($p<0.05$) between genotypes at 0.9 m, 1.2 m and 2.1 m spacing.

Disease rating	Spacing (m)	Poplar genotype and taxon									
		185*	**230***	302	**303***	312	**339***	342	356	373	412
		TD	DM	TD	TD	DD	TD	TD	DD	D	D
Y2	**0.9**	0.10 (0.08)^{b}	0.10 (0.06)^{b}	0.26 (0.07)^{b}	0.00 (0.08)^{c}	0.00 (0.08)^{c}	0.91 (0.07)^{a}	0.10 (0.07)^{b}	0.00 (0.11)^{c}	0.13 (0.11)^{b}	0.22 (0.13)^{b}
	1.2	0.00 (0.07)^{c}	0.03 (0.06)^{c}	0.51 (0.06)^{a}	0.04 (0.07)^{c}	0.07 (0.07)^{c}	0.32 (0.06)^{b}	0.04 (0.06)^{c}	0.13 (0.10)^{bc}	0.00 (0.10)^{c}	0.11 (0.11)^{b}
	2.1	0.11 (0.08)^{b}	0.00 (0.06)^{d}	0.31 (0.07)^{b}	0.04 (0.08)^{b}	0.09 (0.08)^{b}	0.66 (0.06)^{a}	0.24 (0.06)^{b}	0.00 (0.11)^{cd}	0.00 (0.11)^{b}	0.06 (0.13)^{b}
Y3	**0.9**	0.78 (0.14)^{c}	0.91 (0.13)^{c}	0.80 (0.13)^{c}	0.61 (0.15)^{c}	0.00 (0.17)^{d}	2.59 (0.13)^{a}	1.61 (0.13)^{b}	0.82 (0.21)^{c}	0.04 (0.20)^{d}	0.00 (0.25)^{d}
	1.2	1.42 (0.14)^{b}	1.09 (0.12)^{bc}	0.90 (0.12)^{c}	0.98 (0.14)^{c}	0.00 (0.15)^{d}	2.67 (0.12)^{a}	2.51 (0.11)^{a}	1.23 (0.20)^{bc}	0.00 (0.20)^{d}	0.05 (0.20)^{d}
	2.1	1.74 (0.15)^{b}	1.64 (0.12)^{b}	1.63 (0.12)^{b}	1.04 (0.15)^{c}	0.23 (0.15)^{d}	2.72 (0.11)^{a}	2.93 (0.12)^{a}	0.82 (0.21)^{c}	0.00 (0.00)^{d}	0.05 (0.21)^{d}
Y4	**0.9**	2.82 (0.13)^{a}	1.83 (0.12)^{b}	1.52 (0.12)^{bc}	1.46 (0.14)^{c}	0.00 (0.15)^{d}	3.00 (0.12)^{a}	2.95 (0.12)^{a}	0.00 (0.20)^{d}	0.00 (0.19)^{d}	0.00 (0.23)^{d}
	1.2	2.94 (0.11)^{a}	2.38 (0.10)^{b}	1.90 (0.10)^{c}	1.87 (0.12)^{c}	0.07 (0.12)^{d}	3.00 (0.10)^{a}	3.00 (0.09)^{a}	0.00 (0.17)^{d}	0.09 (0.17)^{d}	0.00 (0.17)^{d}
	2.1	3.00 (0.11)^{a}	2.91 (0.08)^{a}	2.39 (0.08)^{b}	1.78 (0.10)^{c}	0.14 (0.10)^{d}	2.93 (0.08)^{a}	3.00 (0.08)^{a}	0.00 (0.15)^{d}	0.09 (0.14)^{d}	0.00 (0.15)^{d}

*indicates the high yielding genotypes in the study

may show product selectivity and lower yield on thermochemical conversion processes caused by undesirable autocatalytic reaction pathways [43].

