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Phytoremediation of landfill leachate using Populus

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Phytoremediation of landfill leachate using *Populus*

by

Jill Annette Zalesny

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Forestry (Forest Biology-Wood Science)

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Iowa State University

Ames, Iowa

2007

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DEDICATION

This dissertation is dedicated to my husband, Dr. Ronald S. Zalesny Jr., for his inspiration, energy, and unwavering confidence. I deeply cherish the time, knowledge, and partnership he shared with me. His support and encouragement were crucial to the accomplishment of my doctoral program.
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CHAPTER 1. GENERAL INTRODUCTION

Introduction

Short rotation woody crops (SRWC) are used for alternative production systems that reduce pressure on native forests. Intensive management utilizes agronomic practices such as site selection and preparation, improved genotypes, pest and pathogen control, and irrigation and fertilization to increase harvest yields over traditional forestry practices. Poplars (Populus spp.) have been extensively studied in SRWC production systems for multiple uses such as fiber, fuel, and environmental benefits (Dickmann, 2001; Isebrands and Karnosky, 2001; Coleman and Stanturf, 2006). Exceptional traits that have contributed to the success of such uses include: ease of rooting, quick establishment, fast growth, and elevated rates of photosynthesis and transpiration (Ceulemans et al., 1992; Pontailler et al., 1999; Zalesny et al., 2006). Broad genetic diversity among poplar genomic groups and selection of specific genotypes within such groups increase the potential enhancement of growth and establishment for various uses across heterogeneous sites (Heilman and Stettler, 1985; Heilman et al., 1994). The combination of appropriate cultural practices and well-suited genotypes helps to maximize poplar performance for improved biomass yields (Buhler et al., 1998; Stanturf et al., 2001).

Environmental benefits have been realized from poplar culture when used as components in riparian buffers along streams (Schultz et al., 2004) and as vegetative filters for phytoremediation applications (Licht and Isebrands, 2005). Several phytoremediation projects utilized wastewater in the form of landfill leachate as an irrigation and fertilization
source for poplar trees (Shrive et al., 1994; Erdman and Christenson, 2000; Zalesny and Bauer, 2007). Proper clonal selection practices must be utilized given the genetic variability within the genus *Populus* (Rajora and Zsuffa, 1990; Eckenwalder, 1996) and the variable concentrations of inorganic and organic components in the leachate (Gettinby et al., 1996).

More testing of genotypes for various phytoremediation applications would be beneficial to ascertain superior clones for specific contaminant problems. Due to the broad variation in expressed traits, which is common among the different genotypes of poplar, selected clones might exhibit elevated phytoremediation capability at specific sites. Additionally, once superior genotypes have been selected from greenhouse and field studies, clones can be asexually propagated with relative ease and efficiency. Overall, plant-based remedial systems are effective technologies from environmental and economical standpoints.

**Objectives and Hypotheses**

The overall objective of my research was to test the phytoremediation potential of current poplar genotypes in order to make recommendations for similar studies and operational projects, where phytoremediation potential included successful establishment, growth, productivity, and nutrient/chemical sequestering ability. I sought to identify poplar genotypes with elevated biomass accumulation and tissue concentration of identified elements by conducting greenhouse (*ex situ*) and field (*in situ*) experiments. My overarching null hypothesis tested in all experiments was that no genotypic differences for phytoremediation capability would be present among the poplar clones tested. The submitted and published research manuscripts from my work (Chapters 2, 3, 4, and 5) detail the
establishment success, growth, productivity, and tissue composition of different poplar genotypes under contrasting irrigation conditions resulting in phenotypic responses that will be useful for site managers in planning future phytoremediation projects.

**Dissertation Organization**

This dissertation contains one manuscript in press in the *International Journal of Phytoremediation* (Chapter 2), one manuscript in press in *Forest Ecology and Management* (Chapter 3), one manuscript submitted for publication to *Environmental Pollution* (Chapter 4), and one manuscript submitted for publication to *Forest Ecology and Management* (Chapter 5). A brief introduction and subsequent literature review (Chapter 1) precedes the manuscripts, while a general conclusion (Chapter 6) following the manuscripts highlights a general discussion, elements of the technology of phytoremediation and its associated benefits, summary of my key research findings, and my recommendations for future research. Additional information that did not fit into any of the chapters but was relevant to my project is provided in the appendices. The format of the manuscripts follows the guidelines of the *International Journal of Phytoremediation, Forest Ecology and Management*, and *Environmental Pollution*. The style of the references at the end of Chapter 1 follows that of the *International Journal of Phytoremediation*. 
Literature Review

The Taxonomy, Evolution, Biology, and Genetics of Populus

The Genus Populus: Six Sections and Numerous Species

The family Salicaceae Mirb. includes the two genera Populus L. and Salix L. Species of Populus are nearly all distributed in the Northern Hemisphere (Dickmann, 2001). In North America, poplars, cottonwoods, and aspens occupy large distributional ranges with abundant genetic variation (Farmer, 1996). Such genetic diversity is a hallmark of Populus, with variation present at the genus, sectional, species, and clonal level (Rajora and Zsuffa, 1990; Eckenwalder, 1996; Stettler et al., 1996). The fast growing, deciduous, single-trunked trees are most notably researched and utilized for intensive management due to ease of rooting, quick establishment, fast growth, and elevated rates of photosynthesis and transpiration, as well as, their ability to be genetically manipulated for improved yield. Based on specific ecological and morphological traits, the genus Populus is divided into six sections: Abaso, Turanga, Leucoides, Aigeiros, Tacamahaca, and Populus (Eckenwalder, 1996). The most important species for short rotation culture are in the sections Aigeiros, Tacamahaca, and Populus. Major barriers to hybridization occur between sections; however, intersectional hybrids of economic significance occur between Aigeiros and Tacamahaca (Zsuffa, 1975; Guries and Stettler, 1976; Gaget et al., 1984; Villar et al., 1987). Breeding and tree improvement strategies often focus on hybridization of species within and between the sections Aigeiros and Tacamahaca; therefore, species and hybrids from these sections are the focus of my research presented in this dissertation.
The six taxonomically-distinct sections described above are generally accepted; however, scientific opinions have differed on the number of species recognized within these sections (Eckenwalder, 1996; Dickmann, 2001). The large geographical ranges, as well as, the broad variation in expressed traits contribute to identity confusion as species separate into variants and subspecies. Also, the production of hybrids through both artificial and natural methods contributes to uncertainty with additional genotypes. For sympatric species, natural hybrids frequently occur in regions of overlap (Farmer, 1996). These hybrids are generally fertile and capable of backcrossing with either parent, thus adding more confusion to the classification of poplars at the species level. My discussion of sections and species follows the conservative approach of Eckenwalder (1996) and Dickmann (2001), who classified fewer genotypes as true species by recognizing 29 worldwide species of *Populus*, one dozen of which are native to North America (Table 1.1). Overall, I provide greater detail about the species that comprised the hybrids utilized in my project (*P. deltoides*, *P. nigra*, *P. trichocarpa*, *P. suaveolens* subsp. *maximowiczii*).

The section *Abaso* (Mexican poplar) contains the sole species *P. mexicana* Wesmael, which is the most southern poplar in North America (Eckenwalder, 1977). This medium-sized riparian species has two described subspecies residing on the east and west coasts of Mexico (Eckenwalder, 1977; Dickmann et al., 2001).

The section *Turanga* (Afro-Asian poplars) includes a total of three species, of which two (*P. euphratica* Oliv. and *P. ilicifolia* (Engler) Roul.) are native to Africa and one (*P. pruinosa* Schr.) is native to China. *Populus euphratica* has shown some tolerance to heat,
drought, and salinity and may offer potential for tree improvement programs with breeding
goals of this nature (Dickmann et al., 2001).

The section *Leucoides* (swamp poplars) includes a total of three species, of which one
(*P. heterophylla* L.) is native to North America and two (*P. lasiocarpa* Oliv. and *P. glauca*
Haines) are native to China. The ecologically-important *P. heterophylla* is commonly found
in riparian areas of the eastern and central United States. *Populus heterophylla* can survive
on heavy clay soils and is one of the most flood tolerant poplars. Neither of the two Chinese
species has economic importance (Dickmann et al., 2001).

The section *Aigeiros* (cottonwoods and black poplar) contains a total of three species,
of which two (*P. deltoides* Bartr. ex Marsh., and *P. fremontii* S. Wats.) are native to North
America and one (*P. nigra* L.) is native to Eurasia. *Populus deltoides* is an economically-
important North American *Populus* species for intensive culture, as well as, being an
ecologically important riparian species. *Populus deltoides* has a large natural range
throughout North America, where it is distributed from the eastern half of the United States
to southern Canada (Figure 1.1). *Populus deltoides* is associated with riparian and upland
habitat where it is shade intolerant and grows in pure stands or in association with other early
successional species. The southwestern *P. fremontii* is a low elevation riparian species that
offers important ecological habitat in riparian areas of this arid region. In earlier times, *P.
nigra* was naturally distributed throughout Europe, Asia, and North Africa (Figure 1.2),
where it grew aggressively and invaded disturbed sites. However, since the introduction and
spread of *P. deltoides* germplasm into Europe centuries ago, concerns over the preservation
of natural populations of *P. nigra* have arisen, and much of its natural range has been reduced (Dickmann et al., 2001).

The section *Tacamahaca* (balsam poplars) includes a total of nine species, of which three (*P. balsamifera* L., *P. trichocarpa* Torr. & Gray, and *P. angustifolia* James) are native to North America and six (*P. suaveolens* Fischer subsp. *maximowiczii*, *P. laurifolia* Ledebour, *P. yunnanensis* Dode, *P. szechuanica* Schneider, *P. simonii* Carrière, and *P. ciliata* Royal) are native to Asia. The North American species occur in ecologically-important riparian habitats and some have commercial significance. *Populus balsamifera* is the most northern growing poplar with the largest distribution of all *Tacamahaca* species in North America, ranging across the northern United States, Alaska, and Canada. *Populus trichocarpa* is a commercially-important species that is common in moist riverine ecosystems along the Pacific Ocean from the northwestern United States to western Canada and Alaska (Figure 1.3). The final North American species, *P. angustifolia*, is a riparian species of the Rocky Mountains and has little economic importance. Of the Asian species, only *P. suaveolens* subsp. *maximowiczii* has economic importance and, therefore, has been utilized as a parent in hybridization work throughout the world. The distribution of *P. suaveolens* subsp. *maximowiczii* was throughout northeastern Asia and Japan (Dickmann, 2001).

The section *Populus* (aspens and white poplars) contains a total of ten species distributed throughout the world, with six species of aspens and four species of white poplars. Only two species of aspen, *P. tremuloides* Michx. and *P. grandidentata* Michx., are found in the United States, and they have tremendous economic and ecological importance. One other aspen species, *P. tremula* L., has a natural distribution throughout Asia, Europe,
and northern Africa, while *P. sieboldii* Miquel, *P. adenopoda* Maxim., and *P. gamblei* Haines are limited to Asia. The Chinese species *P. davidiana* (Dode) Schneider, although with its own binomial status, is considered a subspecies of *P. tremula*. Of the four species of white poplar, *P. alba* L. is native to Europe, Asia, and Africa, while the following three white poplars are found in Mexico: *P. monticola* Brand., *P. guzmanantensis* Vazq. & Cuevas, and *P. simaroa* Rzedo. (Dickmann et al., 2001).

*The Evolution and Fossil Record of Populus*

Sectional representation in the fossil evidence is nearly parallel to sectional primitiveness in the genus *Populus* (Eckenwalder, 1996). Tertiary leaf findings in the fossil record of the first poplars occur in the late Paleocene, 58 million years ago. Those fossils, found in the North Dakota Golden Valley Formation, were related to the present day species *P. mexicana* of section *Abaso*, which is the most primitive section within the genus (Manchester et al., 1986; Collinson, 1992). Fifty-million-year-old fossil evidence of *P. meegsii*, previously *P. cinnamomoides* (Lesquereuz) MacGinitie, also of the section *Abaso*, has been found in the widespread and well-preserved fossil record from the Middle Eocene Green River Formation of Colorado, Wyoming, and Utah (Eckenwalder, 1980; Manchester et al., 1986; Collinson, 1992; Boucher et al., 2003). From this location, one extremely rare twig specimen was found that bore leaves and a fruiting catkin (Manchester et al., 1986; Eckenwalder, 1996). Section *Leucoides* showed up in the late Eocene followed by the last sections to appear in the fossil representation, *Tacamahaca, Aigeiros*, and *Populus*, the three most advanced sections with definite specimens preserved during the Miocene from 10 to 20 million years ago (Eckenwalder, 1996).
Early poplars colonized lowland and riparian areas, which allowed abscised foliage to contribute to annual sediment deposits. Thus, the most complete fossil record of the genus *Populus* was attained through leaf preservation (Eckenwalder, 1980; Collinson, 1992). Two morphological features of *Populus* complicate the correct assignment of fossil evidence. All species of *Populus* exhibit some form of foliar heteromorphism, the ability of an individual to produce different leaf forms during normal development. Foliar heteromorphism occurs in two patterns, heteroblastic leaf development (changes between juvenile and adult leaves) and seasonal heterophylly (changes between early and late leaves) (Eckenwalder, 1980; Eckenwalder, 1996). Other plant parts such as male catkins, pollen, and seed undergo rapid decay and are not expected to be well represented in the fossil record (Birks, 1980; Collinson, 1992).

*The Biology of Populus*

*Ecology.* The survival of riparian cottonwoods in North America (*P. angustifolia, P. balsamifera, P. deltoides, P. fremontii*, and *P. trichocarpa*) is tied to the disturbance regimes of the riverine ecosystems they inhabit. *Populus nigra* and *P. suaveolens* subsp. *maximowiczii* are similar pioneer riparian woodland species distributed throughout Europe and Asia (Major, 1977; Guilloy-Froget et al., 2002). The processes that occur as a result of seasonal floods (snowmelt and storms) produce freshly disturbed sites for seed colonization (Braatne et al., 1996). Natural seed dispersal is timed with intense light and high soil moisture levels that aid in germination and survival. Three stages are crucial for establishment: seed dispersal, seedling emergence, and survival of the cottonwood recruits (Braatne et al., 1996; Guilloy-Froget et al., 2002). In addition to colonization after flood due
to numerous small, wind-blown seed, cottonwoods are capable of vegetative propagation with stem and branch pieces (Braatne et al., 1996).

Fire and other natural disturbances, along with human-induced disturbances such as forest clearing and agriculture, produces open sites for _Populus_ seed colonization on upland landscapes. The riparian cottonwoods _P. deltoides_ and _P. trichocarpa_ have survival strategies of fast growth that are tied to their role as pioneer species (Braatne et al., 1996). In Europe, _P. nigra_ exhibits similar pioneer characteristics on upland and disturbed sites, where _P. nigra_ quickly colonizes given the necessary light and moisture requirements (Guilloy-Froget et al., 2002). As with the riparian ecosystem, the establishment phase of seedlings must be coupled with light and moisture requirements for survival (Braatne et al., 1996). With aspens of the _Populus_ section, seedling establishment is less important than root suckering following disturbance; however, seedling establishment and abundant suckering have been an important means of colonization after large scale clearing following settlement in the Lake States and after fires such as in Yellowstone National Park in 1988 (Zasada et al., 2001).

**Reproduction.** Species of _Populus_ are outcrossers with dioecious trees bearing either male or female pendant catkins (Braatne et al., 1996; Eckenwalder, 1996; Farmer, 1996). The ratio of male to female trees is generally 1:1, but variations exist at low altitudes with pistillate dominance and high altitudes with staminate dominance (Farmer, 1964; Kaul and Kaul, 1984; Farmer, 1996). Early spring production of male and female catkins, prior to leaf emergence, is a common strategy of wind pollinated trees. This early production is important as canopy closure can limit wind dissemination of pollen (Eckenwalder, 1996). The
flowering time of *Populus* species ranges from February to May, with variation in floral phenology due to local environmental conditions (Farmer, 1966; 1993; Boes and Strauss, 1994; Braatne et al., 1996). Anthesis and pistil receptivity last for 1 to 2 weeks, with individual flowers being receptive for only a few days (Braatne et al., 1996). The cottony seed is small, lacks endosperm, and quickly loses viability; however, the seed rapidly germinates in about 24 hours under favorable conditions of light and moisture (Braatne et al., 1996).

Asexual reproduction is prevalent in all sections of *Populus*. Riparian cottonwoods naturally reproduce asexually by branch breakage and crown damage. Branch sprouting and adventitious root formation facilitate tree survival of the broken branches and crown (Braatne et al., 1996). Most species of *Populus* have the ability to coppice, producing new shoots from buds located basally on the stem. However, there is high variability in coppicing ability, which often declines with age (Zasada et al., 2001). Additionally, species of the section *Populus* reproduce clonally by extensive root suckering. It was proposed that an aspen clone of *P. tremuloides* in the Wasatch Mountains of Utah may be the largest organism in the world with nearly 47,000 stems and 43 ha of ground coverage (Mitton and Grant, 1980; Dickmann, 2001).

Tree breeders, silviculturists, and horticulturists take advantage of the propensity for species in the sections *Aigeiros* and *Tacamahaca* to form adventitious roots by using unrooted hardwood stem cuttings as a common and inexpensive propagule in managed systems (Riemenschneider and Bauer, 1997; Zalesny et al., 2003). Short stem pieces, about 30 cm long from one-year-old dormant material, quickly form shoots from dormant buds and
roots from root primordia distributed throughout the stem (Luxova and Lux, 1981a; 1981b; Dickmann, 2001; Stanturf et al., 2001). Additionally, adventitious roots form from callus, a wound-induced parenchymous growth at the base of the cutting (Figure 1.4). Another type of planting stock that is commonly used are rooted cuttings with multiple lateral roots and some residual stems (usually not greater than one meter in height), or rooted cuttings reared in a greenhouse or growth chamber. Either type of rooted cuttings is preferred for genotypes that have poor or erratic rooting (e.g. *P. deltoides* in the Lake States region of the United States). Also, *Populus* whips up to a few meters in length have been used for phytoremediation systems in order to clean up contaminated subsurface plumes, achieve high aboveground productivity in short time, or quickly gain hydraulic control of the site (Licht and Isebrands, 2005; Zalesny et al., 2005b). Furthermore, a second tissue capable of initiating new root growth is softwood cuttings, which offers an alternative propagation method for clones that do not produce roots from hardwood cuttings or do so too slowly or erratically for effective survival (Braatne et al., 1996; Dickmann, 2001).

**Anatomy.** The broad variation in nearly all expressed traits of *Populus* species and hybrids also is exhibited in their leaf shape (e.g. lanceolate, maple-like, deltoid, or rhombic), petiole shape (e.g. flattened or rounded), and teeth on margins (e.g. absence or prominence) (Dickmann, 2001). Poplars mature both preformed and neoformed simple leaves in a growing season, thus exhibiting seasonal heterophyll (Eckenwalder, 1996). During the spring flush only preformed (overwintered) leaves are present. The preformed leaves are texturally tough which is important for survival during low spring temperatures (Dickmann, 1971; Eckenwalder, 1996). Neoformed leaves, meaning leaves newly formed during the
growing season, can differ from preformed leaves for shape, dentition, and texture (Dickmann, 1971; Eckenwalder, 1996).

The mature size of poplar leaves is due to cell number and expansion. For example, *P. trichocarpa* completes cell division early in the development of a leaf, with cell expansion contributing the final 95% of leaf growth. Additionally, *P. trichocarpa* has two layers of palisade mesophyll, stomata located on the lower epidermis, and holds its leaves in a horizontal display. In contrast, *P. deltoides* continually produces and enlarges new cells as the leaf matures. *Populus deltoides* has a double mesophyll with two layers of palisade mesophyll, stomata on both epidermal surfaces, and displays its leaves vertically. Furthermore, the hybrids of these two species are variable for layers of palisade mesophyll and stomatal distribution on upper and lower leaf surfaces. The leaf size of the hybrids is generally larger than either parent due to greater cell division and cell enlargement (Van Volkenburgh and Taylor, 1996).

