Growth of nitrogen-fixing eastern cottonwood (*Populus deltoides*) on reclaimed coal mine soils: a preliminary greenhouse trial of non-GM *P. deltoides*

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Introduction:

Coal mining in the Appalachians has resulted in largescale landscape disturbance of eastern forests, many of which are still in need of reclamation (Wickham et al. 2007, Wickham et al. 2013, Li et al. 2015, Pericak et al. 2018). These disturbances of forest landscapes impact many taxa of terrestrial and aquatic fauna, water quality, and have even been associated with mental health issues (Pond et al. 2014, Evans et al. 2015, Price et al. 2016, Canu et al. 2017, Maigret et al. 2019). Due to all of these impacts across many taxa and components of the ecosystem, reclamation and reforestation are critical in these areas. However, much of the mined land is limiting in plant macronutrients, which is major factor hindering the establishment of resilient plant communities (Lanning & Williams 1981, Li 1991, Li & Daniels 1994). Species capable of nitrogen fixation, including black locust (*Robinia pseudoacacia* (L.)), autumn olive (*Elaeagnus umbellate* Thunb.), and a number of herbaceous legumes, establish readily on reclaimed mine soils (Allan & Steiner 1965, Lanning & Williams 1981, Hardt & Forman 1989, Johnson & Skousen 1990).

Hybrid poplars are used in the production of biofuels, particularly in the Pacific Northwest, and widely studied to improve production efficiency (Sannigrahi et al. 2010, Bryant et al. 2020, Geiselman et al. 2020, Zalesny et al. 2020, Cho et al. 2021). While promising for more widescale energy production, poplar trees will likely need to be grown on marginal soils with low nutrient concentrations in order to meet the energy demands of the future. However, poplars require higher inputs of macronutrients than many other tree species due to poor nutrient use efficiency and may struggle to grow on marginal soils (Stanturf et al. 2001). A research group from the University of Florida, the University of Wisconsin-Madison, Weber State University, and the University of Tennessee has begun the 'NitFix Project', which aims to engineer bioenergy crops, including *Populus spp*., that are able to fix nitrogen (NitFix.org). These efforts would enable the production of a valuable crop with minimal inputs on marginal landscapes, such as reclaimed coal mines. The group has begun development of nitrogen-fixing *Populus deltoides* trees, and they are not ready for testing in a greenhouse or field setting, so we elected to initiate this study by growing non-GM *P. deltoides* on reclaimed soils to ascertain whether these may be viable planting sites. We initiated this investigation with a greenhouse study that would test the performance of several genotypes of *P. deltoides* trees on soils derived from parent materials at the Powell River Project. We focused on the survival, growth, and biomass of trees on soils derived from weathered mine spoil compared to standard prepared growth media (Burger et al. 2005, Skousen et al. 2011, Fields-Johnson et al. 2012).

Our primary research questions are:

1. Are there differences in the rooting ability of *Populus deltoides* among genotypes?
2. How do survival, growth, and biomass of *P. deltoides* differ between the mine spoil and control growth media?
3. Are there differences in growth or biomass of *P. deltoides* among genotypes that are observable when grown on mine spoil or control growth media?

Benefits:

This study was an initial investigation of the ability of *P. deltoides* to grow on reclaimed mine soils. Based on the results of this study, we are planning additional greenhouse and field studies of the GM nitrogen-fixing *P. deltoides* on mine spoils in the future to assess its' efficacy as a viable, relatively low input bioenergy crop for reclaimed mines in southwestern Virginia. The proximity to the hybrid power plant in Wise County could make these trees a valuable crop for these communities on degraded landscapes.

Methods:

Mine soils were collected from the Powell River Project (Harlan Formation) and derived from weathered and unweathered sandstones; these materials further weathered in an outdoor leaching experiment on the Virginia Tech campus from fall 2012 through spring 2022. Control planting media was a mixture of *Sphagnum* peat moss, vermiculite, perlite, Osmocote (20-4-8; 9 month), bone meal, gypsum, dolomite, and Epsom salt. All media were tested for saturated paste pH and SC, % C and % N, as well as concentrations of all major cations (Mehlich I). Coarse fragment content was determined to compare general textural differences in the media.

Cuttings from six different genotypes from five different locales were obtained from collaborators at the University of Florida (Table 1). Cuttings were rooted in the standard potting media generated by Reynold's Homestead (described above) on February 28, 2022 (Figure 1). Cuttings were treated with Zerotol 2.0 to minimize fungal growth and dipped in auxin to facilitate rooting, then monitored twice a week to assess rooting ability and leaf out.

Table 1. *Populus deltoides* genotypes, original locale of genotype, and total number of cuttings planted.

