Available at www.sciencedirect.com

ScienceDirect

http://www.elsevier.com/locate/biombioe

BIOMASS & BIOENERGY

Ex situ growth and biomass of *Populus* bioenergy crops irrigated and fertilized with landfill leachate

Ronald S. Zalesny Jr.*, Adam H. Wiese, Edmund O. Bauer, Donald E. Riemenschneider

US Forest Service, Northern Research Station, Institute for Applied Ecosystem Studies, 5985 Highway K, Rhinelander, WI 54501, USA

ARTICLE INFO

Article history:
Received 30 June 2005
Received in revised form
6 November 2006
Accepted 1 April 2008
Available online 2 June 2008

Keywords:
Populus deltoides
P. nigra
P. maximowiczii
Hybrid poplar
Phytoremediation
Sustainable intensive forestry
Clonal selection

ABSTRACT

Merging traditional intensive forestry with waste management offers dual goals of fiber and bioenergy production, along with environmental benefits such as soil/water remediation and carbon sequestration. As part of an ongoing effort to acquire data about initial genotypic performance, we evaluated: (1) the early aboveground growth of trees belonging to currently utilized *Populus* genotypes subjected to irrigation with municipal solid waste landfill leachate or non-fertilized well water (control), and (2) the above- and below-ground biomass of the trees after 70 days of growth. We determined height, diameter, and number of leaves at 28, 42, 56, and 70 days after planting (DAP), along with stem, leaf, and root dry mass by testing six *Populus* clones (DN34, DN5, I4551, NC14104, NM2, NM6) grown in a greenhouse in a split–split plot, repeated measures design with two blocks, two treatments (whole-plots), six clones (sub-plots), and four sampling dates (sub–sub-plots, repeated measure). Treatments (leachate, water) were applied every other day beginning 42 DAP. The leachate-treated trees exhibited greater height, diameter, and number of leaves at 56 and 70 DAP ($P<0.05$). There was broad variation in clonal responses to leachate treatment for dry mass, with a general trend of leachate-treated trees exhibiting greater stem and leaf dry mass ($P<0.05$), but negligible differences for root dry mass ($P>0.05$). Overall, genotypic responses to the leachate treatment were clone-specific for all traits.

Published by Elsevier Ltd.

1. Introduction

The use of *Populus* for fiber and bioenergy is more established in North America than deployment of these short rotation woody crops for technologies such as phytoremediation [1,2]. Nevertheless, merging traditional intensive forestry with waste management offers dual goals of fiber and bioenergy production, along with environmental benefits such as soil/water remediation and carbon sequestration [3,4]. From a tree improvement standpoint, the broad variation within the genus *Populus* [5–7] promotes the incorporation of traits that are necessary for multiple end-uses into strategic (selection of parental species) or operational (selection within specific genomic groups) breeding plans [8,9]. Such variation supports the opportunity for intra- and inter-specific selection of favorable genotypes, which is important for achieving high levels of success when the trees are established in field-based systems [1,10]. Regardless of the end-uses of the trees, however, field deployment of unfavorable clones often leads to inadequate plantation productivity or even complete failure. In addition, the elevated costs of testing tissue concentration levels and other physiological processes likely are not feasible at the initial stages of plantation management [11].

Therefore, it is vital during early establishment and growth to conduct inexpensive screening trials such as phyto-recurrent selection to evaluate allometric traits that are indicative of field-based performance [11,12], because

*Corresponding author. Tel.: +1 715 362 1132; fax: +1 715 362 1166.
E-mail address: rzalesny@fs.fed.us (R.S. Zalesny Jr.).
0961-9534/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.biombioe.2008.04.012

successful establishment is the first biological requirement for long-term ecological sustainability. Thus, genotypes that exhibit successful early development are more likely than those that are erratic to accumulate adequate biomass for fiber and bioenergy, while serving as biological filters involved with uptake, storage, utilization, or volatilization of contaminants [13–15]. Our initial hypotheses for the current study were two-fold. First, an identical *Populus* genotype will exhibit different phenotypic responses when irrigated with municipal solid waste landfill leachate or non-fertilized well water (i.e. genotype × treatment interactions will exist). Second, phenotypic responses will differ among clones. Thus, as part of an ongoing effort to acquire data about initial genotypic performance, our objectives were to conduct a single cycle of phyto-recurrent selection in the greenhouse and to evaluate: (1) the early aboveground growth of trees belonging to currently utilized *Populus* genotypes subjected to irrigation with landfill leachate or non-fertilized water (control), and (2) the above- and below-ground biomass of the trees after 70 days of growth. Four of the six clones (DN34, DN5, I4551, NM6) tested in the current study were selected and established during June 2001 in a long term, bioenergy/phytoremediation plantation at the Oneida County Landfill in northern Wisconsin.

2. Materials and methods

2.1. Clone selection and tree establishment

Six F_1-hybrid clones were selected across the current range of genotypes from three *Populus* genomic groups in January 2001 based on their growth in earlier studies and anticipated establishment potential. The clones and their parentages were: DN34, DN5, I4551 (*Populus deltoides* Bartr. ex Marsh × *Populus nigra* L.); NC14104 (*P. deltoides* × *Populus maximowiczii* A. Henry); and NM2, NM6 (*P. nigra* × *P. maximowiczii*).