All *Populus* wood is diffuse-porous and contains a variety of cells including vessel elements, fibers, and ray parenchyma. Generally, the density, length, and diameter of vessel elements are taxonomically diagnostic. In the North Central United States, the most common native *Populus* species used for wood products and pulp for paper are *P. tremuloides* and *P. grandidentata*. Concerns over a shortage of suitable aspen in desirable class ranges, especially in this region (Piva, 2006), have led many resource managers and scientists to consider implementing managed plantations (SRWC) of hybrid poplar as an alternative to natural stands (Balatinecz and Kretschmann, 2001). Overall, poplars have the potential to grow nearly eight times faster than native aspen in the North Central United States.
Aboveground stand productivity of poplar is estimated between 27 to 45 m$^3$ ha$^{-1}$ yr$^{-1}$, compared with native aspen stand productivity of 4 to 6 m$^3$ ha$^{-1}$ yr$^{-1}$ (Riemenschneider et al., 2001b; Netzer et al., 2002).

The primary uses of aspen wood include: pulp, lumber, hardboard, oriented strand board (OSB), cordwood, and biomass for energy (Hall et al., 1982; Balatinecz and Kretschmann, 2001). Hybrid poplar offers an alternative for all of these uses. Poplar wood is especially well-suited to the pulp and paper industry and composite wood products. Additionally, poplar may be useful for lumber products, although its wood may not offer the mechanical strength of native aspen, along with having a predisposition to shrink and/or warp (Pliura et al., 2005).

*Populus* is a model tree organism that has been studied extensively (Dickmann and Keathley, 1996; Taylor, 2002). Given the amount of resources necessary for rooting studies and the overall difficulty of acquiring meaningful rooting data (Carlson, 1965; Wiese et al., 2005), the aboveground growth of *Populus* has been studied more relative to belowground processes (Wu and Stettler, 1994; Orlovic et al., 1998). Nevertheless, a great deal of knowledge has been acquired about the growth and physiology of poplar roots (Pregitzer and Friend, 1996; Coleman et al., 2004; Kern et al., 2004), especially as it applies to cutting establishment and early tree development (Zalesny and Wiese, 2006). However, as is the case with most tree species, the majority of this reported information is from field and greenhouse studies, resulting in less overall knowledge about the roots of mature trees.

The poplar root system is fairly complex in that it can develop roots in three ways (Dickmann et al., 2001):
1. Seed roots – the seed root (radicle) emerges,

2. Cuttings or branches – cuttings and branches produce roots from the development and maturation of latent root primordia located under the bark and from the differentiation of roots via callus tissue at wound sites (i.e. at the base of hardwood cuttings) (Figure 1.4) (Luxova and Lux, 1981a; 1981b; Zalesny et al., 2005a), and

3. Suckers – the production of suckers from an existing root system (i.e. the aspens).

Poplar roots mature up to four orders of lateral roots as the tree grows and develops (Dickmann et al., 2001). The distribution of lateral roots is generally 5 to 20 cm below the ground surface and is proportional with tree height (Hansen, 1981; Dickmann et al., 2001). Sinker roots, which are present in all species of *Populus*, branch vertically from lateral roots and can reach soil depths of up to three meters (Friend et al., 1991; Heilman et al., 1994; Dickmann et al., 2001). Fine roots are located near the top 10 cm of the soil surface and function in water and nutrient uptake. These feeder roots require a great deal of carbon and nutrients to support the metabolic functions and mycorrhizal partners of the root system (Dickmann et al., 2001). The production of fine roots is a dynamic process with continual loss and replacement as the roots die and are subjected to herbivory (Pregitzer and Friend, 1996; Dickmann, 2001; Kern et al., 2004).

*The Genetics and Breeding of Populus*

The *Populus* genome was the first tree genome to be sequenced (Tuskan et al., 2006), making *P. trichocarpa* the model forest tree species. *Populus* was selected largely due to the small size of the genome (450 to 550 million base pairs), which is only four times greater
than the size of *Arabidopsis thaliana* L. (100 to 150 million base pairs), the first plant ever sequenced (Taylor, 2002). Other promising plant features of *Populus* include: rapid growth, ease of hybridization, sexual and asexual modes of propagation, and an extensive geographic network of poplar scientists.

Hybridization of *Populus* is common, with hybrid progeny found where compatible species are sympatric (Eckenwalder, 1996; Stettler et al., 1996). This natural process has been applied to the development of hybrids within and among species and sections in order to supply improved genotypes for production systems, especially given heterosis and the potential transfer of favorable traits of interest (Stettler et al., 1996). Many species of *Populus* are relatively easy to artificially hybridize through controlled pollination, following collection of male and female scions (Rajora, 1989). Generally, the success of artificial hybridization has ranged from complete compatibility to complete incompatibility (Stettler et al., 1996). Intersectional crosses between species belonging to the sections *Aigeiros* and *Tacamahaca* have been highly successful and constitute most SRWC breeding efforts (Zsuffa, 1975; Gaget et al., 1984; Villar et al., 1987). However, the success of crosses between the sections *Aigeiros* and *Tacamahaca* are dependent upon the direction in which the cross was made (Hogenboom, 1973), with greater success being obtained when species of *Aigeiros* are used as females with *Tacamahaca* males (Zsuffa, 1975; Guries and Stettler, 1976; Eckenwalder, 2001). Hybrids are common within and among species of the section *Populus*; however, hybrids between this section and others are difficult to obtain (Ronald, 1982; Eckenwalder, 1984). Several mechanisms increase the difficulty with producing hybrids, including pre-fertilization barriers (pistil-pollen incompatibility), post-fertilization
barriers (immature seed and aborted seed), and hybrid inviability (evident during young seedling development) (Guries and Stettler, 1976; Stettler et al., 1996; Zsuffa et al., 1999).

Breeding programs focus on tree improvement to produce superior genotypes for a variety of uses (Riemenschneider et al., 1996; 2001a). One of the primary objectives of such breeding is to select generalist genotypes that perform well over a broad geographic range (over a broad range of contaminants in need of remediation) and/or to select specialist genotypes adapted to local site conditions (used for specific contaminants) (Dickmann and Keathley, 1996; Orlovic et al., 1998; Zalesny et al., 2005a). Overall, it is important these clones exhibit regional fitness and express a number of the following desirable traits (Stettler et al., 1996):

1. produce roots quickly from dormant hardwood cuttings for commercial deployment (Riemenschneider et al., 1996; Zalesny and Wiese, 2006),
2. allocate a large proportion of resources to harvestable biomass (Riemenschneider et al., 2001b),
3. produce sylleptic branches for increased radial growth via greater photosynthetic tissue (Scarascia-Mugnozza et al., 1999; Dickmann, 2001),
4. produce an increased epidermal cell number, cell size, or both for elevated levels of photosynthesis (Ceulemans et al., 1992),
5. optimize the length of the growing season by increasing leaf retention and prolonging the production period (Isebrands et al., 1988),
6. resist or tolerate pest and pathogen attacks (Newcombe, 1996; Newcombe et al., 2001; Coyle et al., 2005), and
7. produce high quality, process-dependent wood (Riemenschneider et al., 1996).

**Phytoremediation Using Populus**

Phytoremediation is a general class of remediation technologies that involves the direct use of plants to clean up contaminated soil, sediment, sludge, or groundwater (Cunningham and Ow, 1996; Cunningham et al., 1997; McIntyre and Lewis, 1997). Phytoremediation includes numerous treatment methods such as contaminant removal through (Figure 1.5) (Anderson et al., 1993; Schnoor et al., 1995; Bañuelos et al., 1999; Vose et al., 2000; Ferro et al., 2001; Mirck et al., 2005):

1. adsorption onto roots or precipitation within the root zone (phytostabilization),
2. filtration or trapping in the root zone (rhizofiltration),
3. degradation of chemical contaminants into less harmful compounds (phytodegradation or enhanced rhizosphere degradation),
4. plant uptake and sequestration in tissues (phytoextraction),
5. volatilization through stomates into a gaseous form (phytovolatilization), and
6. uptake of large volumes of water in order to contain the contaminates in one area or control the migration of the chemicals away from the area (hydraulic control).

Selected genotypes of poplar serve as an ideotype for phytoremediation systems because of their ability to grow fast, produce large plant biomass, grow on heavily contaminated soils and marginal soils that are not suitable for agriculture, produce extensive deep root systems, adapt to riparian sites, grow easily from dormant hardwood cuttings, release plant exudates into the rooting zone to stimulate microbial populations, and transpire
large volumes of water (Dickmann and Stuart, 1983; Jordahl et al., 1997; Vose et al., 2000; Isebrands and Karnosky, 2001; McLinn et al., 2001; Zalesny et al., 2006).

Poplars have been used to remediate sites with contamination from petroleum hydrocarbons (Landmeyer, 2001; Zalesny et al., 2005b), landfill leachates (Erdman and Christenson, 2000; Zalesny and Bauer, 2007; Zalesny et al., 2006; 2007), salts (Shannon et al., 1999), heavy metals (Bañuelos et al., 1999; Schnoor, 2000), fertilizer/pesticides/nitrates (Burken and Schnoor, 1998; 1997; 1996; Gatliff, 1994; O’Neill and Gordon, 1994), and explosives (Thompson et al., 1998a; 1998b). However, very few clones have been used in most of the ongoing field trials to this date. More testing of genotypes for various phytoremediation applications would be beneficial to ascertain superior clones for specific contaminant problems (Zalesny et al., 2007). Due to the broad variation in expressed traits, which is common among the different genotypes of poplar (Eckenwalder, 1984), selected clones might be shown to have elevated phytoremediation capability at specific sites. In one rotation, the trees are capable of providing fiber and wood products, along with biomass for bioenergy and environmental benefits (Heilman, 1999).

**Landfill Leachate Remediation**

Landfills produce leachate from the infiltration of precipitation and internal biological processes (Cureton et al., 1991; Duggan, 2005). The leachate composition of organic compounds, inorganic ions, and heavy metals changes due to the chemical and biological processes that occur during natural degradation of the waste products (Gettinby et al., 1996; Kjeldsen et al., 2002). Although contaminant levels generally decrease with landfill age, leachate treatment is necessary to avoid ground and surface water contamination (Wong and
Leung, 1989). Traditional treatment methods incur costs associated with transportation and treatment. These costs can last for years after landfill closure, and the resulting reduction in revenue can complicate the ability of resource managers to afford traditional leachate processing methods (Duggan, 2005). Economically-sound and environmentally-sustainable options are available to land managers for on-site leachate remediation (Glass, 1999; Schnoor et al., 1995). An alternative treatment that decreases expenditures is to utilize the leachate as a fertilization and irrigation source for plants (Menser et al., 1983; Stephens et al., 2000; Cheng and Chu, 2007). Specifically, these methods have been used for species and interspecific hybrids of the genus *Populus* (Wong and Leung, 1989; Shrive et al., 1994; Erdman and Christenson, 2000).

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Adapted from Eckenwalder (1996) and Dickmann (2001).
Figure 1.1 Natural distribution of *Populus deltoides* Bartr. ex Marsh (dark gray). Adapted from: Little, E.L., Jr., 1971, Atlas of United States trees, volume 1, conifers and important hardwoods; U.S. Department of Agriculture Miscellaneous Publication 1146, 9 p., 200 maps.
Figure 1.2 Natural distribution of *Populus nigra* L. (dark gray). This map was compiled by members of the EUFORGEN *Populus nigra* Network and was published in: van den Broeck, A. 2003. EUFORGEN technical guidelines for genetic conservation and use for European black poplar (*Populus nigra* L.). International Plant Genetic Resources Institute, Rome, Italy, 6 pp.
Figure 1.3 Natural distribution of *Populus trichocarpa* Torr. & Gray (dark gray). Adapted from: Little, E.L., Jr., 1971, Atlas of United States trees, volume 1, conifers and important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146, 9 p., 200 maps.
Figure 1.4 Preformed (lateral) and basal (callus) rooting ontogenies of *Populus*. 
**Phytostabilization**
Plants immobilize contaminants in the soil and groundwater through adsorption onto roots or precipitation within the root zone of the plant. When roots or a dense vegetation of aquatic plants keep water from moving, the term ‘hydraulic control’ is used.

**Phytoextraction**
The uptake and translocation of contaminants by plant roots from the soil into plant parts.

**Rhizofiltration**
The use of plant roots to remove pollutants from water or soil solution. Rhizofiltration is similar to phytoextraction, but the plant species are used primarily to remediate contaminated groundwater rather than soil.

**Phytodegradation**
The breakdown of contaminants taken up by plants through metabolic processes within the plant or the breakdown of contaminants external to the plant through the effect of compounds produced by the plant, such as enzymes.

**Rhizodegradation**
The breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the root zone.

**Phytovolatilization**
The uptake and transpiration of a contaminant by a plant. The contaminant, or a modified form of it, is released into the atmosphere.

**Figure 1.5** Processes of phytoremediation. Definitions from Mirck et al. (2005).
CHAPTER 2. CHOOSING TREE GENOTYPES FOR PHYTOREMEDIATION OF LANDFILL LEACHATE USING PHYTO-RECURRENT SELECTION

A paper in press in the *International Journal of Phytoremediation*¹

Jill A. Zalesny²,⁴, Ronald S. Zalesny Jr.³, Adam H. Wiese³, and Richard B. Hall²

ABSTRACT

Information about the response of poplar (*Populus* spp.) genotypes to landfill leachate irrigation is needed, along with efficient methods for choosing genotypes based on leachate composition. Poplar clones were irrigated during three cycles of phyto-recurrent selection to test whether genotypes responded differently to leachate and water, and to test whether the methodology had merit as a tool for plant selection during remediation. Fifteen belowground and aboveground traits were evaluated. Twenty-five clones were tested in cycle 1, while the


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best 12 genotypes were evaluated in cycles 2 and 3. Eight clones were selected and subsequently tested in an *in situ* landfill study (cycle 4). Results from cycles 1, 2, and 3 are presented here. Overall, clones responded differently to irrigation treatments, with certain genotypes exhibiting better belowground and aboveground growth with water than leachate. However, growth was greater with leachate irrigation for some clones. In addition, differences between treatments within clones decreased with days after planting (DAP). There were no treatment differences for number of leaves, height, and root length at the end of cycle 2 (45 DAP) or cycle 3 (30 DAP). These results detail the extensive variation in clonal responses to leachate irrigation, along with the need and efficacy of using phyto-recurrent selection to choose superior genotypes.

**KEY WORDS:** leachate irrigation, wastewater treatment, chloride stress, clonal selection index, short rotation woody crops, *Populus*, poplar

**INTRODUCTION**

Landfills produce leachate from the infiltration of precipitation and internal biological processes (Duggan, 2005). The leachate composition of organic compounds, inorganic ions, and heavy metals changes due to the chemical and biological processes that occur during natural degradation of the waste products (Gettinby, Sarsby, and Nedwell, 1996). Although contaminant levels generally decrease with landfill age, leachate treatment is necessary to avoid ground and surface water contamination (Wong and Leung, 1989). Economically-sound and environmentally-sustainable options are available to land managers for on-site
leachate remediation (Glass, 1999; Schnoor et al., 1995). Such options reduce costs associated with transportation and treatment. These treatment costs can last for years after landfill closure, and the resulting reduction in revenue can complicate the ability of resource managers to afford traditional leachate processing methods (Duggan, 2005). An alternative treatment that decreases expenditures is to utilize the leachate as a fertilization and irrigation source for species and interspecific hybrids of the genus *Populus* (Erdman and Christenson, 2000).

Hybrid poplars have been utilized in a variety of phytoremediation projects (Bañuelos et al., 1999; Burken, 2001). Selected poplar genotypes are ideal for remediation due to their ability to: establish quickly after planting and produce large plant biomass, produce extensive root systems, transpire large volumes of water, be propagated easily and inexpensively from hardwood cuttings, and grow on marginal lands (Isebrands and Karnosky, 2001). Although a variety of clonal material has been utilized, research efforts have focused on a few commercially available clones which may not offer maximum remedial benefits to researchers and project managers. Breeders across the North Central United States have spent decades developing more than 100,000 new poplar genotypes for multiple uses such as: fiber, bioenergy, riparian stabilization, wood products, cordwood, and now phytoremediation (Heilman, 1999; Riemenschneider et al., 2001; Zalesny et al., 2005a). This untapped supply of different genotypes offers a unique opportunity to study and identify clones that either perform well across most sites or perform well in sites with specific contamination problems (Zalesny, Riemenschneider, and Hall, 2005b), such as elevated salt concentrations in the leachate and/or soil.
Phyto-recurrent selection involves the adoption of crop and tree improvement strategies to identify and select superior performing clones for specific remediation efforts (Zalesny and Bauer, 2007). Specifically, this method involves evaluation, identification, and selection of favorable clones using multiple testing cycles. The length of each cycle increases concurrently with precision of the data that are acquired. Consequently, as the complexity of the data increases, the number of clones tested in each cycle decreases. The identification of such clones is accomplished with experimental procedures such as those outlined below, with adjustments to allow for site-specific features such as soil type and leachate characteristics.

The primary objective was to evaluate the early growth and productivity of different poplar genotypes when irrigated with landfill leachate or water. Crop and tree improvement concepts were utilized to develop a phyto-recurrent selection model that would help identify superior genotypes tailored to specific objectives. Three selection cycles used to choose eight poplar genotypes for an in situ landfill study (cycle 4) are described. Twenty-five clones were tested in cycle 1, while the best 12 genotypes were evaluated in cycles 2 and 3. Fifteen different belowground and aboveground traits were tested. The null hypotheses for each cycle were that clones would not respond differently to leachate and water irrigation, and that clones would not vary for all traits. This information enhances the body of research already conducted using poplars for landfill remediation because there is a general lack of knowledge about clonal comparisons for establishment success, growth, and productivity of poplar genotypes when irrigated with leachate. In addition, the use of crop and tree improvement methodologies and phyto-recurrent selection offers project managers a tool for the
identification and selection of superior clones that may help to increase the success of future projects of this nature.

**MATERIALS AND METHODS**

**Initial Clone Selection and Cutting Preparation**

Twenty-five poplar (*Populus* spp.) clones (Table 2.1) were selected from six genomic groups during January 2005 for phyto-recurrent selection cycle 1, an *ex situ* study testing early root, stem, and leaf growth in an effort to select the best 12 clones for selection cycles 2 and 3. The genomic groups and clones were selected based on current growth in the North Central United States, past clonal screening tests that demonstrated regional growth success (biomass data), representation of hybrids from multiple species (specifically *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P. trichocarpa*), and clonal availability.

Dormant, unrooted cuttings, 25.4-cm long, were processed from whips collected during December 2005. The whips were grown for one growing season in stool beds established at Hugo Sauer Nursery in Rhinelander, Wisconsin, USA (45.6 °N, 89.4 °W). During processing, cuts were 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, and then soaked in water to a height of 15 cm for 3 d before planting. The trees were grown in a greenhouse at the Forestry Sciences Laboratory in Rhinelander with a 16-h photoperiod and a daytime and nighttime temperature of 24 °C and 20 °C, respectively.
**Leachate Description**

Leachate was collected from the Oneida County Landfill on 14 January 2005. The landfill was located 6 km west of Hugo Sauer Nursery. Leachate was collected and sent to Northern Lake Service, Inc. (Crandon, Wisconsin, USA) on 25 January and 23 February 2005 for chemical analysis using approved United States Environmental Protection Agency methods. The leachate was brownish-green in color, with a putrid odor, an electrical conductivity of $10.2 \pm 0.02 \text{ mS cm}^{-1}$ at $25 \degree \text{C}$, and a pH of $8.4 \pm 0.39$. The concentration of nitrogen (N), phosphorus (P), and potassium (K) was $745 \pm 15 \text{ mg N L}^{-1}$ (191 kg N ha$^{-1}$), $2.1 \pm 0.1 \text{ mg P L}^{-1}$ (0.5 kg P ha$^{-1}$), and $450 \pm 30 \text{ mg K L}^{-1}$, (115 kg K ha$^{-1}$). The primary toxicity concern was the relatively high chloride ($\text{Cl}^-$) concentration of $1400 \pm 0 \text{ mg L}^{-1}$ (359 kg Cl$^-$ ha$^{-1}$).

Final closure and capping of the Oneida County Landfill occurred in 2002. Since that time the concentrations of inorganics, organics, and metals have declined annually. Heavy metals and volatile organic compounds were not detectable in the leachate analysis, and therefore, not a concern with respect to plant establishment. Leachate composition varies by local environmental conditions and deposition of waste from residential, commercial, or industrial sources (Kjeldsen et al., 2002), which was exhibited by variable pH, salinity, biological oxygen demand, chemical oxygen demand, and Cl$^-$ concentration since Oneida County Landfill closure (Table 2.2).
Experimental Design

The trees of each selection cycle were arranged in a split-plot design, with blocks (random), treatments (fixed whole plots), and clones (fixed sub plots). Clones were arranged in randomized complete blocks to minimize effects of any potential environmental gradients in the greenhouse. Treatments and clones were considered as fixed in the analysis and, therefore, we evaluated means rather than variances.