Genotype	Genotype locale	Total planted (count)
UF0138-2	**Weakley, TN**	21
UF0048	**Wake, NC**	30
UF0022	**Shelby, TN**	20
UF00049	**Anson, NC**	45
UF0046	**Wake, NC**	30
UF0221	**Lauderdale, TN**	35

Live trees were transplanted into growth media treatments in 300C pots (2.37 L) on June 14, 2022. All pots received 500 mL of 20-10-20 Peat-Lite, 250 ppm N on June 15, 2022. When the experiment began, we had planned to include a nitrogen fertilization treatment, but did not end up with enough trees to be able to do so. Half of trees were transplanted into mine spoils and the remaining half of trees were transplanted into control planting media as a control. Height and groundline diameter were measured again on July 27, 2022, at which point the trees had become so large that we elected to collect biomass the following week on August 2, 2022 before trees became rootbound (Figure 2). Height was remeasured on August 2 prior to biomass collection. Groundline diameter (GLD) was assumed to not have changed significantly since July 27 and was not remeasured before biomass collection. Biomass was separated into leaves (including petioles), stems + branches, and roots. Wet weights were obtained for leaves and stems. Roots were separated from media, which required significant washing (Figure 3), so wet weights were not obtained. Shoot weights were calculated by summing leaf and stem/branch weights. All biomass samples were dried and weighed. Percent water content in leaves, stems, and shoots was calculated by the following formula: ((Wet weight-dry weight)/(wet weight)*100) = % water content.

Figure 1. Planted *Populus deltoides* cuttings in Reynold's Homestead greenhouse.

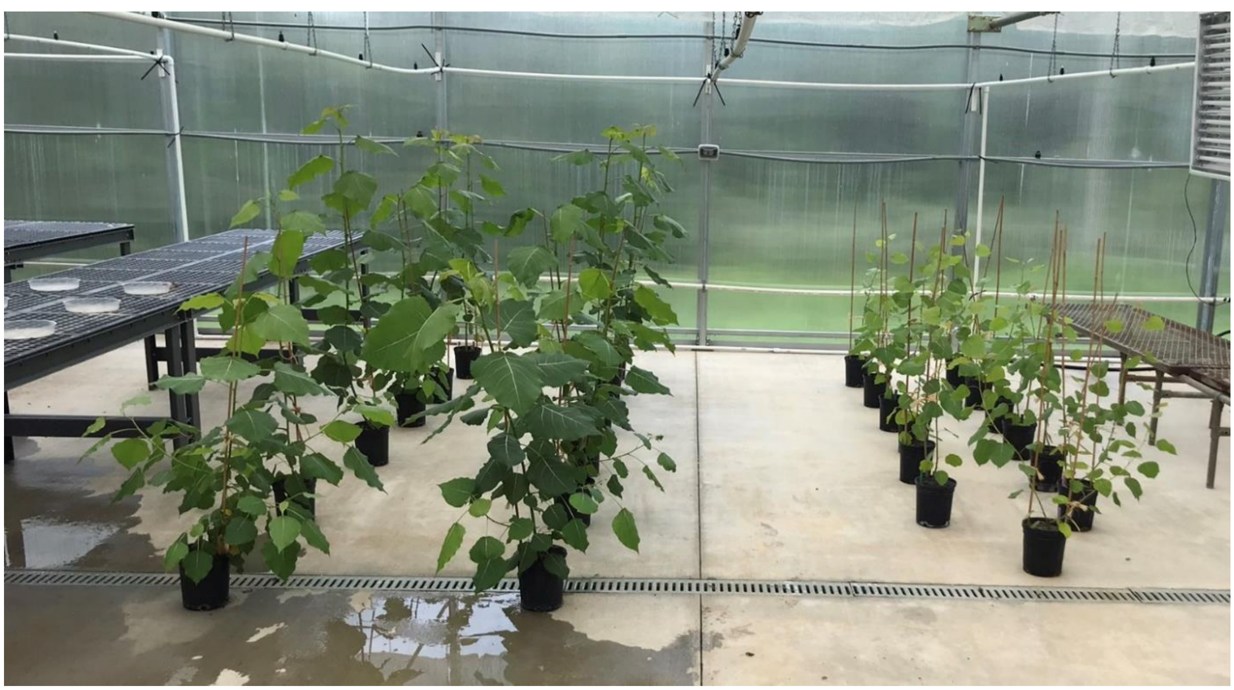

Figure 2. Growth of transplanted *Populus deltoides* trees grown in control media (left) and mine spoil (right).

Figure 3. Separation of *Populus deltoides* roots from growth media. Screen used to collect all fine roots as tree plug broken up and rinsed to remove planting media.

Results:

Soil analysis

Soil analysis showed that the Harlan mine spoil and control planting media were very different materials across almost all analytes (Tables 2 and 3). Notably, the planting media had much higher specific conductance (SC) compared to the mine spoil, due to the amendments of fertilizer, gypsum, and Epsom salt. As the base of planting media was Sphagnum peat moss, this material had much higher organic matter (OM) and carbon (%), as well as higher cation exchange capacity (CEC). All of the soil amendments in the planting media resulted in higher N, and most cations whereas the mine spoil was fairly limiting in most nutrients.

Table 2. Results of mine spoil/media analysis (pH, SC, CEC, overall size fractions, OM, N, C, and C:N).