Shoots were collected from stool beds established at Hugo Sauer Nursery in Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). Hardwood cuttings, 20 cm long, were prepared during January 2001, with cuts made to position at least one primary bud not more than 2.54 cm from the top of each cutting. Cuttings were stored in polyethylene bags at 5 °C until planting in February 2001. Cuttings were soaked in water to a height of 15 cm for 5 days before planting in pots containing medium consisting of equal parts of sand, peat, and vermiculite (v:v:v). The medium per pot had a volume and dry mass of 6600 cm^3 and 3.5 kg, respectively. The trees were grown in a greenhouse at the Institute for Applied Ecosystem Studies (IAES), with a 16-h photoperiod and a constant temperature of 21 °C.

2.2. Experimental design and irrigation treatments

The trees were arranged in a split–split plot, repeated measures design, with two blocks, two treatments, six clones, and four sampling dates. Treatments were considered fixed whole-plots, clones were fixed sub plots, and sampling dates were fixed sub–sub plots. Sampling date was treated as the repeated measure. Clones were arranged in randomized complete blocks in order to minimize effects of any potential environmental gradients in the greenhouse, with four ramets per clone per block × treatment interaction. Treatments, clones, and sampling dates were treated as fixed in the analysis and, therefore, we evaluated means rather than variances.

Table 1 – Composition of landfill leachate and well water (control) used for irrigation treatments in an experiment testing the early growth and biomass of *Populus*

Component	Leachate	Control
pH	8.0	6.3
Electrical conductivity	8.7	0.2
Nitrogen	420.0	0.2
Phosphorus	1.6	0.0
Potassium	420.0	1.1
Sodium	1100.0	2.4
Chloride	1000.0	3.5
Boron	12.0	0.1
Iron	33.0	0.7

Units for electrical conductivity are $m S cm^{-1}$, while those for elemental concentrations are $mg L^{-1}$.

The irrigation treatments, of equal volume, consisted of municipal solid waste landfill leachate from the Oneida County Landfill (OCL) and non-fertilized water (control) from a well at the IAES. The OCL and IAES were located 6 km west and 0.8 km north of Hugo Sauer Nursery, respectively. The composition of the leachate and water, including pH, electrical conductivity, and concentration of nitrogen (N), phosphorus (P), potassium (K), sodium (Na), chloride (Cl), boron (B), and iron (Fe), are listed in Table 1. The primary toxicity concerns were the relatively high Cl and Na concentrations. In addition, the concentrations of B and Fe were of concern, having exceeded United States Environmental Protection Agency maximum concentration limits by 600% and 165%, respectively [16]. Additional inorganic elements, along with organic compounds, generally were not detectable and were, therefore, not a concern with respect to plant establishment.

All trees were irrigated with water every other day for the initial 6 weeks following planting to ensure adequate survival and health. In the remaining 4 weeks of the study, trees were irrigated every other day with equal volumes of leachate or water. The amount of either irrigation treatment needed was calculated by measuring the saturation point of the potting medium as the plants developed. Six non-experimental trees were planted in pots and placed on the greenhouse benches, with one pot per clone studied. The saturation point in these pots was used to determine the amount of leachate and water needed for each clone as the trees developed over time.

2.3. Data collection and analysis

Height, diameter, and number of leaves were determined at 28, 42, 56, and 70 days after planting (DAP). Height was measured at the point of attachment between the stem and the original cutting, and diameter was measured at 2.54 cm from the point of attachment between the stem and the original cutting, in order to reduce experimental error. The number of leaves was used in lieu of leaf area because of the greater relative ease of non-destructively sampling the leaves and overall efficiency. Number of leaves was highly positively correlated ($r = 0.88$, $P < 0.0001$) with leaf area when using pooled data from a study testing the response of poplar cuttings at 14, 31, and 46 DAP to irrigation with similar landfill leachate [12]. In addition, the number of leaves was determined according to the leaf plastochron index (LPI), which is an index of morphological time scale that supports plant comparisons under large environmental and/or developmental variance [17]. Specifically, leaf lamina length of 2 cm was used as a unified reference for plastochron index development. Lamina length was chosen as a reference because it was an arbitrary non-destructive developmental measure. Therefore, LPI 0 was the index leaf of 2 cm, LPI 1, 2, and so on were the leaves above LPI 0 that were not yet 2 cm, and LPI 1, 2, and so on were the leaves below LPI 0 that were greater than 2 cm. Leaves of LPI 0 and greater were used for the analysis.

Stem, leaf, and root dry mass were determined at 70 DAP. Trees were harvested and washed prior to dissection of stems, leaves, and roots. Following dissection, individual plant components were bagged and dried at 70 °C for dry mass determination.