Selection Cycle 1

Tree Establishment and Irrigation Regime. Four blocks, two treatments, and 25 clones (200 experimental units) were tested. The trees were established in folding book planters (4 cells per planter, 10 planters per rack) containing a standard greenhouse potting mix consisting of equal parts of sand, peat, and vermiculite (v:v:v). The irrigation regime was 100-mL treatments of landfill leachate or water (the control) on Monday, Wednesday, and Friday, from 19 January to 2 February 2005.

Data Collection. After 14 d the trees were harvested, washed, and dissected into roots, stems, leaves, and the cutting. In addition, number of roots and leaves were recorded, and leaf area was determined (Li Cor Model 3100 Area Meter). All plant components were oven dried at 70 °C for 72 h, and dry mass was obtained for roots, stems, leaves, and cuttings.

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 (Larson and Isebrands, 1971). Specifically, we used leaf lamina width of 2 cm as a unified reference for plastochron index development.
Lamina width was chosen as a reference because it is 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.

**Data Analysis.** Number of roots and leaves, leaf area, and dry mass data were subjected to analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming the aforementioned split plot design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot). The non-significant ($P > 0.25$) block × clone interaction for all variables was pooled with the three-way interaction into a common error term to increase precision of F-tests (Zalesny et al., 2005b).

Analyses of covariance were conducted to test for the effect of cutting dry mass on all traits because of a broad variation at 14 DAP (1.05 to 7.73 g). Cutting dry mass was a significant covariate for root and top dry mass ($P = 0.0080, P < 0.0001$, respectively), along with number of leaves ($P = 0.0032$); however, cutting dry mass did not have a significant effect on number of roots or leaf area ($P = 0.0567, P = 0.1631$, respectively). Therefore, all means except for number of roots and leaf area were adjusted for the variation in cutting dry mass. Fisher’s protected least significant difference (LSD) was used to compare adjusted and unadjusted means (Chew, 1976).
Selection Cycle 2

Tree Establishment and Irrigation Regime. Four blocks, two treatments, and 12 clones (96 experimental units) were tested. The trees were established in specially-designed rhizotrons that supported two-dimensional, horizontal root growth measurements over time without disturbing aboveground plant growth and without the need for destructive sampling of roots until the final harvest. Wiese, Riemenschneider, and Zalesny (2005) provided a description of the rhizotrons and types of data that can be collected with them. Each rhizotron had a capacity of 6,675 cm$^3$ of soil. The growing medium was sand (rather than the potting mix in cycle 1) to support easier identification of roots during digital root analysis and to supply an inert growing environment. The irrigation regime was 30-mL treatments of leachate or water on Monday, Wednesday, and Friday, from March to June 2005. In addition, drip irrigation with water only was applied for 15 s intervals twice daily. The supplemental irrigation was applied to simulate natural rainfall, along with helping to meet the water demands of the trees.

Data Collection. Digital photographs of the root systems were taken each Monday and Thursday of the experimental period beginning 21 DAP, when roots were present on all clones. The photographs were subjected to digital analysis using WinRHIZO Tron software (Regent Instruments, Inc., Quebec, Canada) to determine total root length at 21, 24, 28, 31, 35, 38, 42, and 45 DAP. Number of leaves (as described above) and tree height was recorded on all photograph dates. Tree height was measured at the point of attachment between the stem and the original cutting in order to reduce measurement error.
After 45 d the trees were harvested, washed, and dissected into roots, stems, leaves, and the cutting. Leaf area was determined (Li Cor Model 3100 Area Meter) and all plant components were oven dried at 70 °C for 72 h to obtain dry mass for roots, stems, leaves, and cuttings.

**Data Analysis.** Number of leaves, height, and root length data were subjected to repeated measures analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming a split plot, repeated measure design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot). The repeated measure was time (i.e. DAP). Given correlated errors associated with DAP, the results from multivariate analyses of variance (MANOVA) were interpreted to provide correct F-variance ratios and to reduce the probability of incorrectly claiming significant differences when, in fact, there were none (i.e. Type I Errors). A pooled error term was used for all traits as in selection cycle 1. Leaf area and dry mass data were subjected to analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming the aforementioned split plot design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot) using a pooled error term. Analyses of covariance were conducted to test for the effect of cutting dry mass on all traits because of a broad variation at 45 DAP (1.41 to 9.04 g). Cutting dry mass was a significant covariate for leaf area, leaf dry mass, stem dry mass, and aboveground dry mass, along with number of leaves (except 24, 42, and 45 DAP) and height throughout the study \( (P < 0.05) \). However, cutting dry mass did not have a significant effect on root dry mass and root length \( (P > 0.05) \). Therefore, all means except for number of leaves at 24, 42, and 45 DAP, along with rooting traits, were adjusted for the variation in cutting dry mass.
Selection Cycle 3

Tree Establishment and Irrigation Regime. Six blocks, two treatments, and 12 clones (144 experimental units) were tested. The trees were established in specially-designed planters constructed of an aluminum framework with plexiglass walls and base. Each planter consisted of four individual tree chambers, each with a capacity of 22,052 cm$^3$ of soil (25.0 cm high × 29.7 cm wide × 29.7 cm deep) (Figure 2.1). Three concentric rings of hardware cloth (0.635 cm × 0.635 cm heavy metal screen) were attached to one another, divided into three layers, and placed in every cell of the planters. Hardware cloth was used to hold roots in place during development and data collection. Cuttings were planted in the middle of the concentric rings. The growing medium was sand to reduce experimental error associated with loss of roots during excavation and to supply an inert growing environment. The irrigation regime was 300-mL treatments of water for a 10-d establishment period, followed by irrigating with 300 mL of leachate or water on Monday, Wednesday, and Friday for the remaining 20 d of the experiment.

Data Collection. Number of leaves and tree height was recorded on all treatment irrigation dates. These traits were determined as described for selection cycle 2. After 30 d the trees were harvested, washed, and dissected into root, stem, leaf, and cutting components. Number of roots was recorded for each layer described above. In addition, leaf area was determined (Li Cor Model 3100 Area Meter) followed by all plant components being oven dried at 70 °C for 72 h. Dry mass was obtained for roots (within each layer), stems, leaves, and the cutting.
Data Analysis. Number of leaves and height data were subjected to repeated measures analyses of variance according to SAS® as described for selection cycle 2. Leaf area, number of roots, and dry mass data were subjected to analyses of variance according to SAS® (SAS Institute Inc., 2004) assuming the aforementioned split plot design with a random block effect and fixed main effects for treatment (whole plot) and clone (sub plot). The block × clone interaction was negligible ($P > 0.25$) for leaf area, number of roots in the first and third layer, total number of roots, and root dry mass in layers one and two. Thus, for these variables, a pooled error term was used.

Analyses of covariance were conducted to test for the effect of cutting dry mass on all traits because of a broad variation at 30 DAP (1.08 to 7.51 g). Cutting dry mass was a significant covariate for leaf area ($P < 0.0001$), leaf dry mass ($P = 0.0070$), stem dry mass ($P = 0.0012$), and aboveground dry mass ($P = 0.0050$), along with root dry mass in the first ($P = 0.0045$) and second layers ($P = 0.0398$). However, cutting dry mass did not have a significant effect on number of leaves, height, or the remaining rooting traits ($P > 0.05$). Therefore, all means except for number of leaves, height, and the remaining rooting traits were adjusted for the variation in cutting dry mass. Fisher’s protected LSD was used to compare adjusted and unadjusted means (Chew, 1976).

Weighted Summation Indices

For the phyto-recurrent selection indices used in cycles 1 to 3, weighted allometric traits (sum of weights = 1) were used based on their relative importance for early establishment and perceived contribution to subsequent phytoremediation. The weights were
multiplied by the adjusted or unadjusted means for the traits of interest, followed by summation of values. In general, roots and leaves each had 40% weight, while stems contributed to 20% of each index value. Favorable genotypes were those that exhibited greater relative index values.

**Selection Cycle 1.** The traits of interest were root number (RN), root dry mass (RDM), leaf number (LN), leaf area (LA), and combined leaf and stem dry mass (LSDM). The following phyto-recurrent selection model was used in the analysis:

\[
\text{Index Value (IV)} = 0.15*RN + 0.25*RDM + 0.1*LN + 0.15*LA + 0.35*LSDM.
\]

**Selection Cycle 2.** The traits of interest were root length (RL), RDM, height (HT), LN, LA, stem dry mass (SDM), and leaf dry mass (LDM). The following model was used:

\[
IV = 0.1*RL + 0.3*RDM + 0.15*HT + 0.05*LN + 0.2*LA + 0.05*SDM + 0.15*LDM.
\]

**Selection Cycle 3.** The traits of interest were root number in layers 1 to 3 (RN\textsubscript{L1}, RN\textsubscript{L2}, RN\textsubscript{L3}), root dry mass in layers 1 to 3 (RDM\textsubscript{L1}, RDM\textsubscript{L2}, RDM\textsubscript{L3}), HT, LN, LA, SDM, and LDM. The following model was used:

\[
IV = 0.025*RN\textsubscript{L1} + 0.075*RN\textsubscript{L2} + 0.1*RN\textsubscript{L3} + 0.025*RDM\textsubscript{L1} + 0.075*RDM\textsubscript{L2} + 0.1*RDM\textsubscript{L3} + 0.15*HT + 0.05*LN + 0.2*LA + 0.05*SDM + 0.15*LDM.
\]

**RESULTS**

**Selection Cycle 1**

Clones responded differently to leachate and water irrigation. Treatment main effects were significant for number of roots (\(P = 0.0005\)), root dry mass (\(P = 0.0050\)), number of
leaves \((P = 0.0003)\), leaf area \((P < 0.0001)\), and combined dry mass of leaves and stems \((P = 0.0001)\). Likewise, clone main effects were significant for these traits \((P = 0.0016\) for root dry mass; \(P < 0.0001\) for all others). Nevertheless, the treatment \(\times\) clone interaction governed these traits \((P = 0.0035\) for root dry mass; \(P = 0.0022\) for number of leaves; \(P < 0.0001\) for all others). Overall, the number of roots across treatments and clones ranged from 0.3 ± 0.3 to 37.5 ± 5.0, with a mean of 12.6 ± 2.4, while root dry mass ranged from 0.0 ± 0.0 to 197.7 ± 17.7 mg, with a mean of 29.4 ± 15.0 mg. Similarly, number of leaves across treatments and clones ranged from 0.0 ± 0.0 to 8.6 ± 0.8, with a mean of 3.0 ± 0.8, while leaf area ranged from 0.0 ± 0.0 to 72.0 ± 4.9 cm\(^2\), with a mean of 15.3 ± 3.5 cm\(^2\). The combined dry mass of leaves and stems across treatments and clones ranged from 7.3 ± 7.3 to 274.0 ± 18.0 mg, with a mean of 96.7 ± 17.9 mg (Figure 2.2). Leachate and water treatments differed for seven of the fourteen backcross clones [(\(P.\) trichocarpa \(\times\) \(P.\) deltoides) \(\times\) \(P.\) deltoides], one pure \(P.\) deltoides clone, and all clones belonging to the remaining genomic groups \((P.\) deltoides \(\times\) \(P.\) maximowiczii; \(P.\) deltoides \(\times\) \(P.\) nigra; \(P.\) nigra \(\times\) \(P.\) maximowiczii). Similar interaction trends existed for all other traits.

**Selection Cycle 2**

Clones responded similarly to leachate and water irrigation. The number of days after planting affected treatment main effects for height \((P_{\text{MANOVA}} = 0.0117)\) and number of leaves \((P_{\text{MANOVA}} < 0.0001)\). From a univariate standpoint, treatment main effects were significant for height at 21 and 24 DAP \((P = 0.0276, P = 0.0310, \text{ respectively})\), and for number of leaves at 21, 24, and 28 DAP \((P = 0.0362, P = 0.0329, P = 0.0487, \text{ respectively})\). The difference
between treatment means at each measurement date decreased over time for these traits (Figure 2.3). Furthermore, clone main effects were significant for height and number of leaves, regardless of DAP ($P < 0.05$), along with leaf area ($P < 0.0001$), stem dry mass ($P = 0.0010$), and leaf dry mass ($P < 0.0001$). In contrast, clone main effects only affected root length at 21 and 24 DAP ($P = 0.0310$, $P = 0.0310$, respectively). Overall, leaf area across treatments and clones ranged from $39.1 \pm 37.6$ to $302.4 \pm 44.7 \text{ cm}^2$, with a mean of $172.0 \pm 38.3 \text{ cm}^2$. Similar clonal variation existed for all other significant traits.

**Selection Cycle 3**

Clones responded differently to leachate and water irrigation for rooting traits, but responded similarly for aboveground traits. The number of days after planting affected clone main effects for height ($P_{\text{MANOVA}} < 0.0001$) and number of leaves ($P_{\text{MANOVA}} = 0.0114$). From a univariate standpoint, clone main effects were significant for all traits ($P < 0.0001$). There was broad variation in clonal responses to treatments for rooting traits at different layers. The treatment $\times$ clone interaction was significant for number of roots in the top layer ($P = 0.0064$), along with root dry mass in top and middle layers ($P = 0.0045$, $P = 0.0398$, respectively). Overall, number of roots in the top layer across treatments and clones ranged from $0.0 \pm 0.0$ to $8.8 \pm 0.6$, with a mean of $4.0 \pm 1.1$. Although mean root dry mass across treatments and clones in the top, middle, and bottom layer was $17.2 \pm 6.3$, $79.0 \pm 22.6$, and $223.4 \pm 46.5$ mg, respectively, significant differences in clonal responses to treatments was present only in the top and middle layers (Figure 2.4).
**Weighted Summation Indices**

A summary of index values and outcomes of the weighted summation indices for each selection cycle is presented in Figure 2.5. The favorable clones of selection cycle 1 belonged to every genomic group except that of pure *P. deltoides* and the F1 hybrids of *P. deltoides*. Nevertheless, we chose *P. deltoides* clone 91.05.02 for selection cycles 2 and 3 because cuttings of the clone ranked eighth (NC13608) were of poor quality, and because we had an academic interest in advancing 91.05.02, which ultimately was not selected for cycle 4. In contrast, the favorable clones of selection cycles 2 and 3 excluded pure *P. deltoides* genotypes. Clone NC13460 was selected over DN182 and NC13475 because there was a discrepancy between the indices for these clones, and because cuttings of NC13475 were of poor quality. Overall, selection cycle 1 took one month to complete (beginning January 2005), while cycles 2 and 3 lasted an additional five months. Selection cycle 4, an *in situ* trial at the Oneida County Landfill in northern Wisconsin, was completed during August 2006, 20 months after the initial planting of cuttings in cycle 1. The most favorable clones are scheduled to be planted during April 2007, 28 months after initial planting.

**DISCUSSION**

The main goals of this study were to test poplar genotypes for differences in belowground and aboveground growth when irrigated with landfill leachate or water, and to develop and evaluate a phyto-recurrent selection model to help researchers and resource managers choose superior clones for phytoremediation field applications. Clones responded differently to treatments for all traits in selection cycle 1. This supports the postulate of
testing clonal compatibility for leachate composition and concentration. Early root and shoot
development is necessary for plant survival, with increased belowground and aboveground
growth indicating genotype-specific tolerance and/or the capability for exclusion of leachate-
imposed stresses (Larcher, 1995). Sensitivity to elevated concentrations of salts in landfill
leachate, such as Cl⁻ in our samples, elicited strong environmental pressure to eliminate
weaker and less-tolerant genotypes. The elimination of unsuccessful genotypes from the
experiment at the earliest selection cycle is imperative to the final goal of field deployment of
well-suited clones. The number of clones depends upon on the size of the planting and
relatedness of the selected genotypes.

Clones responded similarly to treatments during selection cycle 2. Repeated
measurements for number of leaves and height across clones were different early on.
However, treatment differences became more negligible over time. Half of the experimental
genotypes were removed after selection cycle 1, with the remaining clones performing
uniformly during selection cycle 2. The leachate appeared to be more detrimental during the
root initiation stage when young tissues were responsive to osmotic stress. Once established,
the trees were able to tolerate high Cl⁻ concentrations. Overall, the apparent stress had
diminished before the end of the experiment. The trends in Figure 2.3 indicated the potential
for trees receiving leachate to exhibit greater number of leaves and height after 45 DAP.
Thus, it may be beneficial in future studies to test the trees for an additional week, or until the
roots are no longer discernible from one another in the photographs (this depends upon the
exact clones studied).
Generally, treatments did not affect clones for aboveground growth in selection cycle 3. However, clones responded differently to treatments for rooting in the upper two-thirds of the cutting length (i.e. top and middle planter layers). Similar variation in tissue-specific responses across five willow clones belonging to four genomic groups was reported (Dimitriou, Aronsson, and Weih, 2006). Amplified root growth is most likely due to increased nutrients available for exploitation (Rytter and Hansson, 1996). These results are useful to phytoremediation applications because an extensive rhizosphere and its associated microorganisms may offer improved remedial benefits (Anderson, Guthrie, and Walton, 1993). Likewise, root biomass should be positively correlated with phytoremediation capability (Zalesny et al., 2005a).

The usefulness of phyto-recurrent selection lies with balancing the acquisition of meaningful data with successfully identifying and selecting favorable genotypes that can be used for field applications. Although it is next to impossible to simulate field conditions in a greenhouse or growth chamber, our method is a useful technique that includes multiple selection cycles within a short time period and that can be done with limited resources. In the current study, selection cycle 1 lasted one month and five traits were evaluated (Figure 2.5). Selection cycles 2 and 3 were conducted simultaneously for five months, with an additional 7 and 11 traits tested, respectively. Although actual phytoremediation effectiveness was not evaluated in these cycles, a longer (15 month) in situ study (cycle 4) was conducted that involved evaluating approximately 20 traits. The tissue concentration of contaminants in the roots, stems, and leaves, along with soil and leachate concentrations, were tested (J.A. Zalesny, unpublished data). Ultimately, superior clones will be selected for the resource
manager as early as 21 months from the planting of selection cycle 1. The number of clones decreased from 25 in cycle 1 to eight in cycle 4 that will be used for on-site treatment of landfill leachate. As stated above, the number of clones for the phytoremediation system is dependent upon the size of the planting and the parentage of the clones. From a genetics standpoint, it is important to have enough diversity among genotypes to guard against insect/disease outbreaks, changes in soil conditions, and unfavorable genotype × environment interactions (Zalesny and Bauer, 2007).

Short rotation intensive forestry systems using poplars, along with willows (Salix spp.), have provided resource managers with multiple uses and associated products (Heilman, 1999). In addition to phytoremediation, short rotation woody crops provide secondary benefits such as aesthetic improvement and value-added products during short rotation harvests. However, such high-intensity cropping requires optimal plant nutrition for biomass production and for resistance to disease and insect outbreaks (Coyle and Coleman, 2005). Leachate is an excellent source of irrigation and nitrogen fertilization for poplar and willow (Hasselgren, 1992); despite that elevated levels of heavy metals and salts may reduce growth (Stephens, Tyrrel, and Tiberghien, 2000).

The nitrogen application rate of 191 kg N ha⁻¹ in the leachate was within the range of optimal nutrient estimates reported for pure P. deltoides and hybrid genotypes (105 to 276 kg N ha⁻¹) (Heilman and Stettler, 1986; Nelson, Switzer, and Lockaby, 1987). Coleman, Friend, and Kern (2004) reported two-year-old P. deltoides ‘D105’ grown in northern Wisconsin, USA, acquired at most 120 kg N ha⁻¹ yr⁻¹ from synthetic and soil N sources, with trees receiving 50 and 100 kg N ha⁻¹ yr⁻¹ exhibiting near-optimal growth. In contrast, elevated Cl⁻
concentrations impose osmotic stress and associated negative effects on tree growth such as: leaf chlorosis, early leaf abscission, growth inhibition, and increased mortality (Cureton, Groenevelt, and McBride, 1991; Menser, Winant, and Bennett, 1983). In the current study, the elevated leachate Cl⁻ concentration (1400 mg Cl⁻ L⁻¹; 359 kg Cl⁻ ha⁻¹) likely diminished the overall positive effect on growth and productivity associated with N fertilization. Stephens et al. (2000) demonstrated an inverse relationship between leachate Cl⁻ concentrations greater than 2500 mg L⁻¹ and growth/productivity of *Salix viminalis* L. Q683’.