Planting media	pH	SC	Cation exchange capacity (CEC)	Coarse fragments (>2 mm)	Fine particles (≤2 mm)	OM	N	C	C:N ratio
		mS/cm	meq/100g	%					
Harlan mine spoil	7.23	0.357	4.4	48.0	52.0	1.7	0.10	2.62	26.6
Control planting media	5.82	5.66	8.7	5.2	94.8	28.5	0.86	13.50	15.6

Table 3. Concentrations of major cations in the mine spoil and control planting media.

	P	K	Ca	Mg	Zn	Mn	Cu	Fe	B
	ppm								
Harlan mine spoil	59	42	704	93	4.8	32.7	1.4	46	0.1
Control planting media	107	220	668	464	3.2	17.0	1.0	32	0.1

Leaf out

At least one cutting from all six genotypes leafed out after planting cuttings, but by the time of transplant, only four genotypes were still alive (Figure 4; Table 4). Leaf out did not begin within any genotype before 22 days after planting, and took 28.4 ± 5.9 days on average across all genotypes. Overall, less than 40% of cuttings leafed out and about 15% of cuttings were alive at the time of transplant. We did note that the cuttings had dried out somewhat in transit which may have resulted in reduced rooting. In addition, there were limited primary buds so several of the cuttings were relying on accessory buds for new growth, which do not break dormancy as readily as primary buds (Kyle Peer, personal communication, 14 June 2022).

Figure 4. Leaf out of *Populus deltoides* cuttings on April 17, 2022 (left) and May 19, 2022 (right).

Table 4. Leaf-out and survival data for rooted *Populus deltoides* cuttings from all genotypes.

Genotype	Genotype locale	Total leafed out (%)	Average days to leaf-out ± SD	Survival	
				4/25/2022	6/14/2022
UF0138-2	Weakley, TN	42.9	30.9 ± 5.0	2	0
UF0048	Wake, NC	30.0	30.1 ± 8.9	3	3
UF0022	Shelby, TN	5.0	32.0 ± 0.0	0	0
UF00049	Anson, NC	66.7	26.3 ± 3.2	11	8
UF0046	Wake, NC	66.7	31.4 ± 7.8	8	8
UF0221	Lauderdale, TN	45.7	26.1 ± 2.8	10	9

Height and groundline diameter growth

Mean initial height of all trees when transplanted was 33.7 ± 6.6 cm and mean initial heights of the four genotypes ranged from 31.4 ± 4.8 cm in the UF0221 genotype to 37.0 ± 11.1 in the UF0046 genotype (Table 5). Mean initial groundline diameter (GLD) of all trees was 0.58 ± 0.15 cm and ranged from 0.52 ± 0.06 cm in the UF0048 genotype to 0.63 ± 0.23 in the UF0046 genotype. There were no differences in initial height or initial GLD among genotypes, and initial height did not differ between the mine spoil and control media.

Table 5. Initial heights and groundline diameters (means ± SD; measured June 14, 2022) of the *Populus deltoides* trees from the four genotypes.

Genotype	Genotype locale	Initial height	Initial GLD
		Mean ± SD (cm)	
UF0048	**Wake, NC**	32.0 ± 2.8 a*	0.52 ± 0.06 a
UF0221	**Lauderdale, TN**	31.4 ± 4.8 a	0.62 ± 0.15 a
UF0046	**Wake, NC**	37.0 ± 11.1 a	0.63 ± 0.23 a
UF00049	**Anson, NC**	34.8 ± 6.0 a	0.53 ± 0.10 a
*Different letters indicate significant differences among groups.			

After approximately 1.5 months of growth, trees had grown considerably in both of the soil media. Mean final height across all treatments was 97.7 ± 29.5 cm and mean growth across all treatments was 64.0 ± 28.6 cm. Final height ($p<0.001$), height growth ($p<0.001$), final GLD ($p<0.001$), and GLD growth ($p<0.001$) were all significantly greater in the control treatment than the mine spoil treatment (Table 6). Final height and GLD were both approximately 40% greater in the control media treatment than the mine spoil treatment. Height growth and GLD growth in the control treatment were more than double the growth observed in the mine spoil treatment. There were no observed differences in height, height growth, GLD, or GLD growth among genotypes or genotype locales (Tables 7 and 8).

Table 6. Initial, final, and growth of height and GLD means ± SD for the *Populus deltoides* trees grown in the control and mine spoil growth media.

Media treatment	Initial height	Final heightŧ	Height growth	Initial GLD	Final GLDŧ	GLD growth
	Mean ± SD (cm)					
Harlan mine spoil	33.7 ± 6.9 a*	76.7 ± 13.7 b	43.0 ± 10.6 b	0.63 ± 0.10 a	0.90 ± 0.12 b	0.28 ± 0.06 b
Control planting media	33.7 ± 6.7 a	120.5 ± 24.6 a	86.8 ± 24.0 a	0.53 ± 0.18 a	1.45 ± 0.24 a	0.93 ± 0.26 a
*Different letters indicate significant differences among groups. ŧFinal GLD measured on July 27, 2022 and final height measured on August 2, 2022.						