3.2.2. Chemical composition analyses

The primary obstacle in producing biofuels from lignocellulosic biomass, especially in bio-chemical conversions, is the recalcitrance in releasing carbohydrates from the cell wall [44]. Releasing high quantity sugars at low costs from biomass has been the primary goal of many studies [44,45]. Therefore, it is important to assess the chemical composition of biomass, in this case, the amounts of the main structural constituents (e.g., cellulose, hemicellulose, and lignin) of the wood. The wet chemistry results in Table 5 showed that genotype 230 (DM) had the highest cellulose content seemingly offset by the lowest hemicellulose content; genotype 185 (TD) showed the opposite relationship, having one of the lower cellulose content and the highest hemicellulose content. Among all of four genotypes and the two taxa, 230 (DM) had the highest value (61.6%) and 339 the lowest value (57.7%) for total carbohydrates. Altogether, the carbohydrates content is an indicator of the potential yields of fermentable sugars between genotypes; however, other components may influence the recalcitrance and could ultimately result in lower yields of digestible sugars despite higher carbohydrates contents. Ash content ranged between 0.6 and 0.7%, which was not statistically different among the four genotypes. Likewise, total lignin showed no statistical differences between genotypes, as was the case with acid insoluble and soluble lignin and S/G ratio. It is interesting to note that statistically significant differences were observed for the extractives, with genotype 339 having higher content than observed with the other genotypes within and between the two taxa (DM, TD).

3.2.3. Yields of fermentable sugars

Coupled with the chemical analyses, the final objective of this study was to determine differences in the cellulose conversion rate by enzymatic hydrolysis. Before the saccharification, dilute acid pretreatment was conducted with three different severity levels (Table 6a) to assess whether the differences in digestibility between the genotypes, if any, were specific to a single pretreatment condition. The resulting enzymatic hydrolysis data are shown in Table 6b and Fig. 4. Total glucose yields increased with increasing severity of the pretreatment conditions. The cellulose conversion rate after low severity pretreatment showed statistical difference with genotypes 303 and 339 having a higher cellulose conversion compared to genotypes 185 and 230. At moderate and high severity pretreatment levels, genotype 303 showed significantly high conversion rate compared to the three other genotypes. As expected, time course of cellulose conversion rate of the genotypes at low, moderate and high severity pretreatment levels showed steady increase of glucose release (Fig. 4). However, some significant differences in cellulose conversion over time were observed. At low severity, 339 and 303 released much faster glucose than 185 and 230. At 24-h reaction time, nearly 40% more cellulose were converted in 339 and 303 than the other two genotypes. At moderate and high pretreatment severity, 303 continued to release glucose at higher rates than the other genotypes. Interestingly, at moderate severity, 339 and 303 had the exact amount of cellulose conversion until 12 h, however, past this enzymatic time the conversion rate significantly slowed down for 339. Image analysis of the particles showed that there were differences (P value < 0.001) in size with the mean particle image areas for genotypes 230 and 185 being the highest and lowest among the range of values (0.204–0.269 mm^2), respectively (Fig. 5). The mean aspect ratios (particle length divided by particle width) between all genotypes also showed a difference (P value < 0.05) with genotypes 230 and 185 being the highest two values, but within a fairly narrow range (1.92–2.12). Determining the impact of particle size on conversion rate was not in the scope of the current study; however, it is worth noting that the genotypes with the lowest conversion rate when using the lowest severity pretreatment had seemingly higher aspect ratios. Given that the largest and smallest mean particle