Data on height, diameter, and number of leaves were subjected to analyses of variance according to the split–split plot, repeated measures design described above [SAS® PROC GLM; 18]. Likewise, data on stem, leaf, and root dry mass were subjected to analyses of variance according to a split plot design with all classification variables as described above and at one sampling date, 70 DAP [SAS® PROC GLM; 18].

Analyses of covariance were conducted to test for the effect of cutting size on all traits because of a broad variation in cutting dry mass at 70 DAP (2.12±0.26–23.71±0.26 g). Cutting dry mass was a significant covariate for height, diameter, and number of leaves ($P < 0.0001$), along with stem and leaf dry mass ($P < 0.0001$, $P = 0.0001$, respectively); however, cutting dry mass did not have a significant effect on root dry mass ($P = 0.9866$). Therefore, all means except for root dry mass were adjusted for the variation in cutting dry mass. Fisher's protected and unprotected least significant difference (LSD) was used to compare adjusted means for both models [19].

3. Results and discussion

3.1. *Height, diameter, and number of leaves over time*

Clones differed for height, diameter, and number of leaves across treatments and sampling dates ($P < 0.0001$, $P = 0.0004$, $P < 0.0001$, respectively), while the main effect of treatment was significant for diameter ($P = 0.034$) but negligible for height ($P = 0.2703$) and number of leaves ($P = 0.1895$). Trees receiving leachate irrigation exhibited 5% greater diameter than those with water treatment. Despite a negligible interaction between treatment and clone for height, diameter, and number of leaves across sampling dates ($P = 0.6617$,

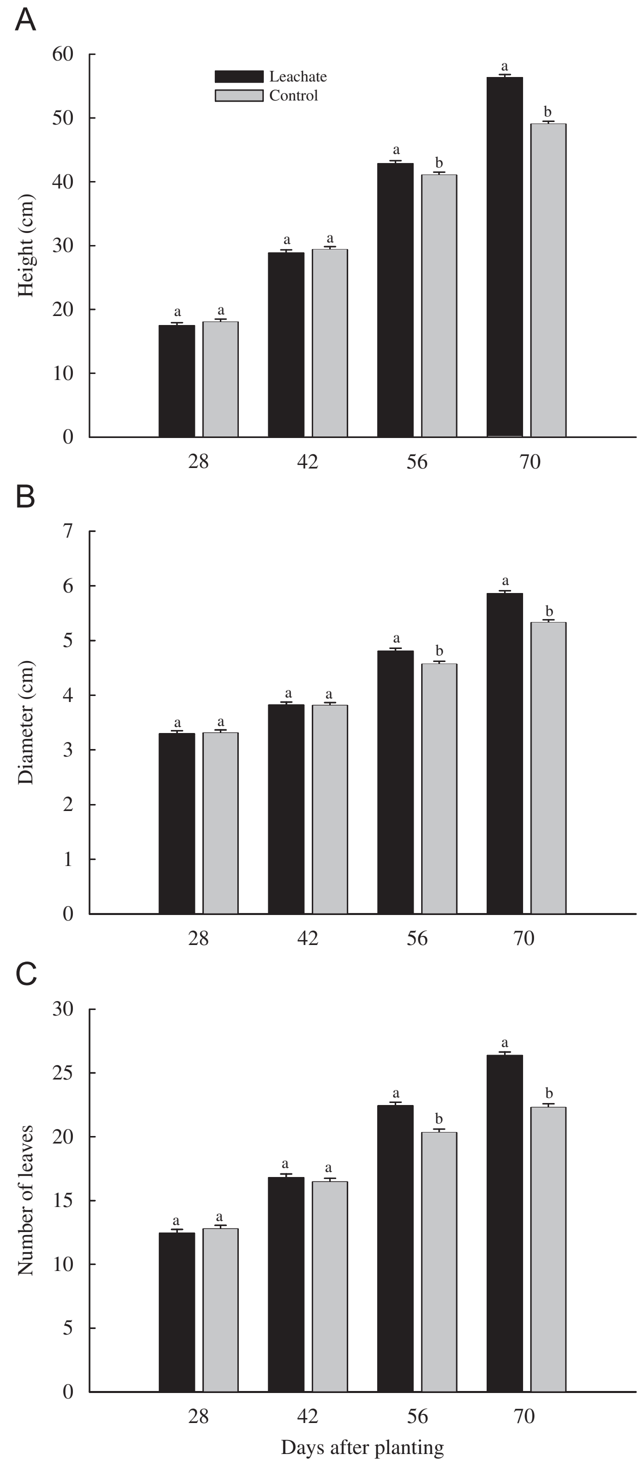

Fig. 1 – Height (A), diameter (B), and number of leaves (C) adjusted for cutting dry mass across six *Populus* clones at 28, 42, 56, and 70 days after planting following landfill leachate irrigation every other day beginning on day 42. The control treatment was application of non-fertilized water only. Each bar represents the mean of 48 trees with one standard error. Bars labeled with different letters within a sampling date were different according to Fisher's protected LSD at $P < 0.05$.