Excessive chloride and elevated electrical conductivity (EC) can be related to increased plant stress and associated reductions in productivity (Neuman et al., 1996; Shannon et al., 1999). The variability in chloride tolerance among the clones used in this study was indicative of the broad genetic variation among *Populus* genotypes at the section, species, and clone level (Rajora and Zsuffa, 1990). Mechanisms of chloride tolerance for poplars are not well understood, however, optimal growth was reported at an EC ranging from 1 to 5 mS cm⁻¹ (Neuman et al., 1996). It has been shown that salt tolerance existed for *P. deltoides × P. nigra* (DN) clones. Aw and Wagner (1993) found that DN clones ranged in sensitivity when irrigated with wastewater with an EC of 3.21 mS cm⁻¹, while Shannon et al. (1999) reported growth reduction at 3.3 mS cm⁻¹. Overall, this corroborated the advancement of clone DN5 to selection cycle 4, while DN182 was removed from the experiment at the end of selection cycle 3, given an EC of the leachate of 10.2 mS cm⁻¹.

Overall, based on our results, there is potential for on-site treatment of landfill leachate using poplars. However, there are concerns with leachate application associated with optimizing plant growth while minimizing environmental impact. In some cases, where
levels of contaminants are toxic, the leachate should be diluted before application to alleviate the potential phytotoxic effects to the trees. For example, Wong and Leung (1989) reported the need to dilute leachate in order for *Brassica* (cabbage) and *Acacia* (acacia) plants to sustain the nutritional advantages of the fertilization without exhibiting phytotoxic impacts. In general, *Brassica* species had greater yields when irrigated with leachate, while species of both genera experienced inhibited root growth from leachate application. Nevertheless, the need for dilution depends upon the specific leachate composition and the genotypes used for remediation. Cheng and Chu (2006) irrigated twelve tree species for 90 days with leachate exhibiting broad differences in chemical properties. Overall, none of the leachate sources caused toxic effects, growth inhibition, or decreased biomass accumulation, but leachate irrigation did increase leaf N concentration. Furthermore, pioneer tree species that shared silvicultural traits with *Populus* and *Salix* withstood harsh conditions imposed by elevated contaminant levels in the leachate. Toxic effects to plants are easier to see and measure than toxic effects to the environment. Application rates need to be carefully considered and monitored to minimize or eliminate contamination to the soil or groundwater.

These methods have important academic and practical implications, assuming there are adequate levels of money and time. However, it is realized that the phyto-recurrent selection techniques used in the current study may be more complicated and time consuming than researchers and resource managers can allocate for genotypic testing. An easier and more-efficient selection effort could involve the use of only one or two of the selection cycles. For example, methods described for selection cycle 1 could be used with larger containers and greater numbers of clones for an extended length of time. In addition, less
complex observations and data collection may be sufficient for selection of the best performing clones tailored to specific project objectives. A second consideration not addressed in the current study is the field deployment of rooted planting stock. Root initiation in this experiment was sensitive to osmotic stress, which might be overcome with selection cycles and field deployment of rooted cuttings. In addition, the use of rooted material would allow land managers access to hundreds of genotypes that otherwise will not survive in the field due to erratic rooting capability. There is a potential for improvement of remedial success because root initiation is essential to cutting survival, but the ability of a cutting to root in the greenhouse compared with the field has no bearing on remediation effectiveness. Overall, the most useful recommendation for researchers and resource managers is to test genotypic material prior to field deployment to make superior clonal selections for enhancement of remedial efforts.

In summary, these results detail the extensive variation in clonal responses to leachate irrigation, along with the need and efficacy of using phyto-recurrent selection to choose superior genotypes. Future landfill leachate remediation research in regions similar to the North Central United States should evaluate rooted cuttings as well as unrooted cuttings, especially for *P. deltoides* genotypes that do not establish well from unrooted cuttings because of erratic rooting. Additionally, there is a need to assess the effect of landfill leachate on macro- and micro-organisms in the rhizosphere.
ACKNOWLEDGMENTS

We thank Bart Sexton (Oneida County Solid Waste Department) for supplying the leachate, along with Steven Ohm, Sherry Otto, and Susan Scobell-Watson (Wisconsin Department of Natural Resources) for assistance obtaining short duration discharge permits for leachate application. In addition, we are grateful to Steven Rock (US EPA) for insightful technical reviews and suggestions. Also, we appreciate review of earlier versions of the manuscript from: Edmund Bauer, Deahn Donner, and Nicanor Saliendra.

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Rajora, O.P. and Zsuffa, L. 1990. Allozyme divergence and evolutionary relationships among *Populus deltoides*, *P. nigra*, and *P. maximowiczii*. *Genome* 33, 44-49.


Table 2.1 Genomic groups and clones of *Populus* irrigated with landfill leachate and municipal water

<table>
<thead>
<tr>
<th>Genomic group</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P. trichocarpa × P. deltoides) × P. deltoides</td>
<td>NC13451, NC13460, NC13475, NC13608, NC13652, NC13661, NC13668, NC13670, NC13672, NC13680, NC13807, NC13850, NC13857, NC14018</td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>P. deltoides × P. deltoides (F₁ hybrid)</td>
<td>80X00601</td>
</tr>
<tr>
<td>P. deltoides</td>
<td>7300501, 8000105, 91.05.02</td>
</tr>
<tr>
<td>P. deltoides × P. maximowiczii</td>
<td>DM115, NC14104, NC14106</td>
</tr>
<tr>
<td>P. deltoides × P. nigra</td>
<td>DN5, DN182</td>
</tr>
<tr>
<td>P. nigra × P. maximowiczii</td>
<td>NM2, NM6</td>
</tr>
</tbody>
</table>

Note: Authorities for the aforementioned species are: *P. deltoides* Bartr. ex Marsh; *P. trichocarpa* Torr. & Gray; *P. nigra* L., *P. maximowiczii* A. Henry
Table 2.2  Leachate composition over time of parameters relevant to the current study compared with those in the published literature

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>pH</th>
<th>Electrical conductivity (mS cm$^{-1}$)</th>
<th>Biological oxygen demand (mg L$^{-1}$)</th>
<th>Chemical oxygen demand (mg L$^{-1}$)</th>
<th>Cl$^-$ (mg L$^{-1}$)</th>
</tr>
</thead>
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<tr>
<td>19 April 2001</td>
<td>8.0</td>
<td>8.7</td>
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<td>2800</td>
<td>1000</td>
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<tr>
<td>9 April 2002</td>
<td>7.9</td>
<td>8.7</td>
<td>270</td>
<td>1300</td>
<td>980</td>
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<tr>
<td>10 October 2002</td>
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<td>10.0</td>
<td>1600</td>
<td>2600</td>
<td>1100</td>
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<tr>
<td>30 April 2003</td>
<td>8.1</td>
<td>6.8</td>
<td>380</td>
<td>1500</td>
<td>1300</td>
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<tr>
<td>28 October 2003</td>
<td>8.6</td>
<td>13.0</td>
<td>690</td>
<td>2300</td>
<td>1600</td>
</tr>
<tr>
<td>6 April 2004</td>
<td>8.1</td>
<td>7.0</td>
<td>69</td>
<td>880</td>
<td>790</td>
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<tr>
<td>15 October 2004</td>
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<td>210</td>
<td>1100</td>
<td>1200</td>
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<tr>
<td>25 January 2005$a$</td>
<td>8.0</td>
<td>10.2</td>
<td>14</td>
<td>1100</td>
<td>1400</td>
</tr>
<tr>
<td>23 February 2005$a$</td>
<td>8.8</td>
<td>10.2</td>
<td>48</td>
<td>1000</td>
<td>1400</td>
</tr>
<tr>
<td>28 April 2005</td>
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<td>5.7</td>
<td>16</td>
<td>670</td>
<td>820</td>
</tr>
<tr>
<td>19 October 2005</td>
<td>8.8</td>
<td>6.6</td>
<td>26</td>
<td>650</td>
<td>750</td>
</tr>
<tr>
<td>Other leachate$b$</td>
<td>4.5 to 9.0</td>
<td>2.5 to 35.0</td>
<td>20 to 57000</td>
<td>140 to 152000</td>
<td>150 to 4500</td>
</tr>
</tbody>
</table>

$a$Leachate used in the current study.

$^b$Ranges based on 14 studies cited in Kjeldsen et al. (2002).
Figure 2.1 Specially-designed planters used to quantify root initiation and development along the length of the cuttings, in addition to aboveground traits. Within each chamber (A), one cutting was planted in the middle of three concentric rings of hardware cloth (B) that were attached to one another and divided into three layers (C).
Figure 2.2 Combined dry mass of the leaves and stems for each combination of treatment (leachate, water) and clone during phyto-recurrent selection cycle 1. Each bar represents the mean adjusted for cutting dry mass with one standard error. Asterisks above bars indicate differences between treatments, according to Fisher’s protected least significant difference (LSD) ($\alpha = 0.05$, $n = 4$, LSD = 51.3 mg). The dashed line represents the overall mean.
Figure 2.3 Number of leaves (A) and height (B) across clones vs. days after planting (DAP) for each treatment (leachate, water) during phyto-recurrent selection cycle 2. Each diamond represents the mean (n = 48) adjusted for cutting dry mass (except number of leaves at 24, 42, and 45 DAP that were unadjusted), with one standard error. Treatments with different letters within a day were different, according to a split-plot, repeated measures ANOVA ($P < 0.05$).
Figure 2.4 Root dry mass in the top (A) and middle (B) layers of specially-designed planters for each combination of treatment (leachate, water) and clone during phyto-recurrent selection cycle 3. Root dry mass in the bottom layer was negligible ($P = 0.1197$). Each bar represents the mean unadjusted (A) and adjusted (B) for cutting dry mass, with one standard error. Treatments with different letters within a clone were different, according to Fisher’s protected least significant difference (LSD) ($\alpha = 0.05$, $n = 6$, $\text{LSD}_{\text{top}} = 20.0$ mg, $\text{LSD}_{\text{middle}} = 60.0$ mg). The dashed line represents the overall mean.
Figure 2.5 Phyto-recurrent selection cycles using weighted summation indices to choose eight *Populus* clones for selection cycle 4, an *in situ* study testing phytoremediation effectiveness following irrigation with landfill leachate or water. See Materials and Methods for quantitative descriptions of the indices.
CHAPTER 3. GROWTH AND BIOMASS OF POPULUS IRRIGATED WITH LANDFILL LEACHATE

A paper in press in Forest Ecology and Management

Jill A. Zalesny, Ronald S. Zalesny Jr., David R. Coyle, and Richard B. Hall

Abstract

Resource managers are challenged with waste disposal and leachate produced from its degradation. Poplar (Populus spp.) trees offer an opportunity for ecological leachate disposal as an irrigation source for managed tree systems. Our objective was to irrigate Populus trees


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with municipal solid waste landfill leachate or fertilized well water (control) (N, P, K) during the 2005 and 2006 growing seasons and test for differences in tree height, diameter, volume, and biomass of leaves, stems, branches, and roots. The trees were grown at the Oneida County Landfill located 6 km west of Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). Eight clones belonging to four genomic groups were tested: NC13460, NC14018 [(P. trichocarpa Torr. & Gray × P. deltoides Bartr. ex Marsh) × P. deltoides ‘BC1’]; NC14104, NC14106, DM115 (P. deltoides × P. maximowiczii A. Henry ‘DM’); DN5 (P. deltoides × P. nigra L. ‘DN’); and NM2, NM6 (P. nigra × P. maximowiczii ‘NM’). The survival rate for each of the irrigation treatments was 78%. The total aboveground biomass ranged from 0.51 to 2.50 Mg ha\(^{-1}\), with a mean of 1.57 Mg ha\(^{-1}\). The treatment × clone interaction was not significant for tree diameter, total volume, dry mass of the stump or basal roots, or root mass fraction \((P > 0.05)\). However, the treatment × clone interaction was significant for height, total tree dry mass, aboveground dry mass, belowground dry mass, and dry mass of the leaves, stems + branches (woody), and lateral roots \((P < 0.05)\). There was broad clonal variation within the BC\(_1\) and DM genomic groups, with genotypes performing differently for treatments. In contrast, the performance of the NM and DN genomic groups was relatively stable across treatments, with clonal response to irrigation being similar regardless of treatment. Nevertheless, selection at the clone level also was important. For example, NC14104 consistently performed better when irrigated with leachate compared with water, while NC14018 responded better to water than leachate. Overall, these data will serve as a basis for researchers and resource managers making decisions about future leachate remediation projects.
Keywords: hybrid poplar; phytoremediation; Populus deltoides; P. maximowiczii; P. nigra; P. trichocarpa; root mass fraction

1. Introduction

Poplars (Populus spp.) have been extensively studied in short rotation woody biomass production systems for multiple uses such as fiber, fuel and environmental benefits (Dickmann, 2001; Isebrands and Karnosky, 2001; Coleman and Stanturf, 2006). Exemplary traits that have contributed to the success of such uses include: ease of rooting, quick establishment, fast growth, and elevated rates of photosynthesis and water usage (Ceulemans et al., 1992; Pontailler et al., 1999; Zalesny et al., 2006). Broad genetic diversity among poplar genomic groups and selection of specific genotypes within such groups increase the potential enhancement of growth and establishment for various uses across heterogeneous sites (Heilman and Stettler, 1985; Heilman et al., 1994). The combination of appropriate cultural practices and well-suited genotypes helps to maximize poplar performance for improved biomass yields (Buhler et al., 1998; Stanturf et al., 2001).

Environmental benefits have been realized from poplar culture when used as components in riparian buffers along streams (Schultz et al., 2004) and as vegetative filters for phytoremediation applications (Licht and Isebrands, 2005). Several phytoremediation projects utilized wastewater in the form of landfill leachate as an irrigation and fertilization source for poplar trees (Shrive et al., 1994; Erdman and Christenson, 2000; Zalesny and Bauer, 2007). Proper clonal selection practices must be utilized given the genetic variability
within the genus *Populus* (Rajora and Zsuffa, 1990; Eckenwalder, 1996) and the variable concentrations of inorganic and organic components in the leachate (Gettinby et al., 1996). Leachate production occurs through natural degradation processes aided by the movement of water through the landfill profile (Christensen and Kjeldsen, 1989). Due to the variation associated with residential, commercial, and industrial waste material, the leachate is highly variable and compositional changes occur seasonally and annually (Shrive et al., 1994; Kjeldsen et al., 2002).

A great deal of information has been reported using poplars for short rotation forestry (Heilman, 1999; Riemenschneider et al., 2001), but there are relatively fewer reports about using poplars for leachate phytoremediation systems. Thus, researchers and resource managers need information that is currently lacking about tree establishment with leachate irrigation. Such information will help increase the success of using poplars for remedial benefits, especially with ecologically-damaging contaminants such as those found in most leachate. Overall, the use of short rotation woody crop management for remediation supports improved environmental quality and secondary benefits such as carbon sequestration, a harvestable product, aesthetic improvements, and erosion control (Isebrands and Karnosky, 2001; Duggan, 2005).

This project expands on our previous work investigating phyto-recurrent selection, which was defined as a method using crop and tree improvement strategies to identify and select superior performing clones for remediation projects (Zalesny et al., 2007). Clonal selections were made after three successive cycles of evaluation (i.e. three separate greenhouse studies) testing 23 traits relating to height growth, leaf development, and root
initiation at 14 (cycle 1; 25 clones), 45 (cycle 2; 12 clones), and 30 (cycle 3; 12 clones) days after planting. The best eight clones were selected for testing in the current *in situ* study (cycle 4) out of the original 25 genotypes belonging to six distinct genomic groups: 1) (*P. trichocarpa* Torr. & Gray × *P. deltoides* Bartr. ex Marsh) × *P. deltoides* ‘BC1’; 2) *P. deltoides* × *P. deltoides* ‘DD’; 3) *P. deltoides* ‘D’; 4) *P. deltoides* × *P. maximowiczii* A. Henry ‘DM’; 5) *P. deltoides* × *P. nigra* L. ‘DN’; 6) *P. nigra* × *P. maximowiczii* ‘NM’.

The overall objective of all phyto-recurrent selection cycles was to test the effectiveness of poplars for uptake of inorganic and organic contaminants found in landfill leachate. More specifically, the objective of the current study was to test for differences in growth and biomass distribution of eight *Populus* clones when irrigated with municipal solid waste landfill leachate or fertilized well water (control) (N, P, K) for two growing seasons. In addition to actual phytoremediation success, tree growth and biomass accumulation are important for evaluating the overall effectiveness of the biological attenuation system. These data will serve as a basis for researchers and resource managers making decisions about future leachate remediation projects.

2. Materials and methods

2.1. Site and leachate description

The study was conducted at the Oneida County Landfill (municipal solid waste) located 6 km west of Rhinelander, Wisconsin, USA (45.6°N, 89.4°W). Temperature,
precipitation, and growing degree days across the experimental period are listed in Table 3.1. The landfill soils are classified as mixed, frigid, coarse loamy Alfic Haplorthods (Padus Loam, PaB), with 0 to 6 percent slopes, and are considered well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash. Soil pH, along with carbon and nitrogen content, is listed in Table 3.2.

Leachate was collected from the Oneida County Landfill and its chemistry was analyzed (Northern Lake Service, Inc., Crandon, Wisconsin, USA) using approved United States Environmental Protection Agency methods. The leachate was brown in color and had a putrid odor. Concentrations of nitrogen (N), phosphorus (P), and potassium (K) were 610 ± 68 mg N L\(^{-1}\) (157 kg N ha\(^{-1}\)), 2.3 ± 0.4 mg P L\(^{-1}\) (0.6 kg P ha\(^{-1}\)), and 450 ± 30 mg K L\(^{-1}\) (115 kg K ha\(^{-1}\)). The primary toxicity concern was the relatively high chloride (Cl\(^{-}\)) concentration of 1114 ± 140 mg L\(^{-1}\) (286 kg Cl\(^{-}\) ha\(^{-1}\)). In contrast, the Cl\(^{-}\) concentration in the well water (control) at the time of harvest was 3.5 mg L\(^{-1}\) (0.9 kg Cl\(^{-}\) ha\(^{-1}\)). Other than Cl\(^{-}\), the leachate concentrations of inorganics, organics, and metals have declined annually since final closure and capping of the landfill in 2002. Heavy metals and volatile organic compounds were not detectable in the leachate analysis, and therefore, not a concern with respect to plant establishment and development. Variation of pH, salinity, biological oxygen demand, chemical oxygen demand, and Cl\(^{-}\) concentration since landfill closure is presented in Table 3.3.
2.2. Clone selection and experimental design

Eight *Populus* clones were selected from 25 original genotypes during three phyto-recurrent selection cycles based on 23 traits relating to height growth, leaf development, and root initiation (Zalesny et al., 2007). The clones and their parentages (i.e. genomic groups) were: NC13460, NC14018 [(*P. trichocarpa* Torr. & Gray × *P. deltoides* Bartr. ex Marsh) × *P. deltoides* ‘BC₁’]; NC14104, NC14106, DM115 (*P. deltoides* × *P. maximowiczii* A. Henry ‘DM’); DN5 (*P. deltoides* × *P. nigra* L. ‘DN’); and NM2, NM6 (*P. nigra* × *P. maximowiczii* ‘NM’). In this paper we use the *Populus* section names as specified by Eckenwalder (1996), but we have retained the species nomenclature for *P. maximowiczii* (Japanese poplar) that has been previously used in the *Populus* literature. *Populus maximowiczii* is currently classified as a subspecies of *P. suaveolens* Fischer (Eckenwalder, 1996; Dickmann, 2001). Throughout this paper, we have qualitatively compared genomic groups because we were interested in evaluating genotypes at the strategic level of selection. However, given the lack of statistically-adequate clonal representation within genomic groups, rigorous testing among genomic groups was not conducted.