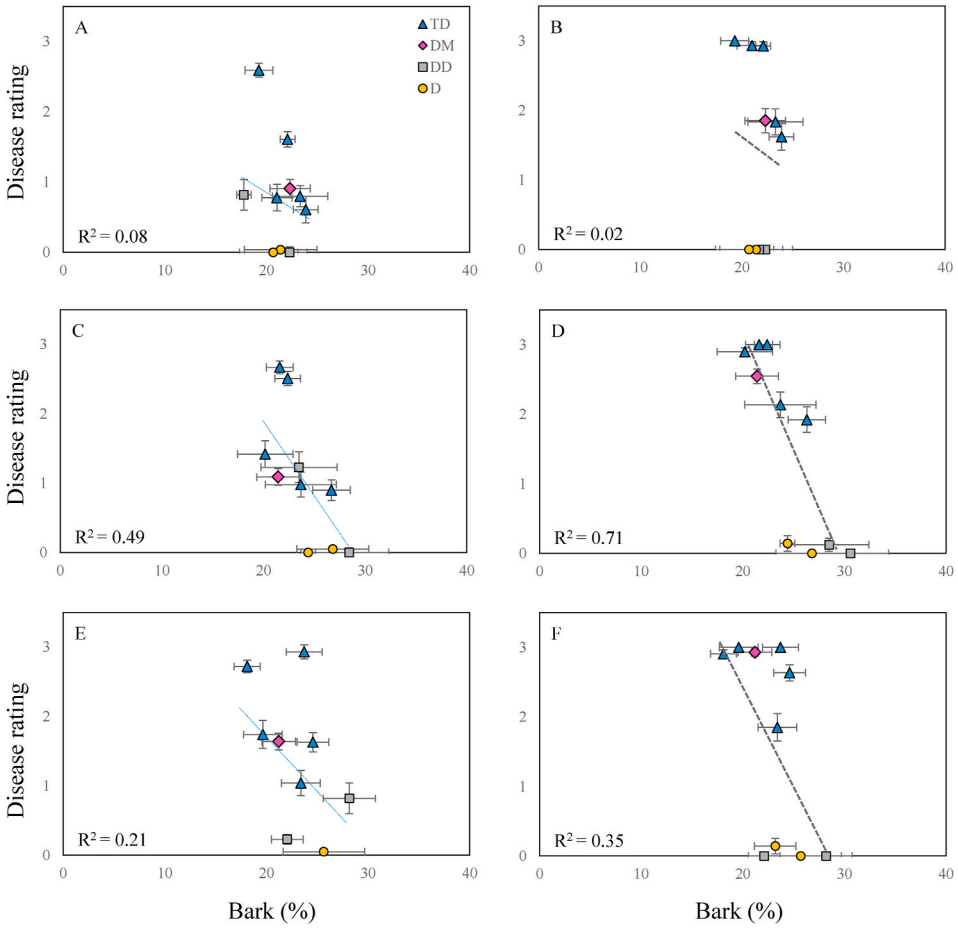

Fig. 3. Correlation between bark and disease among poplar genotypes in year 3 and year 4. The correlation between bark (%) as measured in year 2 of growth and disease rating in year 3 and year 4, when planted at 0.9 m (A, B), 1.2 m (C, D) and 2.1 m (E, F) spacing respectively. Standard error bars denote variability in bark and disease rating within each genotype.

Table 3
Physical properties of bark-wood samples of four genotypes of 2-year-old hybrid poplars grown at 1.2 m spacing. Estimated mean values (n=4 per genotype) and standard errors (in parentheses) are shown. Higher Heating Value (HHV)=0.2949C+0.8250H [40]. Letters indicate significant difference ($p<0.05$) between genotypes.

Genotype (taxon)	Proximate analyses (%)			Ultimate analyses (%)			HHV (MJ/Kg)
	Ash	Volatile matter	Fixed carbon	Carbon	Hydrogen	Nitrogen	
185 (TD)	1.5 (0.1) ^{c}	82.8 (0.3) ^{a}	15.7 (0.3) ^{a}	46.0 (0.3) ^{a}	5.9 (0.0) ^{a}	0.4 (0.1) ^{b}	18.4 (0.0) ^{a}
230 (DM)	2.1 (0.1) ^{a}	82.3 (0.3) ^{a}	15.7 (0.2) ^{a}	46.1 (0.5) ^{a}	5.8 (0.2) ^{a}	0.5 (0.0) ^{a}	18.4 (0.3) ^{a}
303 (TD)	1.9 (0.0) ^{b}	82.8 (0.7) ^{a}	15.3 (0.7) ^{a}	46.3 (0.1) ^{a}	5.9 (0.1) ^{a}	0.4 (0.0) ^{b}	18.5 (0.1) ^{a}
339 (TD)	2.0 (0.2) ^{ab}	82.5 (0.2) ^{a}	15.7 (0.1) ^{a}	46.4 (0.2) ^{a}	6.0 (0.0) ^{a}	0.4 (0.0) ^{ab}	18.6 (0.1) ^{a}

Table 4
Inorganic composition of bark-wood samples of four varieties of 2-year-old hybrid poplar genotypes grown at 1.2 m spacing. AAEM denotes alkali and alkaline earth metals (sum of Ca, K, Mg and Na). Estimated mean values (n=4 per genotype) and standard errors (in parentheses) are shown.