Table 7. Initial, final, and growth of height and GLD means ± SD for the *Populus deltoides* trees from the four genotypes.

Genotype	Genotype locale	Final heightŧ	Height growth	Final GLDŧ	GLD growth
		Mean ± SD (cm)			
UF0048	**Wake, NC**	100.0 ± 25.0 a*	68.0 ± 22.3 a	1.02 ± 0.25 a	0.50 ± 0.30 a
UF0221	**Lauderdale, TN**	79.8 ± 21.1 a	48.4 ± 18.1 a	1.26 ± 0.44 a	0.63 ± 0.43 a
UF0046	**Wake, NC**	103.3 ± 28.1 a	66.3 ± 33.6 a	1.07 ± 0.17 a	0.44 ± 0.32 a
UF00049	**Anson, NC**	113.4 ± 33.5 a	78.7 ± 32.8 a	1.19 ± 0.32 a	0.66 ± 0.40 a
*Different letters indicate significant differences among groups. ŧFinal GLD measured on July 27, 2022 and final height measured on August 2, 2022.					

Table 8. Initial, final, and growth of height and GLD means ± SD for the *Populus deltoides* trees from the three different genotype locales.

Genotype locale	Initial height	Final heightŧ	Height growth	Initial GLD	Final GLDŧ	GLD growth
	Mean ± SD (cm)					
Wake, NC	35.1 ± 8.9 a*	102.1 ± 25.1 a	66.9 ± 28.1 a	0.59 ± 0.19 a	1.05 ± 0.19 a	0.46 ± 0.29 a
Lauderdale, TN	31.4 ± 4.8 a	79.8 ± 21.1 a	48.4 ± 18.1 a	0.62 ± 0.15 a	1.26 ± 0.44 a	0.63 ± 0.43 a
Anson, NC	34.8 ± 6.0 a	113.4 ± 33.5 a	78.7 ± 32.8 a	0.53 ± 0.10 a	1.19 ± 0.32 a	0.66 ± 0.40 a
*Different letters indicate significant differences among groups. ŧFinal GLD measured on July 27, 2022 and final height measured on August 2, 2022.						

Biomass

Populus deltoides trees grown in the control media were visibly larger than those grown in the mine spoil and these observations were reflected in the biomass data (Figures 2, 5, and 6). Across all genotypes, total ($p<0.001$), root ($p<0.001$), stem ($p<0.001$), leaf ($p<0.001$), and shoot ($p<0.001$) biomass were all higher in the control media treatment than the mine spoil treatment (Table 9). Because roots needed to be washed to remove all planting media, a meaningful wet weight couldn't be obtained at harvest, so all root biomass data are presented on a dry weight basis and percent moisture was not calculated. Across all treatments, mean total biomass was 49.9 ± 27.2g; mean root biomass was 13.7 ± 4.7g; mean stem biomass was 14.1 ± 9.3g; mean leaf biomass was 22.1 ± 14.4g; and mean shoot biomass (stems + leaves) was 36.2 ± 23.4g. Mean root-to-shoot ratio (RSR) was 0.52 ± 0.28 (approximately 1:2 root:shoot dry weight). RSR was 0.29 ± 0.06 in the control planting media and 0.74 ± 0.23 in the mine spoil planting media. While root biomass in the control media treatment was nearly twice that of the mine spoil treatment, the observed difference between RSR was driven by a 400% difference in shoot biomass between the control media and mine spoil treatments. There were no significant differences in biomass among genotypes or locales (Table 10 and 11).

Figure 5. *Populus deltoides* leaf biomass collected from trees grown in mine spoil (left) and control media (right).

Table 9. Biomass means ± SD and root-to-shoot ratios of *Populus deltoides* trees grown in mine spoil and control planting media.

Media treatment	**Roots**	**Stems**	**Leaves**	**Shoots (stems + leaves)**	**Total biomass**	**Root:shoot ratio**
	Mean biomass ± SD (g)					
Harlan mine spoil	10.5 ± 1.9 b	6.0 ± 1.8 b	8.8 ± 1.0 b	14.8 ± 2.6 b	25.3 ± 2.9 b	0.74 ± 0.23 a
Control planting media	17.3 ± 4.3 a	22.8 ± 4.8 a	36.5 ± 4.1 a	59.3 ± 7.7 a	76.6 ± 10.5 a	0.29 ± 0.06 b