0.6814, 0.5734, respectively), the main effect of date, as well as, the clone × date and treatment × date interactions, were significant for all traits ($P < 0.0001$). Given that all trees were treated identically through 42 DAP, there was no difference between the leachate and control treatments for any dependent variable throughout this time. However, trees receiving the leachate treatment exhibited greater height, diameter, and number of leaves at 56 and 70 DAP (Fig. 1). The relative growth advantage of leachate-treated trees increased from 56 to 70 DAP. Trees receiving the leachate treatment exhibited 4%, 5%, and 10% greater height, diameter, and number of leaves, respectively, than the control trees at 56 DAP. The leachate-advantage increased to 15%, 10%, and 18% at 70 DAP. This growth advantage of trees receiving leachate exhibited a fertilization effect that most likely was the result of the N content of the leachate versus the unfertilized control. In addition, the confounding effects of P and K fertilization may have been present. However, field studies testing four of the identical clones (DN5, NC14104, NM2, NM6) and leachate from the same source as in the current study have shown similar relative growth increases even when N, P, and K were equalized across irrigation treatments [1,3,10].

Despite the young age of our trees relative to most in the literature (1–7 years old), practical comparisons are possible. The application rate of 107 kg N ha^{-1} in the current study is consistent with reported recommendations of 105 kg N ha^{-1} for *P. deltoides*, while similar estimates for hybrid genotypes have ranged from 168 to 276 kg N ha^{-1} [20,21]. General recommendations for the North Central United States are to apply 85–185 kg N ha^{-1} after canopy closure [22,23]. More specifically, Coleman et al. [24] reported 2-year-old *P. deltoides* 'D105' grown in Rhinelander, Wisconsin, USA, acquired at most 120 kg N ha^{-1} yr^{-1} from native and applied N sources, with trees receiving application rates of 50 and 100 kg N ha^{-1} yr^{-1} exhibiting near-optimal growth.

A major practical implication of the results in the current study was that establishment of the genotypes tested was clone-specific. Thus, selection of genomic groups may not be as effective as selection of specific clones within genomic groups. Analysis of the variability in clonal responses to the leachate treatment in the current study was crucial for the selection of genotypes exhibiting greater establishment potential. Despite negligible treatment × clone × sampling date interactions for each variable ($P < 0.05$), analyses using Fisher's unprotected least significant difference (LSD) at 56 and 70 DAP were conducted to examine the variation in clonal responses to leachate treatment [19]. The phenotypic responses of the six clones studied segregated into three general response groups at 56 and 70 DAP. First, clones exhibited negligible differences between the leachate and control treatments at 56 and 70 DAP. Second, clones exhibited negligible differences between the treatments at 56 DAP and significant differences at 70 DAP. Third, clones exhibited