Variety	Taxon	Inorganic elements (mg/kg)									
		Al	Ca	Fe	K	Mg	Na	P	S	Si	AAEM
185	TD	11.7 (6.4)	3763 (66) ^{c}	27.4 (5.7)	2626 (43) ^{b}	762 (10) ^{c}	31.7 (0.9) ^{a}	588 (11) ^{b}	348 (6.0) ^{b}	129 (15) ^{b}	7183 (298) ^{c}
230	DM	9.9 (0.6)	5803 (153) ^{a}	33.3 (2.8)	2570 (32) ^{b}	1009 (17) ^{b}	18.1 (0.4) ^{b}	654 (8.9) ^{a}	464 (7.2) ^{a}	273 (33) ^{a}	9401 (298) ^{a}
303	TD	8.2 (0.2)	4585 (388) ^{b}	23.8 (1.8)	2784 (226) ^{b}	1042 (71) ^{b}	15.5 (1.1) ^{c}	651 (56) ^{a}	387 (29) ^{b}	234 (36) ^{a}	8426 (298) ^{b}
339	TD	9.9 (1.5)	4764 (287) ^{b}	25.7 (8.6)	3210 (211) ^{a}	1221 (65) ^{a}	8.9 (7.0) ^{b}	658 (22) ^{a}	497 (36) ^{a}	328 (30) ^{a}	9204 (298) ^{ab}

sizes had the lowest conversion rates under the same conditions, it seems unlikely that particle size had an impact our results. Despite the superior cellulose conversion efficiency of genotype 303 at all the pretreatment levels, the low overall wood yield of the genotype, resulted in low sugar yield estimates (Table 6b). Conversely, due to the high wood yield in genotype 230, the sugar yield estimate was the highest in genotype 230, despite low cellulose conversion rates. These results indicate that genotypes with high agronomic yields could overcome their low

Table 5
Chemical composition of wood samples of four genotypes of 2-year-old hybrid poplars grown at 1.2 m spacing. Estimated mean values (n=4 per genotype) and standard errors (in parentheses) are shown. Concentrations were calculated on the % dry basis. Letters indicate significant difference ($p<0.05$) between genotypes.

Variety	Taxon	Chemical composition of wood (% dry basis)								
		Ash	Extractives	Cellulose	Hemicellulose	Total Carb	Total Lignin	Acid Soluble Lignin	Acid Insoluble Lignin	S/G ratio
185	TD	0.6 (0.0) [a]	6.7 (0.4) [b]	40.9 (0.2) [c]	19.9 (0.1) [a]	60.8 (0.2) [b]	26.0 (1.0) [a]	5.2 (0.2) [a]	20.9 (1.0) [a]	1.5 (0.1) [a]
230	DM	0.7 (0.0) [a]	7.0 (0.2) [b]	43.2 (0.7) [a]	18.4 (0.2) [c]	61.6 (0.5) [a]	25.7 (1.3) [a]	5.0 (0.1) [a]	20.7 (1.2) [a]	1.5 (0.1) [a]
303	TD	0.6 (0.0) [a]	7.0 (0.1) [b]	41.7 (0.1) [b]	19.3 (0.0) [b]	61.1 (0.2) [ab]	25.3 (1.2) [a]	5.3 (0.4) [a]	20.0 (0.8) [a]	1.7 (0.1) [a]
339	TD	0.6 (0.4) [a]	8.6 (0.4) [a]	39.5 (0.1) [d]	18.2 (0.0) [d]	57.7 (0.1) [c]	26.8 (1.4) [a]	5.0 (0.3) [a]	21.8 (1.0) [a]	1.6 (0.0) [a]

Table 6a
Dilute acid pretreatment conditions at three different levels of severities.