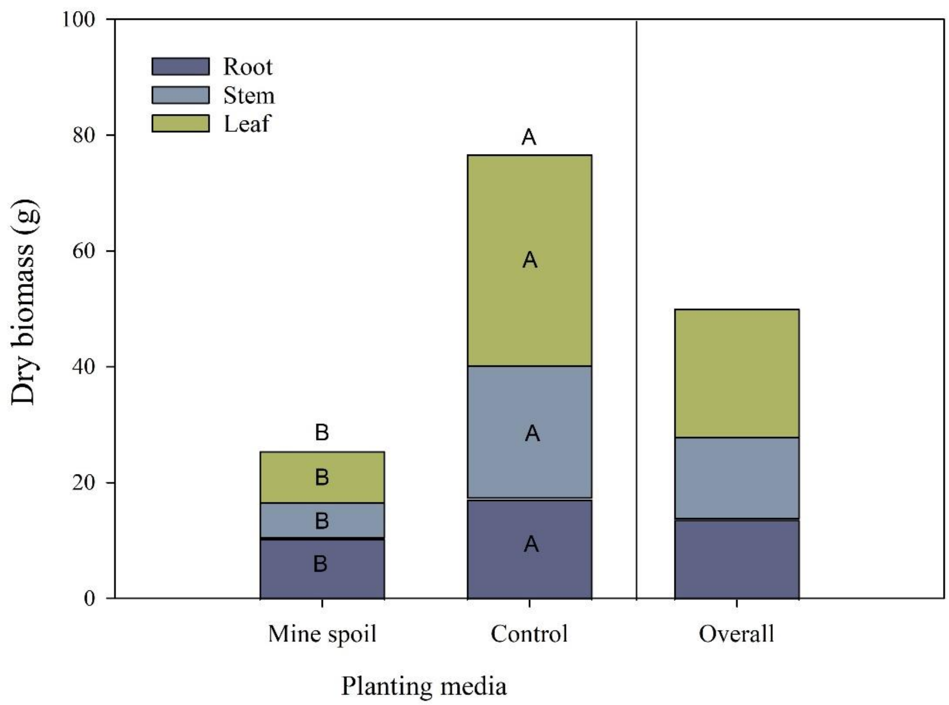

Figure 6. Mean total biomass (separated into roots, stems, and leaves) for all *Populus deltoides* trees in the mine spoil and control planting media treatments, as well as an overall summary for all tree.

Table 10. Biomass means ± SD and root-to-shoot ratios of *Populus deltoides* trees in four genotypes.

Genotype	Genotype locale	Roots	Stems	Leaves	Shoots (stems + leaves)	Total biomass	Root:shoot ratio
		Mean biomass ± SD (g)					
UF0048	**Wake, NC**	9.2 ± 3.4 a	10.7 ± 6.7 ab	17.1 ± 13.9 a	27.7 ± 20.6 a	36.9 ± 23.7 a	0.39 ± 0.15 a
UF0221	**Lauderdale, TN**	15.5 ± 4.2 a	12.8 ± 8.3 b	25.0 ± 15.5 a	37.8 ± 23.7 a	53.3 ± 27.7 a	0.59 ± 0.35 a
UF0046	**Wake, NC**	11.6 ± 2.4 a	13.6 ± 10.5 ab	18.8 ± 15.0 a	32.4 ± 25.5 a	44.1 ± 27.2 a	0.53 ± 0.31 a
UF00049	**Anson, NC**	14.8 ± 5.7 a	17.0 ± 11.1 a	22.8 ± 14.9 a	39.8 ± 26.0 a	54.7 ± 30.7 a	0.49 ± 0.24 a

Table 11. Biomass means ± SD and root-to-shoot ratios of *Populus deltoides* trees grown in three genotypes locales.

Genotype locale	Roots	Stems	Leaves	Shoots (stems + leaves)	Total biomass	Root:shoot ratio
	Mean biomass ± SD (g)					
Wake, NC	10.7 ± 2.9 a*	12.5 ± 8.9 a	18.1 ± 13.6 a	30.7 ± 22.3 a	41.4 ± 24.4 a	0.48 ± 0.26 a
Lauderdale, TN	15.5 ± 4.2 a	12.8 ± 8.3 a	25.0 ± 15.5 a	37.8 ± 23.7 a	53.3 ± 27.7 a	0.59 ± 0.35 a
Anson, NC	14.8 ± 5.7 a	17.0 ± 11.1 a	22.8 ± 14.9 a	39.8 ± 26.0 a	54.7 ± 30.7 a	0.49 ± 0.24 a

*Different letters indicate significant differences among groups.

Across all treatments, mean percent water content for stems, leaves, and shoots were 72.0 ± 6.3%, 69.6 ± 4.2%, and 70.6 ± 4.8%, respectively. Percent water content of leaves ($p<0.001$), stems ($p<0.001$), and shoots ($p<0.001$) were all higher in the control media treatment than the mine spoil treatment (Table 12). Percent water content did not differ among genotypes or genotype locales (Tables 13 and 14).

Table 12. Percent water content of *Populus deltoides* stems, leaves, and shoots in the mine spoil and control planting media treatments.

Media treatment	Stem	Leaf	Shoots (stems + leaves)
	Mean water content (%)		
Mine spoil	67.7 ± 6.0 b	66.3 ± 3.0 b	66.8 ± 3.7 b
Control	76.6 ± 1.7 a	73.2 ± 1.2 a	74.6 ± 1.4 a

Table 13. Percent water content of *Populus deltoides* stems, leaves, and shoots from the four genotypes.