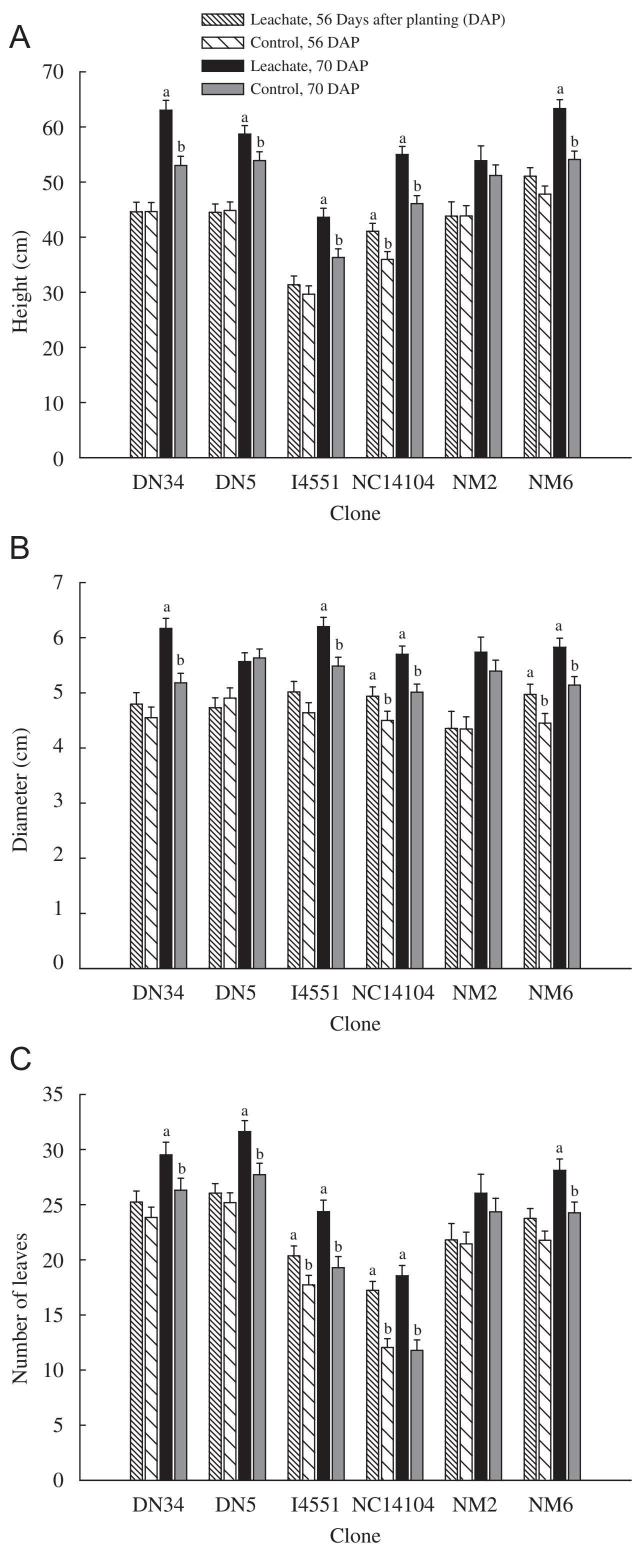

Fig. 2 – Height (A), diameter (B), and number of leaves (C) adjusted for cutting dry mass of six *Populus* clones at 56 and 70 days after planting following landfill leachate irrigation every other day beginning on day 42. The control treatment was application of non-fertilized water only. Each bar represents the mean of eight trees with one standard error. Bars labeled with different letters within a treatment × clone × sampling date interaction were different according to Fisher's unprotected LSD at $P < 0.05$, where all remaining non-labeled treatment comparisons were negligible.

significant differences at 56 and 70 DAP. Differences among clones for height showed NM2 belonged to the first group, while DN34, DN5, I4551, and NM6 belonged to the second group, and NC14104 was in the third group (Fig. 2). Similarly, for diameter, DN5 and NM2 were in the first group, DN34 and I4551 were in the second group, and NC14104 and NM6 belonged to the third group. For number of leaves, clone NM2 was in the first group, DN34, DN5, and NM6 were in the second group, and I4551 and NC14104 belonged to the third group.

Clones NM2 and NM6 have exhibited similar above- and below-ground growth in numerous greenhouse, nursery, and field applications throughout the North Central United States [9,25,26]; however, these clones responded much differently to the leachate treatment in the current study. Trees of clone NM2 did not show significant growth increases with leachate treatment versus the control, whereas NM6 trees responded significantly to the leachate treatment. Likewise, the trees of the F_1 hybrids between *P. deltoides* and *P. nigra* responded differently to the leachate treatment for diameter. Clones DN34 and I4551 exhibited greater diameter growth at 70 DAP with leachate treatment, while such differences were negligible for DN5. In addition, clone I4551 exhibited differences in treatment responses for number of leaves at 56 DAP, while DN34 and DN5 did not show differences between treatments until 70 DAP. Trees of clone NC14104 exhibited differences between treatments at 56 and 70 DAP for all variables, which corroborated the assertion that selection of specific clones is of great importance for increasing the overall establishment potential.

Despite limited information for *Populus*, intra- and inter-specific genotypic variation in growth responses to high levels of Na and Cl have been reported [10]. Similarly, such differences were exhibited among clones in the current study. Some leaf wilting was apparent at early stages following leachate application. We believe negligible height advantages for all clones except NC14104 at 56 DAP, along with a negligible height advantage for clone NM2 at 70 DAP, were the result of osmotic effects associated with elevated salt concentrations that led to short-lived water stress [27]. In contrast, observable toxicity symptoms (i.e. scorched leaf margins, excessive leaf abscission, yellow mottling of leaves, leaf size reduction) associated with excessive accumulation of Na and/or Cl were not evident for any clone.

3.2. Dry mass of stems, leaves, and roots

Clones differed for stem, leaf, and root dry mass ($P<0.0001$, $P<0.0001$, $P=0.05$, respectively), while treatments differed for leaf dry mass ($P=0.0007$) but were negligible for stem and root dry mass ($P=0.0984$ and 0.603, respectively). The leaves exhibited 26% greater dry mass with leachate versus water (Fig. 3). Analyses of the interaction between treatment and clone for stem, leaf, and root dry mass indicated a broad range of variability among clones in their response to the leachate treatment (Fig. 4). Only DN5 did not exhibit differences for stem dry mass, while such differences for root dry mass were negligible for all clones. Furthermore, differences in leaf dry mass were

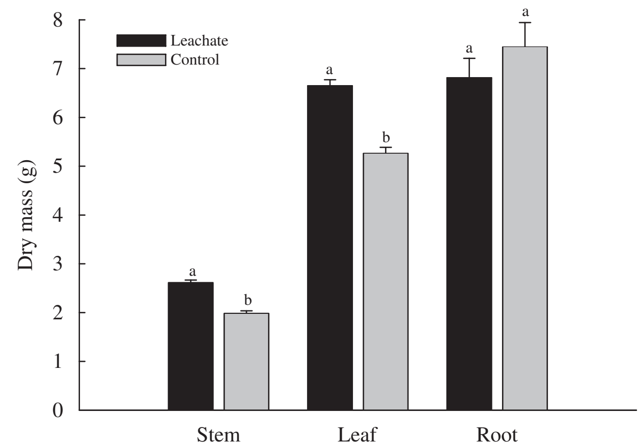

Fig. 3 – Stem and leaf dry mass (adjusted for cutting dry mass) and root dry mass across six *Populus* clones at 70 days after planting following landfill leachate irrigation every other day beginning on day 42. The control treatment was application of non-fertilized water only. Each bar represents the mean of 48 trees with one standard error. Bars labeled with different letters within a plant component were different according to Fisher's protected (leaf) and unprotected (stem and root) LSD at $P<0.05$.