	Pretreatment conditions		
Severity Condition	%H_2SO_4 (W_{acid}/W_{total})	Initial pH	Severity Factor
Low	0.08	1.76	0.6
Moderate	0.2	1.38	0.99
High	1.0	0.82	1.58

Severity equation [58]
Ro"= log(Ro)+|pH-7|.
Ro = (t)(exp (Tr-Tb)/14.75).
t = time of reaction; Tr = temperature of reaction; Tb = baseline temperature (100 °C).

conversion efficiencies to be more favorable to the overall biofuel production. Future studies need to focus on the fine structure of wood chemistry and anatomy to improve our understanding of the factors that contribute to variability in conversion efficiency.

4. Discussion

Several previous studies have assessed poplar genotypic variability either in agronomic traits or in bioenergy production separately [31,46–48]. To improve overall bioenergy production efficiency, it is important to identify genotypic traits that optimize productivity at different stages of biofuel production [36,37]. Therefore, the goal of our study was to integrate information from agronomic performance, compositional data and conversion analyses to identify traits for improving bioenergy productivity. These integrated analyses have revealed that natural variation within poplar genotypes impacts several steps in the bioenergy productivity and that there are potential tradeoffs between critical traits.

Genotypic variability between poplar lines was observed at three different planting densities, although the traits were more variable at wider spacing (Table 1). Trees with wider spacing are able to capture more sunlight for carbon fixation as well as more nutrients and water from the soil [47–49]. Hence, the trees planted at wider spacing likely were able to express their respective genotypic potential that resulted in more variability in traits. It was interesting to note that correlation between DBH and yield was the lowest at 1.2 m spacing due to the presence of outlier genotype(s) that either over- or under-performed compared to others. This implies that the intermediate spacing is more suited for genetic studies to identify over-performing outlier genotypes that can increase poplar productivity.

Correlation analyses revealed that height and DBH were positively correlated with tree mass (Fig. 1) and yield (Supplemental Fig. 2) whereas bark content was negatively correlated with tree mass and disease incidence (Figs. 2 and 3). The positive correlation between DBH and tree mass/yield is well documented and hence, as an easily measured trait, DBH is being used by growers and breeders to identify high-yielding genotypes [37,50,51]. Conversely, the negative correlation between bark amount and yield or disease in poplar genotypes is understudied. In our current study, before coppicing (in Year 3), the genotype 339 with the lowest bark amount had the most disease incidence (Table 2). Evaluation of disease incidence after coppicing (new growth in Year 4) revealed that all the high-yielding genotypes had high disease incidence growth, whereas the low yielding genotypes had low disease incidence (Table 2). Bark tissue protects trees from abiotic and biotic stresses [52,53] and hence the negative correlation between bark amount and disease incidence is expected. The correlation between bark amount and tree growth (and consequently, yield) and the genotypic variability of this response is relatively understudied. Bark may be a considerable sink for carbon and other high-energy compounds (like tannins, hydroxycinnamates and suberin) and this diversion of carbon to bark may negatively impact wood production [52]. The negative correlation between investment in plant defense versus growth is quite well studied [25,54,55] and hence, a causal negative correlation between bark amount and tree yield is plausible. Future studies are needed to decipher whether bark physically restricts tree growth or whether these negative correlations are caused due to physiological tradeoffs in differential investment in defense barriers (bark) or yield (wood). It would also be important to characterize the components within bark that contribute to disease resistance. Although these studies are beyond the scope of our current study, there is a need for detailed characterization of biomass, particularly bark composition and fine structure to understand how bark protects against diseases. Such analyses may enable us to understand whether it is biologically feasible to overcome the tradeoffs and obtain high yielding genotypes with disease tolerance.

Our pretreatment followed by the saccharification experiments identified that genotype 303 had the highest cellulose conversion and genotype 230 the lowest. These differential conversion efficiencies occurred despite the genotypes having only marginal changes in overall chemistry (Tables 3–5). It is possible that several minor differences in

Table 6b
Cellulose conversion and estimates of sugar yields after dilute acid pretreatment at three different levels of severity. Estimated mean values (n=3) of cellulose conversion obtained from the saccharification of wood samples at low, moderate and high level of pretreatment severity are shown. Standard error bars denote variability within each genotype and letters indicate significant difference ($p<0.05$) between genotypes after 72 h of hydrolysis. The conversion rate (%) at 72 h was multiplied with wood yield (t/ha) to obtain estimates of sugar yields at three different levels of severities.