Shoots were collected during dormancy from stool beds established at Hugo Sauer Nursery in Rhinelander. Hardwood cuttings, 20 cm long, were prepared during January 2005, 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 at 5 °C and soaked in water to a height of 15 cm for 3 d before planting on 14 Jun. 2005. Prior to planting, the soil was tilled to a depth of 30 cm. Cuttings were planted in a split plot design with eight blocks, two treatments (whole plots),
and eight clones (sub plots) at a spacing of 1.2 × 2.4 m (i.e. 3472 trees ha⁻¹). Clones were arranged in randomized complete blocks in order to minimize effects of any potential environmental gradients. Two border rows of clone NM2 were established on the perimeter of the planting and between treatment whole plots to reduce potential border effects (Hansen, 1981; Zavitkovski, 1981).

Water (control) from a well located 100 m from the study area was applied to all cuttings via hand irrigation for an establishment period of 14 d. Following establishment, trees were hand irrigated with either leachate or fertilized water, using a low-flow distribution nozzle connected to a garden hose. Fertilizer (N, P, and K) was added to the control treatment during each irrigation application at a rate equal to that of the leachate to eliminate fertilization effects. The 2005 weekly application rate was 3.8 L tree⁻¹ (23.1 mm ha⁻¹ assuming an irrigated soil surface area of 0.16 m² per tree). Given eight applications, a total of 1.9 kL of each treatment was applied across the growing season. Drip irrigation was used to apply treatments during 2006. The treatment application rate for 2006 was increased to 22.7 L tree⁻¹ (34.6 mm ha⁻¹ assuming an irrigated soil surface area of 0.66 m² per tree) because of root system development. Given twelve applications, a total of 17.4 kL of each treatment was applied across the growing season. To prevent substantial leaching from the experimental plot, application of treatments was adjusted based on precipitation events. Irrigation was postponed if greater than 0.5 cm of rainfall occurred within 2 d prior to watering or was expected to occur with a 40% chance or greater for 2 d following watering.

Mechanical and hand weeding were performed weekly in 2005 and 2006 to ensure maximum tree survival. Electric fencing was used to prevent deer browse and injury to the
trees. Polyvinylchloride (PVC) tubing, 15.24 cm in diameter, was installed after leaf senescence in November 2005 on each tree to protect the trunk from girdling by rodents during the winter.

2.3. Data collection and analysis

Height (to the nearest 1.0 cm) and diameter (to the nearest 0.01 mm) were measured on 15 Aug. 2006. Height was measured from ground level to the base of the apical bud on the terminal shoot. To reduce experimental error associated with stump swell, diameter was measured at 10 cm above the soil surface. Volume (cm$^3$) was estimated using the generalized equation: $volume = diameter^2 \times height$, according to Avery and Burkhart (1994).

On 17 Aug. 2006, each tree was rated for presence or absence of sylleptic branches, which are defined as branches that emerge from buds without a period of dormancy (Wu and Stettler, 1998). In addition, one branch from each of the basal, middle, and apical thirds of each tree was randomly chosen for harvest. Total leaf area of the three branches was determined for each tree (Li Cor Model 3100 Area Meter), and the subsampled woody components (stems + branches) and leaves were placed in a drying oven at 70 °C for dry mass determination.

All trees were destructively harvested in two stages on 18 Aug. 2006. First, the aboveground portion of each tree was cut at 10 cm above the soil surface, and woody and leaf components were separated and dried at 70 °C. Woody, leaf, and aboveground (woody + leaf) biomass was determined when dry mass values reached a constant mass. Total tree leaf
area (TTLA) was estimated according to the following equation: \( \text{TTLA} = \frac{\text{area of subsampled leaves}}{\text{dry mass of subsampled leaves}} \times \text{total tree leaf dry mass} \). Second, root systems were excavated using a mechanized tree spade that removed a uniform, conical volume of soil (diameter \( \times \) depth = 0.28 m\(^3\)) for each tree. Root systems were washed and divided into the stump, lateral roots, and basal roots. Lateral and basal root separation was based on organ development from the stump associated with these two primary \textit{Populus} rooting ontogenies. Lateral roots develop from latent root primordia distributed throughout the length of the original cutting, while basal roots develop from callus as a result of wounding at the base of the cutting (Luxova and Lux, 1981; Zalesny et al., 2005). Stump, lateral root, basal root, and belowground (stump + lateral + basal) dry mass was determined identically to shoot components. Root mass fraction was calculated as the ratio between belowground dry mass and total tree dry mass (Coyle and Coleman, 2005).

Data were analyzed using analyses of variance (PROC MIXED; SAS Institute, Inc., 2004) assuming the split plot design described above. Blocks were considered random in the analysis, while treatments were fixed whole plots and clones were fixed sub plots. Therefore, means were evaluated rather than variances. The following linear additive model was used:

\[
Y_{ijk} = \mu + B_i + T_j + BT_{ij} + C_k + TC_{jk} + \text{Pooled Error}
\]

where: \( Y_{ijk} \) = response variable to be analyzed, \( \mu \) = overall mean, \( B_i \) = main effect of \( i^{th} \) block, \( T_j \) = main effect of \( j^{th} \) treatment, \( BT_{ij} \) = effect of interaction between \( i^{th} \) block and \( j^{th} \) treatment, \( C_k \) = main effect of \( k^{th} \) clone, \( TC_{jk} \) = effect of interaction between \( j^{th} \) treatment and
kth clone, and pooled error = error term resulting from pooling of BC_{ik} and BTC_{ijk} terms, defined as: effect of interaction among ith block and kth clone, and effect of interaction among ith block, jth treatment, and kth clone, respectively. Means were considered different at probability values of $P < 0.05$.

3. Results

3.1. Tree growth

The survival rate of the trees at the time of harvest was the same for each treatment at 78% (50/64). Height did not differ between leachate and well water (control) treatments, but there were differences among clones. The treatment × clone interaction was significant (Table 3.4). *Populus nigra* × *P. maximowiczii* ‘NM’ clones NM2 and NM6 had the greatest height across both irrigation treatments (Fig. 3.1). Despite substantial clonal variation among genotypes belonging to the *P. deltoides* × *P. maximowiczii* ‘DM’ (DM115, NC14106, NC14104) and [(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*] ‘BC1’ (NC14018, NC13460) genomic groups, all but one clone exhibited similar performance across treatments. Only clone NC14104 had significantly greater height when irrigated with leachate than water, while significantly greater height for water versus leachate did not exist within any genotype. Overall, the mean height was 149.3 ± 16.0 cm. Treatment and clone main effects, along with their interaction, were negligible for diameter and volume (Table 3.4).
3.2. Biomass distribution

The main effects of treatment and clone for total tree dry mass were not significant, but the treatment × clone interaction was (Table 3.4). The NM clones exhibited the greatest overall total tree dry mass, while the BC₁ and DM genotypes had the most clonal variation (i.e. variation between or among clones within a specific genomic group) (Fig. 3.2). Clones NC13460 and NC14104 had significantly greater total tree dry mass with leachate over water, while clone NC14018 exhibited greater total dry mass with water over leachate. Overall, the mean total tree dry mass was 529.6 ± 189.2 g.

Aboveground and belowground dry mass accumulation was similar among treatments and clones. Treatment and clone main effects for aboveground dry mass were not significant, but the treatment × clone interaction was (Table 3.4). The NM clones exhibited the greatest overall aboveground dry mass, while the BC₁ and DM genotypes had the most clonal variation (Fig. 3.2). Clone NC14104 was the only clone that had significantly greater aboveground dry mass when irrigated with leachate than water. In contrast, clone NC14018 exhibited greater aboveground dry mass with water than leachate. Overall, the mean aboveground dry mass was 453.3 ± 167.2 g. Moreover, treatment and clone main effects were not significant for belowground dry mass, but the treatment × clone interaction was (Table 3.4). A distinct genomic group advantage for overall belowground dry mass was nonexistent, but the BC₁ and DM genotypes had the most clonal variation (Fig. 3.2). Clone NC14104 was the only clone that had significantly greater belowground dry mass when irrigated with leachate than water. In contrast, clones NC14018 and DM115 exhibited
greater belowground dry mass with water than leachate. Overall, the mean belowground dry mass was 76.4 ± 22.7 g.

The main effects of treatment and clone did not differ for leaf dry mass. However, there was a significant treatment × clone interaction (Table 3.4). The NM clones exhibited the greatest overall leaf dry mass, while the DM genotypes had the most clonal variation (Table 3.5). Clone NC14104 was the only clone that had significantly greater leaf dry mass when receiving leachate irrigation compared with water. In contrast, clone NC14018 exhibited greater leaf dry mass when receiving water irrigation compared with leachate. Overall, the mean leaf dry mass was 217.6 ± 73.0 g. There was a highly significant ($P < 0.0001$) linear relationship between leaf area and stem volume and between leaf area and woody dry mass (Fig. 3.3). Leachate treatment did not affect woody dry mass, but there were differences among clones. The treatment × clone interaction was significant (Table 3.4). The NM clones exhibited the greatest overall woody dry mass, while the BC₁ and DM genotypes had the most clonal variation (Table 3.4). Clone NC14104 was the only genotype that had significantly greater woody dry mass when irrigated with leachate versus water, while clone NC14018 exhibited greater stem dry mass with water versus leachate. Overall, the mean woody dry mass was 235.7 ± 94.9 g. The ranking of our genomic groups for relative sylleptic branching from most to least was BC₁:DM:NM:DN.

Treatment and clone main effects, along with their interaction, were not significant for stump dry mass or basal root dry mass (Table 3.4). However, the main effects of treatment and clone, along with the treatment × clone interaction, were significant for lateral root dry mass (Table 3.4). The DM and NM clones exhibited the greatest overall lateral root
dry mass, while the BC\textsubscript{1} genotypes had the most clonal variation (Table 3.5). No clones had significantly greater lateral root dry mass when irrigated with leachate compared with water, but clone NC14018 exhibited greater lateral root dry mass with water compared with leachate. Overall, the mean lateral root dry mass was 25.3 ± 8.2 g.

Treatments did not affect root mass fraction (RMF), but clones were significantly different. There was no treatment × clone interaction for RMF (Table 3.4). The BC\textsubscript{1} clones and DN5 exhibited the greatest overall RMF, while the DM genotypes had the most clonal variation (Fig. 3.4). Overall, genotypes within genomic groups performed similarly, showing a lack of clonal differences. The mean RMF was 0.16 ± 0.01.

4. Discussion

Although leachate irrigation did not enhance tree growth and biomass for most genotypes in the current study, significant productivity reductions associated with the leachate also were not observed. Therefore, there is a great potential for remediation of landfill leachate using \textit{Populus}. Selection within the clonal variation that resulted from variable responses to leachate or well water (control) treatments will serve as a basis for researchers and resource managers making decisions about future leachate remediation projects. Further examinations are needed, however, that test similar responses throughout the entire rotation. The objective of this study was to irrigate \textit{Populus} with landfill leachate or water and to test for differences in height, diameter, and volume, along with biomass of the leaves, stems, branches, and roots. Some of the genomic groups and clones exhibited broad
variation for most traits, while the performance of other genotypes was relatively stable. Specifically, there were two trends in the performance of the four genomic groups. First, broad clonal variation existed within the BC1 and DM genomic groups, with clones performing differently for treatments. Second, the productivity of the NM and DN genomic groups was relatively stable across treatments, with the NM clones having the greatest growth and biomass accumulation for nearly all tissue components. Clone NC14104 was the only genotype to uniformly exhibit greater height and biomass for multiple tissues when irrigated with leachate compared with water, while NC14018 consistently exhibited greater levels of biomass accumulation with water versus leachate.

Irrigation and fertilization effects on *Populus* productivity have been previously tested (Coleman et al., 2004; Brown and van den Driessche, 2005; Coyle and Coleman, 2005). This information is useful for increasing yield when applying an alternative irrigation and fertilizer source such as landfill leachate. Shrive et al. (1994) irrigated NM6 for two seasons with 3.5 mm d⁻¹ of leachate, a volume similar to the current study, and found height to be significantly greater than with the water treatment. In contrast, fertilization effects from the leachate were not present in the current study. We standardized the nutrient content of our water irrigation treatments for N, P, and K (i.e. we added fertilizer at concentrations equal to the leachate for each element) in order to identify impacts resulting from the negative and potentially-toxic chemical constituents of the leachate, without giving the leachate treatment a fertilization advantage. This standardization was important because N is the most limiting factor in short rotation woody crop systems, and N addition is a proven method for increasing overall productivity of the trees (Hansen et al., 1988; Brown and van den Driessche, 2002; Coyle and
Coleman, 2005). In contrast, it has been reported that N-fertilization did not increase growth during establishment. DesRochers et al. (2006) tested growth responses to fertilization of one *P. balsamifera* L. (B) × *P. simonii* Carr. (S) hybrid ‘33 cv. P38P38’ and two *P. deltoides* (D) × *P. × petrowskyana* (P) hybrids ‘24 cv. Walker’ ‘794 cv. Brooks6’ and reported negligible fertilization responses after the second growing season. In addition, variation in fertilization growth responses of *P. tremuloides* Michx. seedlings as a result of different soil pH levels were reported (DesRochers et al., 2003).

The elevated Cl⁻ concentration (1100 mg L⁻¹) and electrical conductivity (EC) (8.3 mS cm⁻¹) was a concern in the current study, considering poplars have been reported to be sensitive to salt and have optimal growth at an EC ranging from 1 to 5 mS cm⁻¹ (Neuman et al., 1996). However, there were no treatment differences for aboveground dry mass. Therefore, the leachate did not negatively impact this trait, which may have been partially due to dilution of the leachate by the soil and/or precipitation. Nevertheless, there was some genetic variation in sensitivity to Cl⁻ and EC among the *Populus* genotypes studied, with minimal productivity losses or increased plant stresses that are common responses related to excessive Cl⁻ and elevated EC (Neuman et al., 1996; Shannon et al., 1999). Aside from NC14018, all clones showed similar or better aboveground biomass with the leachate compared with the water treatment. The elevated Cl⁻ content of the leachate was likely a factor in the clonal sensitivity of NC14018 to leachate irrigation, which was illustrated by the greater biomass of NC14018 when irrigated with water. Thus, proper clonal selection for elevated Cl⁻ and EC is essential for deployment in future systems.
Biological productivity of short rotation woody crops is measured by the combination of aboveground and belowground growth, economic yield, and associated environmental benefits (Dickmann, 2001). Though difficult to quantify, ecological benefits such as carbon sequestration, erosion control, reduced pollution, and improved landscape processes are compelling reasons for the deployment of phytoremediation systems. Economic benefits are relatively easier to quantify and can be obtained from phytoremediation projects by harvesting the aboveground biomass of the trees (i.e. harvestable yield). The total aboveground biomass in the current study ranged from 0.51 to 2.50 Mg ha\(^{-1}\), with a mean of 1.57 Mg ha\(^{-1}\). The NM clones had a clear genomic group advantage, with the greatest overall biomass of 2.50 Mg ha\(^{-1}\) for NM6 and 1.95 Mg ha\(^{-1}\) for NM2. These results were similar to Baker and Blackmon (1977), who reported 2.42 Mg ha\(^{-1}\) of biomass for D after one growing season in Stoneville, Mississippi, USA (33.4 °N, 90.9 °W). This growth from one season in Stoneville (216 frost-free days) is greater than two seasons of growth of our clonal material in northern Wisconsin (103 frost-free days). Therefore, the longer growing season, extending into November, is largely responsible for the greater biomass accumulation in the southern United States versus the North Central region. The 2006 growing season in the current study was shortened given the mid-August harvest. This time frame was used to harvest the trees during their vigorous growth at the end of the leachate applications. Other reports of *Populus* biomass were similar to those in the current study. Pontailler et al. (1999) reported 1.15 to 4.22 Mg ha\(^{-1}\) of aboveground dry mass after one growing season in Orsay, France (49.0 °N, 2.5 °E) for one *P. trichocarpa* (T), one DN, and two *P. trichocarpa × P. deltoides* (TD) genotypes. Likewise, our leaf dry mass (217.6 g tree\(^{-1}\)) was within the range (169 to 235 g
reported by Ceulemans et al. (1996) for second year growth of TD and DN clones. In contrast, our stem dry mass (235.7 g tree\textsuperscript{-1}) was less than the range (504 to 717 g tree\textsuperscript{-1}) reported by Tschaplinski and Blake (1989) for second year growth of three DN clones.

Furthermore, the leaves and woody biomass of the current study each comprised 50% of the total aboveground dry mass, which was relatively similar to the leaves (37%) and stems + branches (63%) for one-year-old D genotypes (Baker and Blackmon, 1977). In addition, leaf and woody biomass components of our study were within the range reported by Friend et al. (1991) for two TD clones after two growing seasons in the Pacific Northwest (PNW) region of the United States (131 frost-free days). In their study, 35% to 81% of aboveground biomass was comprised of stems, while 19% to 65% was in the leaves.

Aboveground biomass of T, D, and two TD clones also was evaluated in the PNW (Scarascia-Mugnozza et al., 1997). After two years of growth, stems + branches comprised 59% to 74% of the aboveground biomass over all clones.

The relationships between leaf area and volume, and between leaf area and aboveground dry mass, are important for phytoremediation given the need for early prediction of potential remedial effectiveness. There was a positive linear relationship for these traits in the current study (Fig. 3.3). Although similar correlations among numerous allometric traits often have been reported for *Populus* (Isebrands and Nelson, 1982; Ridge et al., 1986; Rogers et al., 1989; van den Driessche, 1999), this information remains relevant. Evaluation of specific correlations in any study is necessary, because such correlations may not hold true across studies. For example, the development of sylleptic branching is an important morphometric trait associated with enhanced early productivity and increased
photosynthetic carbon for tree development (Scarascia-Mugnozza et al., 1999; Dickmann, 2001). Well-developed correlations between sylleptic branching and tree yield have been reported (Wu and Stettler, 1998; Scarascia-Mugnozza et al., 1999). However, based on a survey of sylleptic branching in the current study, a positive relationship between sylleptic branching and biomass was not observed. Likewise, Ceulemans et al. (1992) reported a weak correlation between sylleptic branching and stem volume after one and four growing seasons for T female parents, D male parents, and their TD F1 hybrids. Interestingly, they reported the greatest number of sylleptic branches occurred in the T genotypes, with the F1 hybrids exhibiting intermediary scores and the D genotypes the fewest number of sylleptic branches (Ceulemans et al., 1992). The ranking of our genomic groups for relative sylleptic branching from most to least was BC1:DM:NM:DN. Our BC1 clones were the only genomic group with T parentage, but the *P. maximowiczii* (M) males of the DM and NM F1 hybrids also belong to the section *Tacamahaca*. Sylleptic branching was nearly non-existent for DN5, whose parentage is limited to the section *Aigeiros*. Similar intersectional differences have been reported for rooting among these genomic groups (Zalesny and Wiese, 2006).

Indirect selection for a desirable characteristic based on direct selection of an easily-measurable trait can be useful in identification of favorable clones if the intertrait correlation is strong enough. Leaf area is an important trait for many remediation processes, especially given its relationship to photosynthetic productivity (Larson and Isebrands, 1972). Contaminants may either be sequestered and/or degraded in the leaves and other tissues (Burken and Schnoor, 1997; Newman et al., 1997) or be volatilized through leaf stomata and transpired into the atmosphere (Newman et al., 1997; Thompson et al., 1998; Mirck et al.,
2005). However, it is difficult for researchers and resource managers to determine whole-tree leaf area on trees beyond the first growing season. At the time of harvest in the current study, some trees that were sampled for total leaf counts had nearly 2000 leaves. Therefore, there is an ongoing need to identify easily-measurable traits that can be used as predictors of the correlative variables (Larson and Isebrands, 1972; Isebrands and Nelson, 1982; Harrington et al., 1997). If the desired phytoremediation processes involve the direct need for increased leaf area, then simple, non-destructive volume calculations can be used to estimate leaf area. Aboveground dry mass, albeit a destructive method, also would be easier than whole-tree leaf area determinations. Isebrands and Nelson (1982) used similar methods to test whether leaf characteristics could be estimated from less complex variables, with the overall goal of using such information for improving biomass productivity of Populus in short rotation intensive forestry systems. Likewise, Harrington et al. (1997) reported that leaf production (area or mass) was a useful predictor of potential productivity of a TD (11-11) and T (7-75) Populus clone. Given the results of the current study, we believe this type of information can be adapted for similar assessment needs during the establishment phase in almost all phytoremediation settings.