Genotype	Genotype locale	Stem	Leaf	Shoots (stems + leaves)
		Mean water content (%)		
UF0048	**Wake, NC**	68.0 ± 8.2 a	66.2 ± 5.3 a	66.9 ± 6.5 a
UF0221	**Lauderdale, TN**	72.1 ± 7.8 a	69.5 ± 4.7 a	70.4 ± 5.5 a
UF0046	**Wake, NC**	72.2 ± 4.6 a	70.4 ± 3.2 a	71.2 ± 3.2 a
UF00049	**Anson, NC**	73.3 ± 5.3 a	70.5 ± 3.9 a	71.8 ± 4.5 a

Table 14. Percent water content of *Populus deltoides* stems, leaves, and shoots from the three genotype locales.

Genotype locale	Stem	Leaf	Shoots (stems + leaves)
	Mean water content (%)		
Wake, NC	70.6 ± 6.0 a	68.8 ± 4.3 a	69.6 ± 4.7 a
Lauderdale, TN	72.1 ± 7.8 a	69.5 ± 4.7 a	70.4 ± 5.5 a
Anson, NC	73.3 ± 5.3 a	70.5 ± 3.9 a	71.8 ± 4.5 a
*Different letters indicate significant differences among groups.			

Summary and conclusions:

Initially, we observed some differences among genotypes in the number of cuttings that leafed out and survived to be repotted. In particular, UF00049, UF0046, and UF0221 rooted and survived well. We did note that many of the cuttings had dried out during transit, which may have resulted in reduced rooting of poplar cuttings. Once trees were repotted into the planting media treatments, only planting media resulted in significant differences in height, GLD, and biomass metrics, and no differences were observed among genotypes or genotype locales. No trees died after repotting in the growth media treatments. It is possible that the particular metrics we were studying do not inherently have large differences among the particular genotypes we were testing.

Trees within the control planting media were visibly larger and, anecdotally, the foliage of trees in control pots appeared greener and leaves were larger (visible in Figures 2 and 5). Biomass allocation between roots and shoots differed between the planting media, but as discussed above, this was largely driven by much higher shoot biomass in the control planting media. Mean RSR in the control planting media was similar to RSR observed in numerous field and greenhouse studies of *Populus spp*., while RSR in the mine spoil treatment was considerably higher than other studies (Elferjani et al. 2014, Phillips et al. 2014, Krabel et al. 2015, Rogers et al. 2019, Li et al. 2020). Li et al. observed that RSR was higher in N-deficient soils (no added fertilizer) than under a normal fertilization regime, which could explain the observed higher RSR in the low N mine spoil (2020).

There were clear differences in growth between the control planting media and the mine spoil trees, however there were also several differences in soil characteristics between the planting media that are related to reduced growth. Specifically, the control planting media had higher P, K, N, Mg, C, OM, and CEC than the mine spoil, and the control media was also lower in pH and much lower in coarse fragment content. Because of the many differences between the media types, it is not possible at this time to definitively conclude why trees grew more in the control treatment. However, it has been established that *Populus spp*. have poor nutrient use efficiency and therefore have higher requirement for nutrients, such as N, than other tree species (Stanturf et al. 2001). Studies showing that additions of N, N+P, and N+P+K+S fertilizers resulted in increased growth of four-year old hybrid poplars on plantation sites, suggesting that higher macronutrient concentrations in the control media were a significant factor in this study (van den Driessche et al. 2008). Rogers et al. observed lower growth of hybrid poplars grown on marginal soils of landfills compared to control soils (2019). In this study, we observed chlorosis in some of the leaves in the mine spoil treatment (visible in Figure 5), which may be indicative of some nutrient limitations. Phillips et al. noted that unevenly textured soils, such as the Harlan mine spoil that was nearly 50% coarse fragments, can result in reduced root growth in hybrid poplars (2014). In this study, root biomass was 75% higher in the control media treatment while shoot biomass was approximately 300% higher in the control media treatment. So while the smaller roots in the mine spoil may have been a contributing factor to overall lower biomass in the mine spoil treatment, shoot biomass contributed more to overall biomass. However, smaller roots would have limited nutrient and water uptake, affecting growth, so this variable should not be discounted.

Lower water content in leaves, stems, and shoots was also observed in trees grown in the mine spoil treatment compared to the control planting media. Given the much lower OM and coarser texture of the Harlan mine spoil, we would expect water holding capacity to be much lower in this material compared to the control planting media. Water holding capacity has been shown to increase in mined alfisols in China, even over relatively short timescales, as OM% increases (Yuan et al. 2019). Certainly lower water content would have directly affected plant growth, but it is possible that lower water content also translated to lower nutrient uptake, indirectly affecting plant growth (Lara-Villa et al. 2022).