	Conversion (%)			Wood yield (t/ha)	Sugar yield (t/ha)		
Genotype	Low	Mod	High		Low	Mod	High
185	17.2 (0.3)[b]	25.5 (0.8)[b]	51.9 (0.4)[b]	13.0[ab]	2.2	3.3	6.7
230	15.1 (1.2)[b]	24.8 (0.5)[b]	42.5 (4.2)[b]	19.2[a]	2.9	4.8	8.2
303	24.1 (0.1)[a]	38.2 (0.5)[a]	62.8 (1.8)[a]	6.8[b]	1.6	2.6	4.3
339	21.1 (0.8)[a]	23.9 (0.1)[b]	45.9 (0.2)[b]	10.6[b]	2.2	2.5	4.9

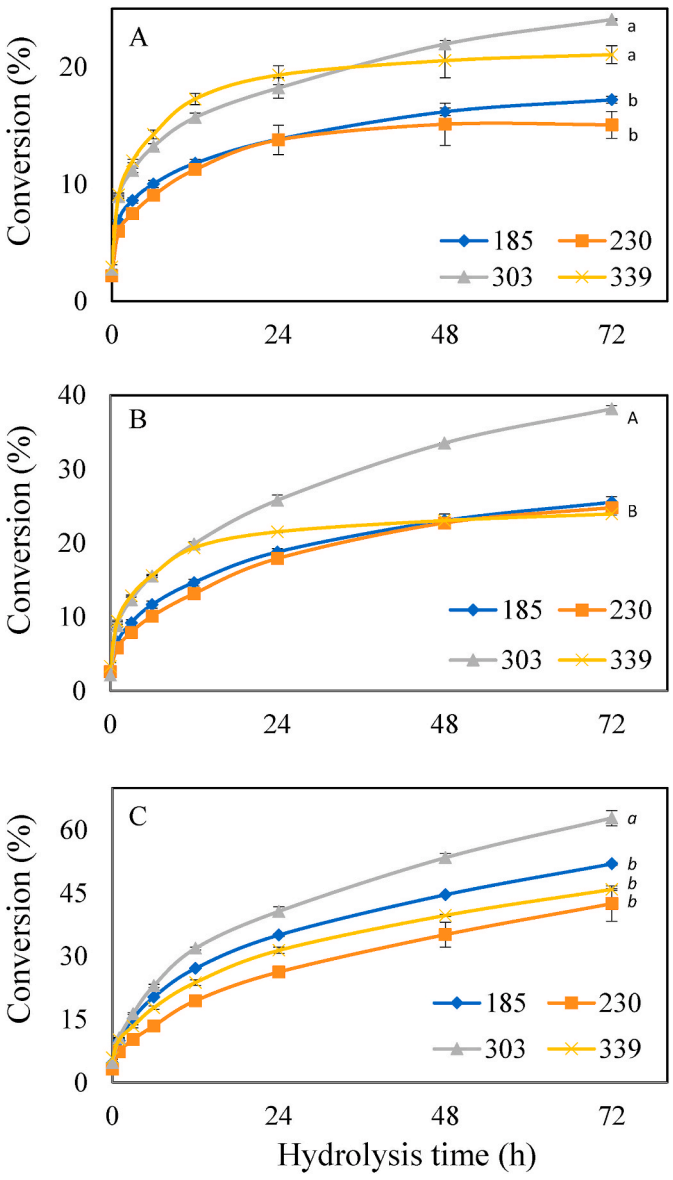

Fig. 4. Cellulose conversion rate over a 72 h period after dilute acid pretreatment at three different levels of severity. Estimated mean values (n = 3) of cellulose conversion obtained from the saccharification of wood samples at mild (A), moderate (B) and high (C) of pretreatment severity are shown. Standard error bars denote variability within each genotype and letters indicate significant difference ($p < 0.05$) between genotypes after 72 h of hydrolysis.