5. Conclusion

Overall, given that every leachate source should be regarded as unique, there is an essential need for initial genotype screening followed by the establishment and evaluation of test plots to ascertain clonal performance prior to large scale deployment. The lack of overall
differences in response to treatments in the current study was a result of extensive genotypic screening during phyto-recurrent selection cycles 1 to 3 that reduced the variability among the clones deployed, relative to the original 25 genotypes (Zalesny et al., 2007). However, from a practical standpoint, the variation that was observed was useful for further selection of clones that could be used in a large-scale system. For example, clone NC14018 would not be suitable for further deployment if irrigated with the leachate used in the current study, but NC14104 would be an ideal candidate relative to the other clones. Thus, similar tree-based bioremediation technologies can be beneficial for the reduction of environmental damage resulting from such pollution (Mirck et al., 2005). Phytoremediation merges the science of plantation forestry with environmental clean-up methodologies to achieve the following important ecological benefits: 1) phytoremediation utilizes natural plant processes whereby the leachate can be biologically cleansed to remove many of the excessive nutrients and chemicals; 2) depending on the contaminants, phytoremediation plantations may be harvested in 8 to 10 years for fiber or energy, utilizing short rotation forestry to offset demand and conserve natural forest stands (Gladstone and Ledig, 1990); 3) when plants remove and sequester excess nutrients and chemicals found in the leachate, it prevents the unwanted leaching of potentially harmful contaminants into nearby watersheds.
Acknowledgements

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Table 3.1
Mean temperature, total precipitation, and total number of growing degree days (GDD; base = 10°C) from May to October during 2005 and 2006 in Rhinelander, Wisconsin, USA (45.6 °N, 89.4 °W)

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
<th>Precipitation (cm)</th>
<th>GDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>10</td>
<td>12</td>
<td>4.44</td>
</tr>
<tr>
<td>June</td>
<td>16</td>
<td>17</td>
<td>4.51</td>
</tr>
<tr>
<td>July</td>
<td>20</td>
<td>21</td>
<td>13.04</td>
</tr>
<tr>
<td>August</td>
<td>16</td>
<td>18</td>
<td>5.43</td>
</tr>
<tr>
<td>September</td>
<td>12</td>
<td>naa</td>
<td>3.90</td>
</tr>
<tr>
<td>October</td>
<td>5</td>
<td>na</td>
<td>3.44</td>
</tr>
</tbody>
</table>

*a* Not applicable because trees were harvested 18 Aug. 2006.
Table 3.2
Soil pH (n = 3), along with carbon and nitrogen content (n = 4), at a depth of 0 to 30 cm at nine sampling points for each treatment. The control treatment was well water applied at a volume equal to that of the leachate.

<table>
<thead>
<tr>
<th>Sampling point</th>
<th>pH (Control)</th>
<th>pH (Leachate)</th>
<th>C (g kg⁻¹) (Control)</th>
<th>C (g kg⁻¹) (Leachate)</th>
<th>N (g kg⁻¹) (Control)</th>
<th>N (g kg⁻¹) (Leachate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.21 ± 0.03</td>
<td>6.07 ± 0.04</td>
<td>7.03 ± 0.21</td>
<td>22.35 ± 2.44</td>
<td>0.60 ± 0.06</td>
<td>1.73 ± 0.20</td>
</tr>
<tr>
<td>2</td>
<td>5.68 ± 0.07</td>
<td>6.15 ± 0.02</td>
<td>7.43 ± 1.36</td>
<td>36.25 ± 2.00</td>
<td>0.67 ± 0.19</td>
<td>3.05 ± 0.17</td>
</tr>
<tr>
<td>3</td>
<td>5.54 ± 0.07</td>
<td>5.71 ± 0.02</td>
<td>5.83 ± 0.84</td>
<td>24.40 ± 1.25</td>
<td>0.50 ± 0.07</td>
<td>2.00 ± 0.11</td>
</tr>
<tr>
<td>4</td>
<td>5.31 ± 0.07</td>
<td>6.21 ± 0.09</td>
<td>10.23 ± 0.76</td>
<td>45.70 ± 2.23</td>
<td>0.88 ± 0.06</td>
<td>3.80 ± 0.17</td>
</tr>
<tr>
<td>5</td>
<td>5.93 ± 0.04</td>
<td>6.32 ± 0.03</td>
<td>16.83 ± 1.80</td>
<td>51.30 ± 5.45</td>
<td>1.55 ± 0.10</td>
<td>4.53 ± 0.52</td>
</tr>
<tr>
<td>6</td>
<td>6.35 ± 0.02</td>
<td>6.25 ± 0.02</td>
<td>42.50 ± 3.77</td>
<td>49.55 ± 2.24</td>
<td>3.60 ± 0.32</td>
<td>4.35 ± 0.22</td>
</tr>
<tr>
<td>7</td>
<td>5.70 ± 0.03</td>
<td>6.37 ± 0.01</td>
<td>33.63 ± 2.47</td>
<td>50.23 ± 2.57</td>
<td>2.95 ± 0.19</td>
<td>4.38 ± 0.18</td>
</tr>
<tr>
<td>8</td>
<td>6.16 ± 0.03</td>
<td>6.11 ± 0.03</td>
<td>5.03 ± 0.39</td>
<td>39.03 ± 1.30</td>
<td>0.53 ± 0.05</td>
<td>3.45 ± 0.10</td>
</tr>
<tr>
<td>9</td>
<td>5.86 ± 0.05</td>
<td>6.35 ± 0.00</td>
<td>11.80 ± 0.43</td>
<td>41.85 ± 1.17</td>
<td>1.05 ± 0.05</td>
<td>3.75 ± 0.10</td>
</tr>
<tr>
<td>Overall</td>
<td>5.75 ± 0.21</td>
<td>6.17 ± 0.12</td>
<td>15.82 ± 2.25</td>
<td>40.07 ± 1.89</td>
<td>1.39 ± 0.19</td>
<td>3.45 ± 0.18</td>
</tr>
</tbody>
</table>
Table 3.3
Oneida County Landfill leachate composition over time of parameters relevant to the current study compared with those in the published literature

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>pH</th>
<th>Electrical conductivity (mS cm⁻¹)</th>
<th>Biological oxygen demand (mg L⁻¹)</th>
<th>Chemical oxygen demand (mg L⁻¹)</th>
<th>Cl⁻ (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 Apr. 2001</td>
<td>8.0</td>
<td>8.7</td>
<td>1600</td>
<td>2800</td>
<td>1000</td>
</tr>
<tr>
<td>9 Apr. 2002</td>
<td>7.9</td>
<td>8.7</td>
<td>270</td>
<td>1300</td>
<td>980</td>
</tr>
<tr>
<td>10 Oct. 2002</td>
<td>7.7</td>
<td>10.0</td>
<td>1600</td>
<td>2600</td>
<td>1100</td>
</tr>
<tr>
<td>30 Apr. 2003</td>
<td>8.1</td>
<td>6.8</td>
<td>380</td>
<td>1500</td>
<td>1300</td>
</tr>
<tr>
<td>28 Oct. 2003</td>
<td>8.6</td>
<td>13.0</td>
<td>690</td>
<td>2300</td>
<td>1600</td>
</tr>
<tr>
<td>6 Apr. 2004</td>
<td>8.1</td>
<td>7.0</td>
<td>69</td>
<td>880</td>
<td>790</td>
</tr>
<tr>
<td>15 Oct. 2004</td>
<td>8.9</td>
<td>3.4</td>
<td>210</td>
<td>1100</td>
<td>1200</td>
</tr>
<tr>
<td>25 Jan. 2005</td>
<td>8.0</td>
<td>10.2</td>
<td>14</td>
<td>1100</td>
<td>1400</td>
</tr>
<tr>
<td>23 Feb. 2005</td>
<td>8.8</td>
<td>10.2</td>
<td>48</td>
<td>1000</td>
<td>1400</td>
</tr>
<tr>
<td>28 Apr. 2005</td>
<td>8.8</td>
<td>5.7</td>
<td>16</td>
<td>670</td>
<td>820</td>
</tr>
<tr>
<td>19 Oct. 2005</td>
<td>8.8</td>
<td>6.6</td>
<td>26</td>
<td>650</td>
<td>750</td>
</tr>
<tr>
<td>12 Apr. 2006</td>
<td>8.2</td>
<td>9.6</td>
<td>190</td>
<td>1100</td>
<td>1200</td>
</tr>
</tbody>
</table>

Reported leachate

- pH 4.5 to 9.0
- Electrical conductivity 2.5 to 35.0
- Biological oxygen demand 20 to 57000
- Chemical oxygen demand 140 to 152000
- Cl⁻ 150 to 4500

Table adapted from Zalesny et al. (2007).

*Ranges based on 14 studies cited in Kjeldsen et al. (2002).*
Table 3.4
Probability values from analyses of variance comparing growth and biomass traits of eight *Populus* clones (see Materials and Methods for descriptions) irrigated once-weekly with treatments of fertilized well water (control) or landfill leachate during the 2005 and 2006 growing seasons. Significant values are in bold.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Trait</th>
<th>Treatment</th>
<th>Clone</th>
<th>Treatment × Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height (cm)</td>
<td>0.1642</td>
<td><strong>0.0094</strong></td>
<td><strong>0.0494</strong></td>
</tr>
<tr>
<td></td>
<td>Diameter (cm)</td>
<td>0.2552</td>
<td>0.1027</td>
<td>0.1368</td>
</tr>
<tr>
<td></td>
<td>Volume (cm³)</td>
<td>0.1336</td>
<td>0.1504</td>
<td>0.0910</td>
</tr>
<tr>
<td>Dry mass (g)</td>
<td>Total tree</td>
<td>0.4965</td>
<td>0.0620</td>
<td><strong>0.0397</strong></td>
</tr>
<tr>
<td></td>
<td>Aboveground</td>
<td>0.5987</td>
<td>0.0550</td>
<td><strong>0.0464</strong></td>
</tr>
<tr>
<td></td>
<td>Belowground</td>
<td>0.0956</td>
<td>0.0921</td>
<td><strong>0.0146</strong></td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.3767</td>
<td>0.1495</td>
<td><strong>0.0400</strong></td>
</tr>
<tr>
<td></td>
<td>Woody (stem + branch)</td>
<td>0.8124</td>
<td><strong>0.0180</strong></td>
<td><strong>0.0515</strong></td>
</tr>
<tr>
<td></td>
<td>Stump</td>
<td>0.2954</td>
<td>0.0716</td>
<td>0.0971</td>
</tr>
<tr>
<td></td>
<td>Basal root</td>
<td>0.2355</td>
<td>0.4944</td>
<td>0.0616</td>
</tr>
<tr>
<td></td>
<td>Lateral root</td>
<td><strong>0.0185</strong></td>
<td><strong>0.0102</strong></td>
<td><strong>0.0119</strong></td>
</tr>
<tr>
<td></td>
<td>Root mass fraction</td>
<td>0.1031</td>
<td>&lt;0.0001</td>
<td>0.9099</td>
</tr>
</tbody>
</table>
Table 3.5
Dry mass (g) of tree components for each combination of clone and treatment (n = 3 to 8) 14 months after planting following once-weekly landfill leachate irrigation during the 2005 (3.8 L tree⁻¹ week⁻¹) and 2006 (22.7 L tree⁻¹ week⁻¹) growing seasons. The control treatment was water applied at a volume equal to that of the leachate. See Materials and Methods for genotypic descriptions. Means within each component labeled with different letters were different at \( P < 0.05 \). The treatment × clone interaction was negligible for stump (\( P = 0.0971 \)) and basal root (\( P = 0.0616 \)) dry mass. Aboveground, belowground, and total tree biomass are illustrated in Fig. 3.2.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Treatment</th>
<th>Leaf</th>
<th>Stem + Branch</th>
<th>Stump</th>
<th>Lateral root</th>
<th>Basal root</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC13460</td>
<td>Control</td>
<td>49.4 ± 100.7  d</td>
<td>18.1 ± 130.8 d</td>
<td>7.7 ± 12.3</td>
<td>5.7 ± 11.5  d</td>
<td>10.5 ± 13.4</td>
</tr>
<tr>
<td></td>
<td>Leachate</td>
<td>119.3 ± 87.5  cd</td>
<td>107.3 ± 113.7 cd</td>
<td>18.9 ± 10.7</td>
<td>9.6 ± 9.9  cd</td>
<td>11.6 ± 11.6</td>
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<tr>
<td>NC14018</td>
<td>Control</td>
<td>368.5 ± 87.5  a</td>
<td>428.0 ± 113.7 ab</td>
<td>40.6 ± 10.7</td>
<td>61.0 ± 9.9  a</td>
<td>35.2 ± 11.6</td>
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<tr>
<td></td>
<td>Leachate</td>
<td>128.3 ± 62.8  cd</td>
<td>135.7 ± 81.8  c</td>
<td>21.6 ± 7.6</td>
<td>12.9 ± 7.0  cd</td>
<td>15.7 ± 8.2</td>
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<td>NC14104</td>
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<td>153.3 ± 66.9  bcd</td>
<td>127.5 ± 87.1  c</td>
<td>16.1 ± 8.2</td>
<td>19.9 ± 7.5  bcd</td>
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<td>373.3 ± 62.8  a</td>
<td>404.8 ± 81.8  ab</td>
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<td>Control</td>
<td>246.9 ± 62.8  abcd</td>
<td>189.0 ± 81.8  bc</td>
<td>25.4 ± 7.6</td>
<td>30.3 ± 7.0  bc</td>
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<td>78.6 ± 113.7  cd</td>
<td>14.2 ± 10.7</td>
<td>10.9 ± 9.9  cd</td>
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<td>DM115</td>
<td>Control</td>
<td>272.6 ± 72.0  abcd</td>
<td>267.6 ± 93.7  abc</td>
<td>38.3 ± 8.8</td>
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<td>127.8 ± 102.2 c</td>
<td>17.1 ± 9.6</td>
<td>20.4 ± 8.9  bcd</td>
<td>8.9 ± 10.4</td>
</tr>
<tr>
<td>DN5</td>
<td>Control</td>
<td>230.8 ± 66.9  abcd</td>
<td>272.9 ± 87.1  abc</td>
<td>41.6 ± 8.2</td>
<td>26.1 ± 7.5  bcd</td>
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<td>143.7 ± 66.9  bcd</td>
<td>184.4 ± 87.1  bc</td>
<td>28.7 ± 8.2</td>
<td>9.3 ± 7.5  d</td>
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<td>NM2</td>
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<td>262.1 ± 66.9  abcd</td>
<td>275.4 ± 87.1  abc</td>
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<td>324.5 ± 87.1  abc</td>
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<td>354.2 ± 81.8  ab</td>
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<td>41.7 ± 8.2</td>
<td>40.0 ± 7.5  ab</td>
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Fig. 3.1. Height of eight *Populus* clones (with genomic groups listed in parentheses) 14 months after planting following once-weekly landfill leachate irrigation during the 2005 (3.8 L tree⁻¹ week⁻¹) and 2006 (22.7 L tree⁻¹ week⁻¹) growing seasons. The control treatment was water applied at a volume equal to that of the leachate. Each bar represents the mean of 3 to 8 trees with one standard error. Bars labeled with different letters were different at $P < 0.05$. 
Fig. 3.2. Above- and below-ground biomass of eight *Populus* clones (with genomic groups listed in parentheses) 14 months after planting following once-weekly landfill leachate irrigation during the 2005 (3.8 L tree\(^{-1}\) week\(^{-1}\)) and 2006 (22.7 L tree\(^{-1}\) week\(^{-1}\)) growing seasons. The control (C) treatment was water applied at a volume equal to that of the leachate (L). Zero on the y-axis denotes the groundline. Each bar represents the mean of 3 to 8 trees with one standard error. Bars labeled with different lowercase [aboveground {above 0} and belowground {below 0}] and uppercase (total tree biomass) letters were different at \(P < 0.05\).
Fig. 3.3. Leaf area versus stem volume (A) and woody (stem + branch) dry mass (B), per tree (n = 100 for each).

Leaf area = 0.8054 + 0.0012 Volume
\[ r^2 = 0.76 \]
\[ P < 0.0001 \]

Leaf area = 0.5267 + 0.0074 Dry mass
\[ r^2 = 0.90 \]
\[ P < 0.0001 \]
Fig. 3.4. Root mass fraction across leachate and water (control) irrigation treatments of eight *Populus* clones (with genomic groups listed in parentheses) 14 months after planting. Each bar represents the mean of 7 to 15 trees with one standard error. Bars labeled with different letters were different at $P < 0.05$. 

[Graph showing root mass fraction across different clones and their respective genomic groups with statistical differentiation.]
CHAPTER 4. SODIUM AND CHLORIDE ACCUMULATION IN LEAF, WOODY, AND ROOT TISSUE OF POPULUS AFTER IRRIGATION WITH LANDFILL LEACHATE

A paper submitted to *Environmental Pollution*¹

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Bart Sexton⁴, and Richard B. Hall²

**Abstract**

The response of *Populus* to irrigation sources containing elevated levels of sodium

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(Na\(^+\)) and chloride (Cl\(^-\)) is poorly understood. We irrigated eight Populus clones with fertilized well water (control) (N, P, K) or municipal solid waste landfill leachate weekly during 2005 and 2006 in Rhinelander, Wisconsin, USA (45.6 °N, 89.4 °W). During Aug. 2006, we tested for differences in total Na\(^+\) and Cl\(^-\) concentration in preplanting and harvest soils, and in leaf, woody (stems + branches), and root tissue. The leachate-irrigated soils at harvest had the greatest Na\(^+\) and Cl\(^-\) levels. Genotypes exhibited elevated total tree Cl\(^-\) concentration and increased biomass (clones NC14104, NM2, NM6), elevated Cl\(^-\) and decreased biomass (NC14018, NC14106, DM115), or mid levels of Cl\(^-\) and biomass (NC13460, DN5). Leachate tissue concentrations were 17 (Na\(^+\)) and four (Cl\(^-\)) times greater than water. Sodium and Cl\(^-\) levels were greatest in roots and leaves, respectively.

Capsule: Sodium and chloride supplied via landfill leachate irrigation is accumulated at high concentrations in tissues of Populus.

Keywords: Hybrid Poplars; Short Rotation Woody Crops; Phytoaccumulation; Salts; Waste Management

1. Introduction

There is a need for environmental practices that merge intensive forestry with waste management (Mirck et al. 2005). For example, leachate remediation is accomplished in situ when used as an irrigation source for Populus trees (i.e. poplars) and other short rotation
woody crops (SRWC) (Shrive et al. 1994). However, leachate application has been detrimental when applied to SRWC systems, with negative impacts on plant tissues including: leaf chlorosis and necrosis, decreased biomass accumulation, and increased mortality (Stephens et al. 2000). Such impacts are exacerbated when excessive salt levels in the leachate irrigation cause osmotic stress (Duggan 2005), in addition to nutrient imbalance and toxic effects in the plant tissues (Lessani and Marschner 1978). The chemical composition of most leachate is highly variable (Kjeldsen et al. 2002). Therefore, leachate chemistry needs to be evaluated to determine potential phytotoxic effects resulting from such elevated ionic concentrations, and to prevent reductions in photosynthesis, leaf area, height, and diameter of *Populus* genotypes (Fung et al. 1998; Stephens et al. 2000).

Poplars have shown potential for phytoremediation projects involving landfill leachate and high salinity environments (Shrive et al. 1994; Bañuelos et al. 1999; Shannon et al. 1999; Erdman and Christenson 2000). Traits that make poplars suitable for such uses include: quick establishment, large biomass accumulation, extensive and deep root systems, high rates of transpiration, ease of asexual propagation, and exceptional growth on marginal lands (Isebrands and Karnosky 2001; Zalesny et al. 2005). However, there are few reports in the literature about the response of different genomic groups and clones of *Populus* to elevated levels of sodium (Na⁺) and chloride (Cl⁻), or the variation in salt tolerance and tissue composition of such genotypes over multiple growing seasons in field settings. Therefore, field trials of a mixture of genotypes representing numerous genomic groups offers an opportunity to identify and select clones that exhibit broad variation in tolerance to salt environments. Such information is important for making recommendations to resource
managers that will help to increase the successful utilization of landfill leachate as a fertilization and irrigation source for species and interspecific hybrids of the genus *Populus* (Erdman and Christenson 2000; Zalesny and Bauer 2007). Additionally, such information is useful for establishing field-scale Na\(^+\) and Cl\(^-\) thresholds for *Populus*.