While we were unable to incorporate a fertilization treatment in this study, we are planning future work that would compare growth of wildtype *P. deltoides* trees under a series of N fertilization treatments with growth of transgenic, N-fixing *P. deltoides* trees, allowing us to further investigate the importance of the other soil characteristics, such as coarse fragment content. Unfortunately, the timeline for the availability of the N-fixing *P. deltoides* trees is unclear, but we look forward to comparing fertilization treatments against N-fixing abilities.

Relationship to PRP Strategic Plan:

Dr. Jason Holliday is an early career researcher in the Department of Forest Reclamation and Environmental Considerations. He is involved in a number of projects related to forest population genetics and adaptive evolution in the face of anthropogenic climate change. Mine reclamation in the Anthropocene will benefit from an increased understanding of the association between genotypes and the resulting phenotypes.

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References:

Allan, Philip F. and W.W. Steiner. (1965). Autumn olive for wildlife and other conservation uses. Leaflet No. 458 [Revised]. Washington, DC: U.S. Department of Agriculture. 8 p. [44936]

Brown, J.H. and C.J. Greenberg. (1998). Growth of cottonwood and sycamore in relation to age, soils and topography on pre-1972 Ohio strip mine law sites. *International Journal of Surface Mining, Reclamation, and the Environment* **12(**2): 91-96. DOI: 10.1080/09208118908944029

Bryant N.D., Y. Pu, T.J. Tschaplinski, G.A. Tuskan, W. Muchero, U.C. Kalluri, C.G. Yoo, and A.J. Ragauskas. (2020). Transgenic Poplar Designed for Biofuels. *Trends in Plant Science* **25**(9):881-896. doi: 10.1016/j.tplants.2020.03.008. Epub 2020 May 29. PMID: 32482346.

Burger J.A., D. Graves, P. Angel, V. Davis, and C. Zipper. (2005). The forestry reclamation approach. U.S. Office of Surface Mining, Department of Interior, Washington. Forest Reclamation Advisory 2.

Canu, W.H., J.P. Jameson, E.H. Steele, and M. Denslow. (2017). Mountaintop removal coal mining and emergent cases of psychological disorder in Kentucky. *Community Mental Health Journal* **53** (7): 802-810.

Cho, J.S., M.H. Kim, E.K. Bae, Y.I. Choi, H.W. Jeon, K.H. Han, and J.H. Ko. (2021). Field evaluation of transgenic hybrid poplars with desirable wood properties and enhanced growth for biofuel production by bicistronic expression of *PdGA20ox1* and *PtrMYB3* in wood-forming tissue. Biotechnology for *Biofuels* **14**:177.

Daniels, W.L., L.W. Zelazny, and C.J. Everett. (1987). Virgin hardwood forest soils of the southern Appalachian Mountains: II. weathering, mineralogy, and chemical properties. *Soil Science Society of America Journal* **51**: 730-738.

Elferjani, R., A. Desrochers, and F. Tremblay. (2014). Effects of mixing clones on hybrid poplar productivity, photosynthesis, and root development in northeastern Canadian poplulations. *Forest Ecology and Management* **327**: 157-166.

Evans, D.M., C.E. Zipper, E.T. Hester, and S.H. Schoenholtz. (2015). Hydrologic effects of surface coal mining in Appalachia (U.S.). *Journal of the American Water Resources Association* **51**(5): 1436-1452.

Fields-Johnson, C.W. (2011). Appalachian surface mine reforestation techniques: effects of grading, cultural treatments, and species selection. M.S. Thesis; Virginia Tech, Blacksburg, VA.

Fields-Johnson C.W., C.E. Zipper, J. Burger, and D. Evans. (2012). Forest restoration on steep slopes after coal surface mining in Appalachian USA: Soil grading and seeding effects. *Forest Ecology and Management* **270**: 126–134.

Geiselman, G.M., J. Kirby, A. Landera, P. Otoupal, G. Papa, C. Barcelos, E.R. Sundstrom, L. Das, H.D. Magurudeniya, M. Wehrs, A. Rodriguez, B.A. Simmons, J.K. Magnuson, A. Mukhopadhyay, T.S. Lee, A. George, and J.M. Gladden. (2020). Conversion of poplar biomass into high-energy density tricyclic sesquiterpene jet fuel blendstocks. *Microbial Cell Factories*, **19**:208.

Hardt, R.A. and R.T.T. Forman. (1989). Boundary form effects on woody colonization of reclaimed surface mines. *Ecology* **70**(5): 1252-1260.

Johnson, C. D.; Skousen, J. G. (1990). Tree species composition, canopy coverage, and importance on several AML sites in northern West Virginia. *Proceedings of the 1990 mining and reclamation conference and exhibition*; 1990 April 23-26; Charleston, WV. Morgantown, WV: West Virginia University, Agricultural and Forestry Experiment Station: 545-553.

Krabel, D., M. Meyer, A. Solger, R. Muller, P. Carvalho, and J. Foulkes. (2015). Early root and aboveground biomass development of hybrid poplars (Populus spp.) under drought conditions. *Canadian Journal of Forest Research* **45**: 1289-1298.