clone-specific. For example, despite an absolute advantage of 10%, there was no difference in leaf dry mass between trees of clone DN5 when treated with the leachate versus the control (5.93 ± 0.32, 5.39 ± 0.33 g, respectively; $LSD_{0.05}=0.92$, $n=8$), but the leaf dry mass of the leachate-treated trees of DN34 (37%) and I4551 (33%) was significantly greater than that of the control trees (DN34: 6.73 ± 0.37, 4.90 ± 0.35 g, respectively; I4551: 7.35 ± 0.34, 5.53 ± 0.32 g, respectively). Likewise, leaf dry mass of the leachate and control trees was similar (only 2% greater for leachate trees) for clone NM2 (5.55 ± 0.56, 5.44 ± 0.40 g, respectively), but that of NM6 was significantly greater (26%) when treated with leachate versus the control (7.12 ± 0.34, 5.65 ± 0.31 g, respectively). The advantage for leaf dry mass with leachate treatment versus the control was greatest (55%) for clone NC14104 (7.20 ± 0.30, 4.65 ± 0.30 g, respectively). Likewise, Moffat et al. [13] reported significant biomass increases for two poplar genotypes (*P. trichocarpa* × *P. deltoides* 'Beaupre' and *P. trichocarpa* 'Trichobel') following irrigation with wastewater effluent during the last two years of a 3-year rotation, compared with no irrigation.

A lack of the aforementioned leachate response for root dry mass contrasts strongly with our observation of a positive leachate response for stem and leaf dry mass. These results are similar to those of Pregitzer et al. [28], who reported greater shoot growth relative to root growth as the level of N applied to the soil was increased. Other researchers have reported similar shifts in biomass from roots to shoots with increasing N levels [29]. However, when considering developmental effects on the allometric relationships between above- and below-ground biomass accumulation, increased levels of soil resources may not support relatively greater shoot growth