The current study expands on our previous work evaluating ex situ genotype selection for phytoremediation projects (Zalesny et al. 2007a), along with testing the in situ growth and biomass accumulation of eight *Populus* clones when irrigated with landfill leachate for two growing seasons (Zalesny et al. 2007b). However, specific levels of Na\(^+\) and Cl\(^-\) accumulation of the trees were not evaluated in either of these previous experiments. Therefore, the primary objective of the current study was to test the uptake and distribution of Na\(^+\) and Cl\(^-\) into leaf, woody (stems + branches), and root tissue of eight *Populus* genotypes that were irrigated with fertilized well water (control) (N, P, K) or municipal solid waste landfill leachate for two growing seasons. Our hypotheses were that clones would respond differently to water and leachate irrigation, and that clones would vary for tissue concentration of Na\(^+\) and Cl\(^-\) in leaf, woody, and root tissue. This information is useful to SRWC biomass production for environmental benefits, because there is a general lack of knowledge about the response of *Populus* genotypes to Na\(^+\) and Cl\(^-\) concentrations in landfill leachate, especially when used as an irrigation source in field trials.
2. Materials and methods

2.1. Site description

The study was conducted at the Oneida County Landfill located 6 km west of Rhineland, Wisconsin, USA (45.6 °N, 89.4 °W). Temperature, precipitation, and growing degree days across the experimental period were described previously (Zalesny et al. 2007b). The landfill soils are classified as mixed, frigid, coarse loamy Alfic Haplorthods (Padus Loam, PaB), with 0 to 6 percent slopes, and are considered well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash.

2.2. Clone selection

Eight Populus clones were selected from 25 original genotypes, based on aboveground and belowground traits, after being irrigated with leachate in a series of greenhouse experiments that constituted three phyto-recurrent selection cycles (Zalesny et al. 2007a). The clones and their parentages (i.e. genomic groups) were: NC13460, NC14018 [(P. trichocarpa Torr. & Gray × P. deltoides Bartr. ex Marsh) × P. deltoides ‘BC1’]; NC14104, NC14106, DM115 (P. deltoides × P. maximowiczii A. Henry ‘DM’); DN5 (P. deltoides × P. nigra L. ‘DN’); and NM2, NM6 (P. nigra × P. maximowiczii ‘NM’). In this paper we use the Populus section names as specified by Eckenwalder (1996), but we have
retained the species nomenclature for *P. maximowiczii* (Japanese poplar) now classified as a subspecies of *P. suaveolens* Fischer (Eckenwalder 1996; Dickmann 2001).

### 2.3. Tree establishment and experimental design

Shoots were collected during dormancy from stool beds established at Hugo Sauer Nursery in Rhinelander. Hardwood cuttings, 20 cm long, were prepared during January 2005, 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 at 5 °C and soaked in water to a height of 15 cm for 3 d before planting on 14 Jun. 2005. Prior to planting, the soil was tilled to a depth of 30 cm. Cuttings were planted in a split plot design with eight blocks, two irrigation treatments (whole plots), and eight clones (sub plots) at a spacing of 1.2 × 2.4 m (i.e. 3472 trees ha⁻¹). Clones were arranged in randomized complete blocks in order to minimize effects of any potential environmental gradients. Two border rows of clone NM2 were established on the perimeter of the planting and between treatment whole plots to reduce potential border effects (Hansen 1981; Zavitkovski 1981). Mechanical and hand weeding were performed weekly in 2005 and 2006 to ensure maximum tree survival. Electric fencing was used to prevent deer browse and injury to the trees. Polyvinylchloride (PVC) tubing, 15.24 cm in diameter, was installed after leaf senescence in November 2005 on each tree to protect the trunk from girdling by rodents during the winter.
2.4. Treatment application

Water (control) from a non-impacted well located 100 m from the study area was applied to all cuttings via hand irrigation for an establishment period of 14 d. Following establishment, trees were hand irrigated with either fertilized water or municipal solid waste landfill leachate that was collected weekly, using a low-flow distribution nozzle connected to a garden hose. Fertilizer (N, P, and K) was added to the control treatment during each irrigation application at a rate equal to that of the leachate to eliminate fertilization effects of these macronutrients. The 2005 weekly application rate was 3.8 L tree\(^{-1}\) (23.1 mm ha\(^{-1}\) assuming an irrigated soil surface area of 0.16 m\(^2\) per tree). Given eight applications, a total of 1.9 kL of each treatment was applied across the growing season. Drip irrigation was used to apply treatments during 2006. The treatment application rate for 2006 was increased to 22.7 L tree\(^{-1}\) (34.6 mm ha\(^{-1}\) assuming an irrigated soil surface area of 0.66 m\(^2\) per tree) because of root system development and increased water usage as the trees developed. Given twelve applications, a total of 17.4 kL of each treatment was applied across the growing season. To prevent substantial leaching from the experimental plot, application of treatments was adjusted based on precipitation events. Irrigation was postponed if greater than 0.5 cm of rainfall occurred within 2 d prior to watering or was expected to occur with a 40% chance or greater for 2 d following watering.
2.5. Sampling and measurements

2.5.1. Well water (control) and municipal solid waste landfill leachate

Water and leachate from the same source as the irrigation treatments were sampled from the Oneida County Landfill during April and October of 2005 and 2006. The water and leachate chemistry was analyzed (Northern Lake Service, Inc., Crandon, Wisconsin, USA) using approved United States Environmental Protection Agency methods. The leachate was brown in color and had a putrid odor. The composition of the water and leachate, including pH, electrical conductivity, biological oxygen demand, chemical oxygen demand, and Na\(^+\) and Cl\(^-\) concentration, are given in Table 4.1. The rate per application of Na\(^+\) and Cl\(^-\), expressed on a kg ha\(^{-1}\) basis, is given in Table 4.2. Heavy metals and volatile organic compounds were not detectable in the leachate analysis, and therefore, not a concern with respect to plant establishment and development.

2.5.2. Soil

Using a 5-cm diameter hand auger, nine soil samples at a depth of 0 to 30 cm were collected from each irrigation treatment plot one day before planting (13 Jun. 2005) and harvesting (17 Aug. 2006). For each date, soil from three sampling points was bulked, and three bulked samples were sent to the University of Wisconsin Soil & Plant Analysis Laboratory (Madison, Wisconsin, USA) for analysis of pH using a Fisher Scientific Accumet Model No. AR25 pH meter with combination reference-glass electrode (Orion, Ross\(^\circledR\) Sure-Flow\(^\text{TM}\) combination, epoxy body Model No. 8165), electrical conductivity using a VWR
Model No. 23226-523 digital conductivity meter with automatic temperature compensation, and Na⁺ concentration using inductively coupled plasma optical emission spectrometry (ICP-OES). Identical samples were sent to the Iowa State University Soil & Plant Analysis Laboratory (Ames, IA, USA) for analysis of Cl⁻ concentration using a modified mercury thiocyanate method with a Lachat Flow Injection Analysis Auto-Analyzer. The composition of the soil, including pH, electrical conductivity, and Na⁺ and Cl⁻ concentration, are given in Table 4.3.

2.5.3. Plant tissues

All trees were destructively harvested in two stages on 18 Aug. 2006. First, the aboveground portion of each tree was cut at 10 cm above the soil surface, and leaf and woody (stems + branches) components were separated and dried at 70 °C. Leaf and woody biomass was determined when dry mass values reached a constant mass. Second, root systems were excavated using a mechanized tree spade that removed a uniform, conical volume of soil (diameter × depth = 0.28 m³) for each tree. Root systems were washed and dry mass was determined identically to shoot components. Leaf, woody, and root samples of three replications for each irrigation treatment × clone interaction were sent to A & L Great Lakes Laboratories, Inc. (Fort Wayne, Indiana, USA) for analysis of Na⁺ (ICP-OES) and Cl⁻ (ion chromatography).
2.6. Data analysis

Soil Na⁺ and Cl⁻ data were analyzed using analyses of variance (PROC GLM; SAS Institute, Inc. 2004) assuming a completely random design with a fixed main effect for soil sample (preplanting, harvest control, and harvest leachate).

Tissue Na⁺ and Cl⁻ data were analyzed using analyses of variance (PROC GLM; SAS Institute, Inc. 2004) assuming a split split plot design with a random block effect and fixed main effects for irrigation treatment (whole plot), clone (sub plot), and plant tissue (sub sub plot). Where appropriate, non-significant ($P > 0.25$) interaction terms that included the block main effect were pooled into a common error term to increase precision of $F$-tests (Zalesny et al. 2005). Given the fixed main effects in both models, means were evaluated rather than variances. Fisher’s protected least significant difference (LSD) was used to compare means of soil and tissue data. Principal component analyses (PROC PRINCOMP; SAS Institute, Inc. 2004) were used to assess irrigation $\times$ clone interactions for total tree Cl⁻ concentration (Manly 1986).

3. Results

The Na⁺ and Cl⁻ application rate in the leachate increased from 2005 to 2006, given the increased volume of leachate applied. The application rate of Na⁺ in the leachate was 2.5 times greater in 2006 than 2005 (Table 4.2), while that during the 2006 irrigation season was 500 times greater than the fertilized well water (control). Likewise, the Cl⁻ application rate
was 1.7 times greater in 2006 than 2005, with the leachate treatment increasing the Cl⁻ application 693 times over the water treatment in 2006. Similar results were observed for concentrations of Na⁺ and Cl⁻ in the soil when comparing preplanting levels with those at harvest for both irrigation treatments (Table 4.3). The soil Na⁺ concentration of the leachate treatment was nearly three times greater than at preplanting and 24 times greater than the control. The leachate soil Cl⁻ concentration was 4.7 times greater than at preplanting and three times greater than the control. Soil pH was significantly greater for the leachate treatment than the control, but neither irrigation pH differed from the preplanting level (Table 4.3). Electrical conductivity differed among all three soil groups, with preplanting levels being the greatest and control levels the least (Table 4.3).

Treatment effects of water and leachate irrigation differed for Na⁺ and Cl⁻ concentration across clones and tissues (Table 4.4). In contrast, clones and the irrigation × clone interaction were not significant. However, the Na⁺ and Cl⁻ concentration differed among tissues and for the irrigation × tissue interaction and the clone × tissue interaction. Nevertheless, the irrigation × clone × tissue interaction was most important, influencing the distribution of Na⁺ and Cl⁻ in leaf, woody (stems + branches), and root tissue.

Sodium concentration was greatest in trees irrigated with leachate, along with being most concentrated in root tissue and least concentrated in woody tissue (Fig. 4.1). Leaf Na⁺ concentration was similar for genomic groups, except for NC14018 of the BC₁ genomic group [(P. trichocarpa × P. deltoides) × P. deltoides]. Clone NC14018 exhibited greater leaf Na⁺ concentration than all other genotypes. The Na⁺ concentration in woody tissue was not different among genomic groups and clones. In contrast, broad genotypic variation existed
for Na$^+$ concentration in the roots. The DM ($P.~deltoides \times P.~maximowiczii$) and NM ($P.~nigra \times P.~maximowiczii$) genomic groups performed similarly to one another and had greater root Na$^+$ concentration than the BC$_1$ genotypes and clone DN5 ($P.~deltoides \times P.~nigra$). Clones NM2 and NM6 of the NM genomic group differed, with NM6 having greater Na$^+$ in the roots. Overall, clonal ranking for total tree Na$^+$ concentration varied by treatment, with only one clone performing similarly regardless of being irrigated with water or leachate. Specifically, NM6 sequestered the greatest amount of Na$^+$ across tissues in both the water (0.72 g Na$^+$ kg$^{-1}$) and leachate (8.32 g Na$^+$ kg$^{-1}$) treatments. The total tree Na$^+$ concentration of the remaining clones irrigated with leachate was: NC14018 (8.23 g kg$^{-1}$), NC14104 (7.68 g kg$^{-1}$), NC14106 (7.17 g kg$^{-1}$), NM2 (7.05 g kg$^{-1}$), DM115 (6.57 g kg$^{-1}$), NC13460 (5.49 g kg$^{-1}$), and DN5 (3.87 g kg$^{-1}$).

Chloride concentration was greatest in trees irrigated with leachate, along with being most concentrated in leaf tissue and least concentrated in woody tissue (Fig. 4.2). Broad variation existed among genomic groups and clones for leaf Cl$^-$ concentration. The DM and NM genomic groups performed similarly to one another and had greater leaf Cl$^-$ concentration than DN5. For the BC$_1$ clones, NC14018 exhibited greater leaf Cl$^-$ concentration than NC13460, which was similar to the DM/NM clones (NC14018) and DN5 (NC13460). The Cl$^-$ concentration in woody tissue was not different among genomic groups and clones. Similarly, there were no differences among genomic groups for Cl$^-$ in the roots. However, for the BC$_1$ clones, NC14018 sequestered more Cl$^-$ in the roots than NC13460. Overall, clonal ranking for total tree Cl$^-$ concentration varied by irrigation treatment, with clones NC13460 (decreased Cl$^-$) and NM6 (increased Cl$^-$) ranking similarly regardless of
water or leachate application. In contrast, clone NC14106 had the broadest Cl\(^-\) concentration range across tissues, with the greatest for leachate (19.07 g kg\(^{-1}\)) and the least for water treatment (3.17 g kg\(^{-1}\)). The total tree (leaf + woody + root) Cl\(^-\) concentration of the remaining clones irrigated with leachate was: NM6 (19.03 g kg\(^{-1}\)), DM115 (17.97 g kg\(^{-1}\)), NC14018 (17.33 g kg\(^{-1}\)), NC14104 (17.10 g kg\(^{-1}\)), NM2 (16.47 g kg\(^{-1}\)), DN5 (9.90 g kg\(^{-1}\)), and NC13460 (8.07 g kg\(^{-1}\)).

The distributional trends in the percent of total Cl\(^-\) allocated to leaf, woody, and root tissues was similar among genomic groups and clones (Fig. 4.3). With the exception of DN5, leachate-irrigated trees exhibited greater relative distribution of Cl\(^-\) into the leaves compared with those irrigated with water. The increased relative percent of Cl\(^-\) distributed to leaves was most apparent for clones NM2 and NM6. Although NM6 sequestered 16% more Cl\(^-\) into the combination of all plant tissues compared with NM2, the relative percent allocation to tissues was nearly identical.

There was broad clonal variation in the relationship between tissue Cl\(^-\) concentration and biomass production (Fig. 4.4). The range in total tree Cl\(^-\) concentration was narrow for clones irrigated with water, while total tree biomass was highly variable. Clones irrigated with leachate segregated into three response groups: 1) NC14104, NM2, and NM6 had elevated levels of total tree Cl\(^-\) concentration along with increased biomass; 2) NC14018, NC14106, and DM115 exhibited elevated levels of total tree Cl\(^-\) concentration along with decreased biomass; 3) NC13460 and DN5 exhibited mid levels of total tree Cl\(^-\) concentration and biomass. Principal component analyses corroborated these univariate results, with the first two principal components accounting for 100% of the variation in the irrigation × clone interaction.
interaction data for total tree Cl\textsuperscript{\textendash} concentration. A plot of the 16 irrigation × clone combinations substantiated the clustering of the three response groups described above (Fig. 4.5). Similar univariate and multivariate trends were exhibited for leaf, woody, and root tissue.

4. Discussion

The enhanced distribution of Na\textsuperscript{+} and Cl\textsuperscript{\textendash} in leaf, woody, and root tissue when irrigated with municipal solid waste landfill leachate versus well water (control) was evidence of successful clone-specific elemental uptake using Populus. The results of the current study are important for using Populus genotypes for environmental benefits, because there is a lack of information about the response of such genotypes to elevated levels of Na\textsuperscript{+} and Cl\textsuperscript{\textendash} in irrigation sources such as landfill leachate. The 2006 leachate levels of 1200 ± 0 mg Na\textsuperscript{+} L\textsuperscript{\textendash} and 1250 ± 50 mg Cl\textsuperscript{\textendash} L\textsuperscript{\textendash} in the current study were six times (Na\textsuperscript{+}) and 1.9 times (Cl\textsuperscript{\textendash}) greater than commonly-accepted maximum concentration limits of 200 mg Na\textsuperscript{+} L\textsuperscript{\textendash} and 650 mg Cl\textsuperscript{\textendash} L\textsuperscript{\textendash} as constituents of irrigation water (Peavy et al. 1985). However, the broad variation among genomic groups and clones for Na\textsuperscript{+} and Cl\textsuperscript{\textendash} concentrations in the tissues substantiated the need for extensive genotypic screening prior to large-scale deployment (McCutcheon and Schnoor 2003). As expected, the higher concentrations of Na\textsuperscript{+} and Cl\textsuperscript{\textendash} in the leachate over the water irrigation treatment significantly increased the concentrations of these elements in leaf, woody, and root tissue. Across all genotypes, Na\textsuperscript{+} levels were greatest in the roots, and Cl\textsuperscript{\textendash} levels were greatest in the leaves.
Furthermore, the addition of equivalent rates of N, P, and K fertilization to the water treatment was utilized to eliminate the effect of increased fertilization of the trees receiving the leachate irrigation versus the water. Therefore, conclusions of the current study have been related to the elevated Na\(^+\) and Cl\(^-\) in the leachate. Specifically, the three-way interaction among irrigation treatment, clone, and tissue was evaluated to address the hypotheses of the study: 1) clones would respond differently to water and leachate irrigation; 2) clones would vary for tissue concentration of Na\(^+\) and Cl\(^-\) in leaf, woody, and root tissue.

The specific responses to and adaptations for salt stress of Populus species has not been well documented (Neuman et al. 1996). The visual damage to vegetative growth at the time of harvest in the current study ranged from no apparent salt stress to heavy defoliation, with an overall mortality rate of 22% that ranged from 6% (NM6) to 56% (NC13460). This variation in the response to salt stress is similar to that reported for other non-halophytes (Greenway and Munns 1980; Munns and Termaat 1986). In addition, horticultural and agricultural crop species used for the production of food and forage have been evaluated for salt tolerance more often than forest species, including SRWC, and have exhibited similar variability (Allen et al. 1994).

The genetic variation present in the genus Populus influences the ability of different genotypes to tolerate elevated levels of Na\(^+\) and Cl\(^-\) in the rhizosphere. Variation in tissue Na\(^+\) and Cl\(^-\) concentrations has resulted from the ability of the trees to exclude, compartmentalize, or translocate salts in an effort to reduce negative effects on growth (Neuman et al. 1996). In general, the initial tree responses to increased tissue Na\(^+\) and Cl\(^-\) levels were decreased leaf growth and a corresponding increased root:shoot ratio (Munns and
Sodium levels were greatest in the root tissue of all but one clone tested in the current study, which had the greatest proportion of Na\(^+\) in the leaves. Clone NC14018 \([(P.\ trichocarpa \times P.\ deltoides) \times P.\ deltoides]\) had the following tissue Na\(^+\) allocations: leaf (46%), woody (13%), and root (41%). All other clones allocated the greatest percent of total tree Na\(^+\) to root tissue, with clones in the DM genomic group \((P.\ deltoides \times P.\ maximowiczi)\) having the highest Na\(^+\) percent in root tissue: NC14104 (75%), NC14106 (67%), and DM115 (71%). All eight clones allocated 18% or less of the total tree Na\(^+\) into the woody tissue. Likewise, Stewart et al. (1990) reported that D \((P.\ deltoides)\) and DN \((P.\ deltoides \times P.\ nigra)\) clones allocated the greatest percent of total Na\(^+\) to root tissue when irrigated with municipal wastewater.

Similar variability in Na\(^+\) distribution to that in the current study also has been reported. For example, after 30 days of irrigation with 300 mL NaCl solution (300 mM), \(P.\ deltoides \times P.\ nigra\) cv. Italica had the highest concentration of Na\(^+\) in leaf tissue, followed by root and stem tissue. \(Populus\ popularis\) (unknown authority) and \(P.\ euphratica\) Oliv., both species from the section \(Turanga\), allocated the greatest amount of Na\(^+\) to roots, followed by leaves and stems (Chen et al. 2002). Differences in salt tolerance of these three genotypes was likely the result of salt exclusion. \(Populus\ euphratica\) expressed the greatest ability to restrict Na\(^+\) movement in the xylem, which is an important mechanism for the salt tolerance of woody species (Maas 1993). Likewise, Chen et al. (2003) irrigated \(P.\ euphratica\) and \(P.\ tomentosa\) Carrière, section \(Populus\), with NaCl for 20 days and reported the following total tree Na\(^+\) allocation: \(P.\ euphratica\), leaf (40%), stem (27%), and root (33%); \(P.\ tomentosa\), leaf (36%), stem (30%), and root (34%).
A substantial long-term response to increased tissue Na⁺ levels includes the abscission of older leaves that generally have higher salt concentrations than younger leaves, which was observed to some extent in the current study. Leaf abscission in response to long-term translocation of salts may be the cause of reduced biomass, which is often a yield-limiting factor due to decreased assimilation of carbon (Munns and Termaat 1986). For this reason, an increase in salt tolerance is often related to decreased amounts of salts being translocated to leaves, whereby the plant is able to preserve more biomass in photosynthetic tissue. Thus, the whole plant response supports the assimilation of carbon for growth with the production of new leaves at a higher rate than the loss of old leaves (Munns and Termaat 1986).