Lanning, S. and S.T. Williams. (1981). Nitrogen and land reclamation. *Environmental Pollution (Series B)* **2**: 179-191.

Lara-Villa, M.A., J.L. Flores-Flores, F. Alatriste-Mondragon, and R. Mata-Gonzalez. (2022). Plant production in semiarid shrubland communities amended with biosolids in Central Mexico. *Canadian Journal of Soil Sciences* **102**: 719-731.

Li, R. (1991). Nitrogen cycling in young mine soils in southwest Virginia. PhD Dissertation, Virginia Tech, Blacksburg.

Li, R.S. and W.L. Daniels. (1994). Nitrogen accumulation and form over time in young mine soils. *Journal of Environmental Quality* **23**: 166-172.

Li, J., C.E. Zipper, P.F. Donovan, R.H. Wynne, and A.J. Oliphant. (2015). Reconstructing disturbance history for an intensively mined region by time-series analysis of Landsat imagery. *Environmental Monitoring and Assessment* **187** (9): 1-17.

Li, Y., J. Kang, Z. Li, H. Korpelainen, and C. Li. (2020). Ecophysiological responses of two poplar species to intraspecific and interspecific competition under different nitrogen levels. *Journal of Plant Ecology* **13**: 693-703.

Maigret, T.A., J.J. Cox, and J. Yang. (2019). Persistant geophysical effects of mining threaten ridgetop biota of Appalachian forests. *Frontiers in Ecology and the Environment* **17**(2): 85-91.

Nussbaumer, Y., M.A. Cole, C.E. Offler, and J.W. Patrick. (2016). Identifying and ameliorating nutrient limitations to reconstructing a forest ecosystem on mined land. *Restoration Ecology* **24**(2): 202-211.

Phillips, C.J., M. Marden, and L.M. Suzanne. (2014). Observations of root growth of young poplar and willow planting types. *New Zealand Journal of Forestry Science* **44**:15.

Pond, G.J., M.E. Passmore, N.D. Pointon, J.K. Felbinger, C.A. Walker, K.J.G. Krock, J.B. Fulton, and W.L. Nash. (2014). Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia. *Environmental Management* - **54** (4): 919-933.

Price, S.J., B.L. Muncy, S.J. Bonner, A.N. Drayer, and C.D. Barton. (2016). Effects of mountaintop removal mining and valley filling on the occupancy and abundance of stream salamanders. *Journal of Applied Ecology* **53** (2): 459-468.

Rogers, E.R., R.S. Zalesny Jr., R.A. Hallett, W.L. Headlee, and A.H. Wiese. (2019). Relationships among root-shoot ratio, early growth, and health of hybrid poplar and willow clones grown in different landfill soils. *Forests* **10**:49. doi:10.3390/f10010049

Sannigrahi, P., A.J. Ragauskas, and G.A. Tuskan. (2010). Poplar as a feedstock for biofuels: A review of compositional characteristics. *Biofuels, Bioproducts, and Biorefining* **4**: 209-226.

Skousen J., C.E. Zipper, J. Burger, C. Barton, P. Angel. 2011. Selecting materials for mine soil construction when establishing forests on Appalachian mine sites. U.S. Office of Surface Mining, Department of Interior, Washington. Forest Reclamation Advisory 8.

Stanturff, J.A., C. van Oosten, D.A. Netzer, M.D. Coleman, C.J. Portwood. (2001). Ecology and silviculture of poplar plantations. In: Dickmann, D.I., J.G. Isebrands, J.E. Eckenwalder, J. Richardson (eds) Poplar Culture in North America. NRC Research Press, Ottawa, ON, p.397.

Van den Driessche, R., B.R. Thomas, and D.P. Kamelchuk. (2008). Effects of N, NP, and NPKS fertilizers applied to four-year old hybrid poplar plantations. *New Forests* **35**: 221-233.

Wickham, J.D., K.H. Riitters, T.G. Wade, M. Coan, and C. Homer. 2007. The effect of Appalachian mountaintop mining on interior forest. *Landscape Ecology* **22**: 179-187.

Wickham J.D., P.B. Wood, M.C. Nicholson, W. Jenkins, D. Druckenbrod, et al. (2013). The overlooked terrestrial impacts of mountaintop mining. *Bioscience* **63**:335–348.

Yuan, Y., X. Li, D. Xiong, H. Wu, S. Zhang, L. Liu, and W. Li. (2019). Effects of restoration age on water conservation function and soil fertility quality of restored woodlands in phosphate mined-out areas. *Environmental Earth Sciences* **78**:653. doi: 10.1007/s12665-019-8671-8

Zelasny Jr., R.S., J.Y. Zhu, W.L. Headlee, R. Gleisner, A. Pilipovic, J. Van Acker, E.O. Bauer, B.A. Birr, and A.H. Wiese. (2020). Ecosystems services, physiology, and biofuels recalcitrance of poplars grown for landfill phytoremediation. *Plants* **9**(10): 1357. doi: 10.3390/plants9101357. PMID: 33066487; PMCID: PMC7602285.