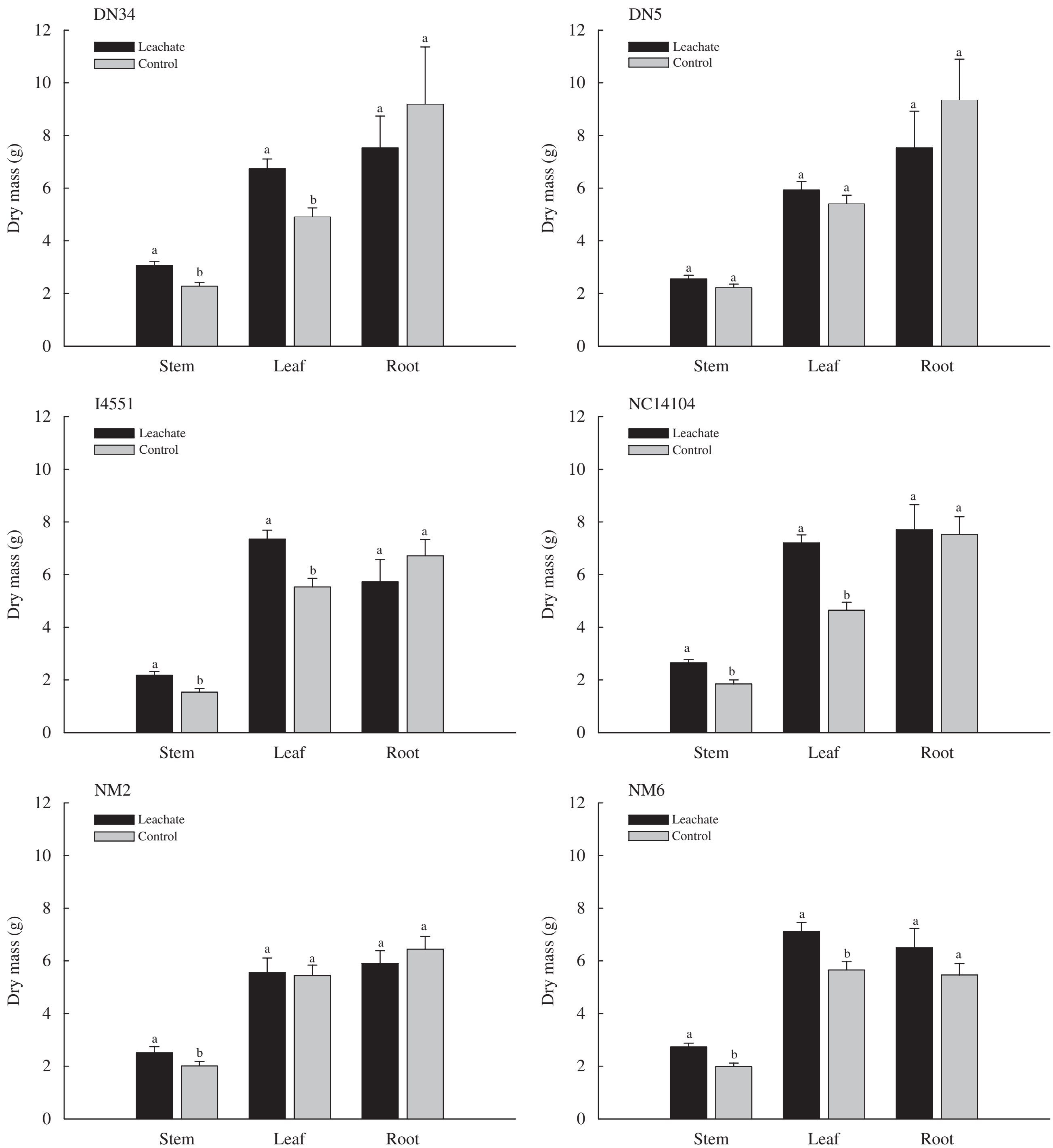

Fig. 4 – Stem and leaf dry mass (adjusted for cutting dry mass) and root dry mass of six *Populus* clones at 70 days after planting following landfill leachate irrigation every other day beginning on day 42. The control treatment was application of non-fertilized water only. Each bar represents the mean of eight trees with one standard error. Bars labeled with different letters within a plant component were different according to Fisher's unprotected LSD at $P<0.05$.

[24,30,31]. Furthermore, there are reports of decreasing belowground biomass along with increasing aboveground biomass of *P. deltoides* 'S7C15' following a combination treatment of irrigation with fertilization compared with either treatment alone [31]. However, our results did not support this assertion of organ-specific phytotoxicity, nor did our data indicate preferential accumulation of roots in the fertilized soil [32]. In contrast, the leachate exhibited no effect on root growth relative to the non-fertilized control treatment. Hence, the leachate caused a fertilization effect for stems and leaves but failed to impact or influence root growth.

4. Conclusions

The overarching practical implication of our results was that specific clones of *Populus* exhibited great establishment potential (i.e. elevated growth and biomass at 70 DAP) following repeated irrigation with landfill leachate versus water. We used a multiplicative summation index with equal weighting to rank the six genotypes based on the six aforementioned traits. The clonal ranking beginning with the greatest establishment potential was DN34, NM6, DN5, NM2, NC14104, and I4551, with DN34 exhibiting a selection value 30% greater than I4551. Overall, these data serve as a basis for making clonal selections that may lead to effective merging of traditional intensive forestry with waste management to achieve dual goals of fiber and bioenergy production, along with environmental benefits such as soil/water remediation and carbon sequestration. Such integrated systems can be used to provide alternative energy sources while recycling wastewaters that otherwise are expensive to transport and treat.

Acknowledgements

We are indebted to Dr. Jill A. Zalesny (Wisconsin Department of Natural Resources) for endless hours of consultation about the physiology and genetics associated with using wastewaters as fertigation for our trees. We thank Bart Sexton (Oneida County Solid Waste Department) for providing the leachate and insight into the field of waste management. We are grateful to the following people for review of earlier versions of this manuscript: William Berguson, David Coyle, Rob Doudrick, William Headlee, and Jill Zalesny.