the physical, anatomical or chemical properties between the genotypes could act synergistically to give different degrees of performance in terms of conversion yield. In this context, the high amount of Ca in the genotype 230 along with relatively low levels of hemicellulose could indicate that there is more Ca per unit hemicellulose in the cell walls of genotype 230. Whether these interactions are causally related to recalcitrance in the cell walls of genotype 230 remains to be determined. While our results demonstrate that chemical analyses alone may be useful in screening poplar varieties for use in a fermentation-based biofuel operation, they are not a direct drop-in replacement for direct determination of the conversion rates. Aside from the biomass chemical composition impacting the conversion rates, physical properties should also be taken into consideration; for instance differences in specific gravity of the wood, could impact conversion by denser wood particle being less accessible to the digestion reagents, or the density of the wood causing different particle size distributions/shapes [56,57]. Overall,

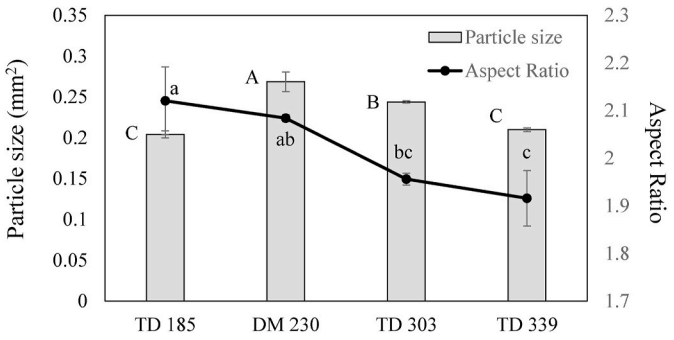

Fig. 5. Estimated mean values (n = 3) of particle size (mm^2) and aspect ratio (particle length divided by particle width) of four best performing poplar genotypes obtained from ImageJ analysis. Standard error bars denote variability within each genotype. Significant differences ($p < 0.05$) between genotypes in particle size (capital letters) and aspect ratio (small letters) are indicated.

these results suggest that it is important to conduct more detailed wood structural and compositional analyses and to optimize the biomass from different genotypes for bioconversion processes.

Although genotype 303 showed the highest cellulose conversion efficiency, given its low biomass yield, the estimated sugar yields were quite low compared to the high biomass yielding genotype 230 (Table 6b). Similar results were observed in another study that showed a TD hybrid poplar genotype with low field productivity had higher sugar release and a DM hybrid poplar genotype with high field productivity had lower sugar release [36]. These findings demonstrate the complexity of biomass feedstock production and the need for similar comprehensive investigations across bioenergy production pipeline to guide the selection of the optimal genotypes for fermentation-based biofuel operations. Nevertheless, breeders and producers still have to contend with tradeoffs between desirable traits such as high-biomass yield, disease resistance and bioconversion efficiency. Future studies need to focus on detailed understanding of the biology of tree development, factors involved in disease development and biomass conversion efficiencies to assess whether these tradeoffs can be genetically separated to obtain desirable genotypes for increased biomass productivity. New technological developments in the field of genetics, genomics, phenomics and bioconversion present new avenues for improving our understanding of the genetic variability in poplar and will further assist in developing sustainable feedstocks for bio-derived fuels and products.

5. Conclusions

Simultaneous assessment of poplar genotypes for increased productivity across the stages of biomass production and bioconversion revealed that there are tradeoffs between yields at different stages. Among the genotypes tested, the genotype 303 (TD) with the highest cellulose conversion efficiency had the lowest estimated sugar yields due to its low biomass yield, whereas the genotype 230 (DM) with the lowest conversion efficiency had the highest estimated sugar yields due to its high biomass yield. Genotypes had differential conversion efficiencies of enzymatic saccharification despite having only marginal changes in overall chemistry. Future studies need to focus on conducting more detailed wood structural analyses to optimize the biomass for bioconversion processes and to assess whether tradeoffs between agronomic yield and yield from bioconversion can be genetically separated to maximize overall biomass feedstock production efficiency.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a

potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2021.106259.

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