REFERENCES

[1] Zalesny JA, Zalesny Jr. RS, Coyle DR, Hall RB. Growth and biomass of *Populus* irrigated with landfill leachate. Forest Ecology and Management 2007;248:143–52.
[2] Isebrands JG, Karnosky DF. Environmental benefits of poplar culture. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. Poplar culture in North America, Part A, Chapter 6. Ottawa, ON, Canada: NRC Research Press, National Research Council of Canada; 2001. p. 207–18.
[3] Zalesny JA, Zalesny Jr. RS, Wiese AH, Sexton B, Hall RB. Distribution of macro- and micro-nutrients in leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate. Journal of Sustainable Forestry [in press].
[4] Mirck J, Isebrands JG, Verwijst T, Ledin S. Development of short-rotation willow coppice systems for environmental purposes in Sweden. Biomass and Bioenergy 2005;28:219–28.
[5] Orlovic S, Guzina V, Krstic B, Merkulov L. Genetic variability in anatomical, physiological and growth characteristics of hybrid poplar (*Populus* × *euramericana* Dode (Guinier)) and eastern cottonwood (*Populus deltoides* Bartr.) clones. Silvae Genetica 1998;47:183–90.
[6] Eckenwalder JE. Systematics and evolution of *Populus*. In: Stettler RF, Bradshaw Jr. HD, Heilman PE, Hinckley TM, editors. Biology of *Populus* and its implications for management and conservation, Part I, Chapter 1. Ottawa, ON, Canada: NRC Research Press, National Research Council of Canada; 1996. p. 7–32.
[7] Rajora OP, Zsuffa L. Allozyme divergence and evolutionary relationships among *Populus deltoides*, *P. nigra*, and *P. maximowiczii*. Genome 1990;33:44–9.
[8] Zalesny Jr RS, Bauer EO. Evaluation of *Populus* and *Salix* continuously irrigated with landfill leachate. II. Soils and early tree development. International Journal of Phytoremediation 2007;9:307–23.
[9] Zalesny Jr. RS, Riemenschneider DE, Hall RB. Early rooting of dormant hardwood cuttings of *Populus*: analysis of quantitative genetics and genotype × environment interactions. Canadian Journal of Forest Research 2005;35:918–29.
[10] Zalesny JA, Zalesny Jr. RS, Wiese AH, Sexton B, Hall RB. Sodium and chloride accumulation in leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate. Environmental Pollution 2007, in press, doi:10.1016/j.envpol.2007.10.032.
[11] Zalesny Jr. RS, Bauer EO. Selecting and utilizing *Populus* and *Salix* for landfill covers: implications for leachate irrigation. International Journal of Phytoremediation 2007;9:497–511.
[12] Zalesny JA, Zalesny Jr. RS, Wiese AH, Hall RB. Choosing tree genotypes for phytoremediation of landfill leachate using phyto-recurrent selection. International Journal of Phytoremediation 2007;9:513–30.
[13] Moffat AJ, Armstrong AT, Ockleston J. The optimization of sewage sludge and effluent disposal on energy crops of short rotation hybrid poplar. Biomass and Bioenergy 2001;20:161–9.
[14] Erdman JA, Christenson S. Elements in cottonwood trees as an indicator of ground water contaminated by landfill leachate. Ground Water Monitoring and Remediation 2000;20:120–6.
[15] Hasselgren K. Use of municipal waste products in energy forestry: highlights from 15 years of experience. Biomass and Bioenergy 1998;15:71–4.
[16] US EPA. Guidelines for water reuse. EPA/625/R-04/108. Table 2–7, 2004, p. 25.
[17] Larson PR, Isebrands JG. The plastochron index as applied to developmental studies of cottonwood. Canadian Journal of Forest Research 1971;1:1–11.
[18] SAS Institute Inc. SAS/STAT™ User's Guide. Version 9. Cary, NC: SAS Institute, Inc.; 2004.
[19] Chew V. Comparing treatment means: a compendium. HortScience 1976;11:348–57.
[20] Heilman PE, Stettler RF. Nutritional concerns in selection of black cottonwood and hybrid clones for short rotation. Canadian Journal of Forest Research 1986;16:860–3.
[21] Nelson LE, Switzer GL, Lockaby BG. Nutrition of *Populus deltoides* plantations during maximum production. Forest Ecology and Management 1987;20:25–41.
[22] Stanturf JA, van Oosten C, Netzer DA, Coleman MD, Portwood CJ. Ecology and silviculture of poplar plantations. In: Dickmann DI, Isebrands JG, Eckenwalder JE, Richardson J, editors. Poplar culture in North America, Part A, Chapter 5. Ottawa, ON, Canada: NRC Research Press, National Research Council of Canada; 2001. p. 153–206.
[23] Hansen EA. A guide for determining when to fertilize hybrid poplar plantations. Research Paper NC-319. St. Paul, Minnesota: USDA Forest Service, North Central Forest Experiment Station; 1994. 7p.
[24] Coleman MD, Friend AL, Kern CC. Carbon allocation and nitrogen acquisition in a developing *Populus deltoides* plantation. Tree Physiology 2004;24:1347–57.
[25] Riemenschneider DE, Berguson WE, Dickmann DI, Hall RB, Isebrands JG, Mohn CA, et al. Poplar breeding and testing strategies in the north-central US: demonstration of potential yield and consideration of future research needs. The Forestry Chronicle 2001;77:245–53.

[26] Zalesny Jr. RS, Hall RB, Bauer EO, Riemenschneider DE. Shoot position affects root initiation and growth of dormant unrooted cuttings of *Populus*. Silvae Genetica 2003;52:273–9.
[27] Lessani H, Marschner H. Relation between salt tolerance and long-distance transport of sodium and chloride in various crop species. Australian Journal of Plant Physiology 1978;5:27–37.
[28] Pregitzer KS, Dickmann DI, Hendrick R, Nguyen PV. Whole-tree carbon and nitrogen partitioning in young hybrid poplars. Tree Physiology 1990;7:79–93.
[29] Dickmann DI, Nguyen PV, Pregitzer KS. Effects of irrigation and coppicing on above-ground growth, physiology and fine-root dynamics of two field-grown hybrid poplar clones. Forest Ecology and Management 1996;80:163–74.
[30] Coleman MD, Dickson RE, Isebrands JG. Growth and physiology of aspen supplied with different fertilizer addition rates. Physiologia Plantarum 1998;103:513–26.
[31] Coyle DR, Coleman MD. Forest production responses to irrigation and fertilization are not explained by shifts in allocation. Forest Ecology and Management 2005;208:137–52.
[32] Rytter RM, Hansson AC. Seasonal amount, growth and depth distribution of fine roots in an irrigated and fertilized *Salix viminalis* L. plantation. Biomass and Bioenergy 1996;11:129–37.