In general, several negative effects on plant growth occur due to increased Na⁺ and Cl⁻, including osmotic effects and water stress, nutrient and ion imbalance, and toxic effects on plant processes such as decreased photosynthesis and stomatal conductance (Lessani and Marschner 1978; Neuman et al. 1996). One or more of these impacts may dominate and cause a physiological stress within the plant. However, some plants have adaptations to decrease the impact from the associated increase in ions. For example, a common stress response is the movement of salt ions into vacuoles in order to compartmentalize and translocate salts to aerial portions of the plant (Lessani and Marschner 1978).

Of the movement of Na⁺ and Cl⁻ in the plant, Na⁺ translocation is generally under tighter root regulation than Cl⁻. In contrast, Cl⁻ generally dominates in the shoot (Lessani and Marschner 1978). In a study of seven crop species, applications of NaCl increased the foliar concentration of Cl⁻ over foliar Na⁺ levels in all but one species [sugar beet (Beta vulgaris]
L.), which had a 1:1 ratio of Na$^+$ to Cl$^-$ (Lessani and Marschner 1978). Likewise, all clones in the current study sequestered the majority of Cl$^-$ in the leaf tissue. Leaves of clone NM2 had the highest percent of total tree Cl$^-$ (73%) and NM6 had the second highest percent (70%), despite that the total amount of Cl$^-$ in NM6 leaf tissue was 10% greater than that of NM2. Likewise, Zalesny and Bauer (2007) reported the greatest leaf Cl$^-$ concentration for NM (P. nigra × P. maximowiczii) clones relative to DN genotypes. Similar trends in Cl$^-$ distribution to that exhibited in the current study also have been reported. For example, after 30 days of irrigation with 300 mL NaCl solution (300 mM), Italica had the highest concentration of Cl$^-$ in leaf tissue, followed by root and stem tissue. Populus popularis and P. euphratica allocated the greatest amount of Cl$^-$ to leaves, followed by roots and stems (Chen et al. 2002). Likewise, Chen et al. (2003) irrigated P. euphratica and P. tomentosa with NaCl for 20 days and reported the following total tree Cl$^-$ allocation: P. euphratica, leaf (54%), stem (27%), and root (19%); P. tomentosa, leaf (55%), stem (29%), and root (16%).

Salt tolerance did not appear to be strictly related to salt uptake and distribution or biomass accumulation. There were three broad response categories of the eight clones for the relationship between total tree Cl$^-$ concentration and total tree biomass. First, NC14104, NM2, and NM6 had elevated levels of total tree Cl$^-$ concentration along with increased biomass. This relationship may have been the result of high ionic concentrations not reducing growth of these clones in relation to the other clones or in relation to the control, which might have occurred by compartmentalization and sufficient growth rates that replaced abscised foliage. Second, NC14018, NC14106, and DM115 exhibited elevated levels of total tree Cl$^-$ concentration along with decreased biomass, suggesting that high concentrations of
Cl\(^{-}\) did have a negative impact on growth compared to the other clones and the control. This impact most likely was due to osmotic effects and water stress, nutrient and ion imbalance, and/or toxic effects on plant processes. Additionally, premature leaf abscission may have reduced photosynthetic area and the ability of the plant to produce carbon compounds necessary for biomass accumulation. Third, NC13460 and DN5 exhibited mid levels of total tree Cl\(^{-}\) concentration and biomass, suggesting the ability of the plant to cope with the stress imposed by salts on plant processes at a cost of carbohydrates or growth inhibiting processes such as decreased photosynthesis and stomatal conductance.

The broad genetic variation that is the hallmark of the genus *Populus* may offer opportunities for introducing salt tolerance into breeding programs. However, despite variation among *Populus* genotypes in salt tolerance, the specific physiological response mechanisms are poorly understood. Therefore, there is an overwhelming need for genotypic screening among genetically distinct genomic groups and clones in order to determine levels of tolerance to salinity that are highly positively correlated with measurements of growth and yield, as well as, tissue concentrations of ions (Allen et al. 1994). Proper genotypic selection is necessary in order to select clones that perform well over a broad range of contaminants or that exhibit elevated phytoaccumulation potential for specific elements (Zalesny and Bauer 2007). Selection of favorable clones is important for managed forests from economical and biological standpoints. A failed plantation depletes valuable resources associated with time, personnel, travel, and materials and supplies, while lengthening the time period to effective site remediation. Overall, differences that occur among clones are due to rate of uptake, salt
retention in the roots, restricted translocation to the shoots (basipetally via xylem), and retranslocation back to the roots (acropetally via phloem) (Lessani and Marschner 1978).

Biomass production is generally increased with irrigation. However, negative impacts to plant tissues and soil health need to be considered when utilizing a waste product such as high-salinity landfill leachate as the irrigation source (Neuman et al. 1996). The soil data collected before planting and at the time of harvest in the current study illustrated the importance of monitoring soil impacts from irrigation with leachate. Given that salt additions have the potential to alter the chemical, physical, and biological quality of soil after irrigation for lengthy periods (Bañuelos et al. 1999), it is especially meaningful in future studies to assess the amount of Na\(^+\) and Cl\(^-\) that is lost through leaching, which may impact groundwater, to perform deeper soil sampling, and to test the release of salt into the soil from abscised leaves. Similar concerns with heavy metal concentrations in leaves also have been reported (Laureysens et al. 2004).

5. Conclusion

The impacts of soil salinity on ecosystem health are not as widespread in Wisconsin relative to other areas of North America. However, human activities have introduced increased salts into areas desired for plant growth, such as roadsides impacted from deicing salts (Sucoff et al. 1975), areas where Cl\(^-\) contributes to pollution due to agricultural irrigation (Stites and Kraft 2001), and sites utilizing specialized irrigation regimes such as municipal solid waste landfills. Projects of this nature will benefit from Populus clones that
are able to tolerate and sequester high amounts of Na⁺ and Cl⁻ in leaf, woody, and root tissue. Clones NC14104, NM2, and NM6 exhibited high salt concentrations and biomass growth over two growing seasons, thereby expressing the necessary economical (woody biomass) and environmental (uptake) response for managed experimental plantations. Given the genetic variability among Populus clones, similar phytoaccumulation effectiveness is possible on other sites and with other high-salinity inputs.

Acknowledgements

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References


Research Council of Canada, Ottawa, pp. 7-32.


Tolerance of hybrid poplar (Populus) trees irrigated with varied levels of salt, selenium,
Continued


Zalesny, R.S., Jr., Bauer, E.O., 2007. Evaluation of Populus and Salix continuously irrigated


Table 4.1. Composition (mean ± standard error, n = 2) of well water (control) and leachate from the Oneida County Landfill (Rhinelander, Wisconsin, USA) during the 2005 and 2006 growing seasons.

<table>
<thead>
<tr>
<th>Component</th>
<th>2005</th>
<th>2006</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Leachate</td>
<td>Control</td>
<td>Leachate</td>
</tr>
<tr>
<td>pH</td>
<td>6.2 ± 0.1</td>
<td>8.8 ± 0.0</td>
<td>6.3 ± 0.2</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>Electrical conductivity (mS cm⁻¹)</td>
<td>0.2 ± 0.1</td>
<td>6.2 ± 0.5</td>
<td>0.1 ± 0.0</td>
<td>9.4 ± 0.2</td>
</tr>
<tr>
<td>Biological oxygen demand (mg L⁻¹)</td>
<td>naₐ</td>
<td>21 ± 5</td>
<td>na</td>
<td>108 ± 83</td>
</tr>
<tr>
<td>Chemical oxygen demand (mg L⁻¹)</td>
<td>ndₐ</td>
<td>660 ± 10</td>
<td>na</td>
<td>1050 ± 50</td>
</tr>
<tr>
<td>Na⁺ (mg L⁻¹)</td>
<td>na</td>
<td>690 ± 10</td>
<td>2.4₇</td>
<td>1200 ± 0</td>
</tr>
<tr>
<td>Cl⁻ (mg L⁻¹)</td>
<td>nd</td>
<td>1093 ± 178</td>
<td>1.8 ± 1.8</td>
<td>1250 ± 50</td>
</tr>
</tbody>
</table>

ₐNot available.
₇Not detectable.
₇One sample collected at harvest.
Table 4.2. Rate per application of sodium (Na$^+$) and chloride (Cl$^-$) in well water (control) and leachate from the Oneida County Landfill (Rhineland, Wisconsin, USA) during the 2005 and 2006 growing seasons.

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Leachate</th>
<th>Control</th>
<th>Leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>na$^c$</td>
<td>163.88</td>
<td>0.83</td>
<td>412.73</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>0.00</td>
<td>259.59</td>
<td>0.62</td>
<td>429.92</td>
</tr>
</tbody>
</table>

$^a$Eight applications total. Rate based on an application volume of 3.8 L tree$^{-1}$ and an irrigated soil surface area of 0.16 m$^2$ tree$^{-1}$.

$^b$Twelve applications total. Rate based on an application volume of 22.7 L tree$^{-1}$ and an irrigated soil surface area of 0.66 m$^2$ tree$^{-1}$.

$^c$Not available.
Table 4.3. Soil pH, electrical conductivity (EC), and concentration of sodium (Na⁺) and chloride (Cl⁻) (mean ± standard error, n = 3) before planting and at whole-tree harvest after irrigating for the 2005 and 2006 growing seasons with well water (control) and leachate from the Oneida County Landfill (Rhinelander, Wisconsin, USA).

<table>
<thead>
<tr>
<th>Harvest</th>
<th>pH</th>
<th>Lecture</th>
<th>Control</th>
<th>Leachate</th>
<th>LSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td></td>
<td>Preplanting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.9 ± 0.1</td>
<td>ab</td>
<td>5.7 ± 0.1</td>
<td>b</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>EC (mS cm⁻¹)</td>
<td>2.78 ± 0.17</td>
<td>a</td>
<td>0.28 ± 0.01</td>
<td>c</td>
<td>1.39 ± 0.18</td>
</tr>
<tr>
<td>Na⁺ (mg kg⁻¹)</td>
<td>72.5 ± 0.9</td>
<td>b</td>
<td>8.5 ± 0.3</td>
<td>c</td>
<td>203.0 ± 21.9</td>
</tr>
<tr>
<td>Cl⁻ (mg kg⁻¹)</td>
<td>19.5 ± 3.4</td>
<td>b</td>
<td>30.0 ± 3.9</td>
<td>b</td>
<td>90.8 ± 12.5</td>
</tr>
</tbody>
</table>

*Means for each row followed by different letters were different at P < 0.05, according to Fisher’s least significant difference (LSD).
Table 4.4. Probability values from analyses of variance comparing the concentration of sodium ($\text{Na}^+$) and chloride ($\text{Cl}^-$) across two irrigation treatments [well water (control) and landfill leachate], eight $\textit{Populus}$ clones (see materials and methods for descriptions) and three tissues (leaf, woody, and root). Significant values are in bold.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Na$^+$</th>
<th>Cl$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation</td>
<td>0.0192</td>
<td>0.0035</td>
</tr>
<tr>
<td>Clone</td>
<td>0.8190</td>
<td>0.2038</td>
</tr>
<tr>
<td>Irrigation $\times$ Clone</td>
<td>0.8741</td>
<td>0.3245</td>
</tr>
<tr>
<td>Tissue</td>
<td>$&lt;$0.0001</td>
<td>$&lt;$0.0001</td>
</tr>
<tr>
<td>Irrigation $\times$ Tissue</td>
<td>$&lt;$0.0001</td>
<td>$&lt;$0.0001</td>
</tr>
<tr>
<td>Clone $\times$ Tissue</td>
<td>0.0090</td>
<td>0.0007</td>
</tr>
<tr>
<td>Irrigation $\times$ Clone $\times$ Tissue</td>
<td>0.0240</td>
<td>0.0073</td>
</tr>
</tbody>
</table>
Fig. 4.1. Concentration of sodium for each combination of irrigation treatment [well water (control) and landfill leachate], Populus clone, and tree tissue (leaf, woody, and root). Error bars represent one standard error of the mean (n = 3). Bars labeled with the same letter were not different, according to Fisher’s protected least significant difference (LSD).
Fig. 4.2. Concentration of chloride for each combination of irrigation treatment [well water (control) and landfill leachate], *Populus* clone, and tree tissue (leaf, woody, and root). Error bars represent one standard error of the mean (n = 3). Bars labeled with the same letter were not different, according to Fisher’s protected least significant difference (LSD).
Fig. 4.3. Percent of total chloride allocated to leaf, woody, and root tissue of eight *Populus* clones irrigated with well water (control) or landfill leachate for two growing seasons.
Fig. 4.4. Total tree chloride concentration versus biomass production of eight *Populus* clones irrigated with well water (control) or landfill leachate for two growing seasons (*n* = 9).
Fig. 4.5. Plot of first two principal components ($Z_1, Z_2$; 100% of variation) for total tree chloride concentration of eight $Populus$ clones irrigated with well water (control) or landfill leachate for two growing seasons ($n = 9$).
CHAPTER 5. DISTRIBUTION OF MACRO- AND MICRO-NUTRIENTS
IN LEAF, WOODY, AND ROOT TISSUE OF POPULUS AFTER
IRRIGATION WITH LANDFILL LEACHATE

A paper submitted to Forest Ecology and Management

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Abstract

Information about macro- and micro-nutrient uptake and distribution into tissues of

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Populus genotypes irrigated with landfill leachate helps to maximize biomass production and to understand impacts of leachate chemistry on tree health. We irrigated eight Populus clones (NC13460, NC14018, NC14104, NC14106, DM115, DN5, NM2, NM6) with fertilized well water (control) (N, P, K) or municipal solid waste landfill leachate weekly during 2005 and 2006 in Rhinelander, Wisconsin, USA (45.6 °N, 89.4 °W). During Aug. 2006, we tested for differences in total N, P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Pb concentration in preplanting and harvest soils, and in leaf, woody (stems + branches), and root tissue. Other than N ($P = 0.0191$), leachate did not increase the soil concentration of elements relative to preplanting levels ($P > 0.05$). There was broad variation among genomic groups and clones for tissue element concentrations, along with clone-specific uptake for most elements. The concentration of N, P, K, Ca, Mg, S, B, and Mn was greatest in leaves and least in woody tissue, while that of Fe, Cu, and Al was greatest in roots and least in leaves and woody tissue. Overall, there was successful uptake of macro- and micro-nutrients without detrimental impact to tree health, which validated the use of landfill leachate as an irrigation and fertilization source for Populus.

Keywords: hybrid poplar; phytoremediation; Populus deltoides; P. maximowiczii; P. nigra; P. trichocarpa; waste management
1. Introduction

Using *Populus* for environmental benefits requires selection of genotypes that are matched to local environments and specific contaminants (Isebrands and Karnosky 2001; Zalesny and Bauer 2007b). The intensive management of *Populus* requires irrigation and fertilization to increase biomass production (Brown and van den Driessche 2002; Coyle and Coleman 2005; DesRochers et al. 2006). Landfill leachate used as an irrigation and fertilization source may supply water and elemental nutrient requirements to *Populus* trees (i.e. poplars) grown in short rotation woody crop (SRWC) systems at a lower cost than traditional sources (Shrive et al. 1994; Erdman and Christenson 2000; Zalesny and Bauer 2007a). However, the leachate chemistry and movement varies due to variation in the waste materials received at the facility and seasonal changes in waste decomposition (Shrive et al. 1994; Kjeldsen et al. 2002). Therefore, it is necessary to evaluate leachate chemistry in order to determine its potential nutritive value to the trees, especially as it relates to providing fertilization rates for optimal biomass production (Fung et al. 1998; Stephens et al. 2000).

There are few reports about the specific plant tissue responses to macro- and micro-nutrients available in landfill leachate. Thus, to maximize environmental benefits it is necessary to combine the knowledge of *Populus* species and clones in SRWC systems for remedial benefits following uptake and distribution of macro- and micro-nutrients (Mirck et al. 2005). Overall, there is a need to compare growth and tissue concentration of field-grown *Populus* trees with those irrigated and fertilized with traditional methods. Understanding macro- and micro-nutrient accumulation and distribution in leaf, woody, and root tissue of
Populus irrigated with landfill leachate is important for maximizing biomass production during a growing season, along with understanding the phytotoxic impacts of excessive levels of nutrients on tree health, soil health, and groundwater quality.

This study expands on our previous work evaluating the selection of clonal material (Zalesny et al. 2007a), growth and biomass accumulation (Zalesny et al. 2007b), and salt accumulation (Zalesny et al. 2007c) of Populus clones irrigated with landfill leachate. However, uptake of nutrients into the trees was not evaluated in those studies. Therefore, the primary objective of the current study was to test the uptake and distribution of macro- and micro-nutrients into leaf, woody (stems + branches), and root tissue of eight Populus genotypes that were irrigated with fertilized well water (control) (N, P, K) or municipal solid waste landfill leachate for two growing seasons. Our hypotheses were that clones would respond differently to water and leachate irrigation, and that tissue concentrations of macro- and micro-nutrients in leaf, woody, and root tissues would vary among clones. This information is useful to SRWC management, because there is a general lack of knowledge about elemental nutrient concentration in the tissues of Populus genotypes when irrigated with landfill leachate in the field.

2. Materials and methods

Zalesny et al. (2007b) provided details about site description, clone selection, tree establishment, experimental design, and treatment application. In summary, the study was conducted at the Oneida County Landfill located 6 km west of Rhinelander, Wisconsin, USA.
(45.6 °N, 89.4 °W). Temperature, precipitation, and growing degree days across the experimental period were described previously (Zalesny et al. 2007b). The landfill soils are classified as mixed, frigid, coarse loamy Alfic Haplorthods (Padus Loam, PaB), with 0 to 6 percent slopes, and are considered well to moderately well drained with loamy deposits underlain by stratified sand and gravel glacial outwash.

Eight _Populus_ clones were selected from 25 original genotypes, based on aboveground and belowground traits, after being irrigated with leachate in a series of greenhouse experiments that constituted three phyto-recurrent selection cycles (Zalesny et al. 2007a). The clones and their parentages (i.e. genomic groups) were: NC13460, NC14018 [( _P. trichocarpa_ Torr. & Gray × _P. deltoides_ Bartr. ex Marsh) × _P. deltoides_ ‘BC’]; NC14104, NC14106, DM115 ( _P. deltoides_ × _P. maximowiczii_ A. Henry ‘DM’); DN5 ( _P. deltoides_ × _P. nigra_ L. ‘DN’); and NM2, NM6 ( _P. nigra_ × _P. maximowiczii_ ‘NM’).

Although _P. maximowiczii_ is currently classified as a subspecies of _P. suaveolens_ Fischer, we have retained the species nomenclature for _P. maximowiczii_ (Japanese poplar) that has been previously used in the _Populus_ literature (Eckenwalder 1996; Dickmann 2001).

Shoots were collected during dormancy from stool beds established at Hugo Sauer Nursery in Rhinelander. Hardwood cuttings, 20 cm long, were prepared during January 2005, 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 at 5 °C and soaked in water to a height of 15 cm for 3 d before planting on 14 Jun. 2005. Prior to planting, the soil was tilled to a depth of 30 cm. Cuttings were planted in a split plot design with eight blocks, two irrigation treatments (whole plots), and eight clones (sub plots) at a spacing of 1.2 × 2.4 m (i.e. 3472 trees ha⁻¹).
Clones were arranged in randomized complete blocks in order to minimize effects of any potential environmental gradients. Two border rows of clone NM2 were established on the perimeter of the planting and between treatment whole plots to reduce potential border effects (Hansen, 1981; Zavitkovski, 1981).

Water (control) from a non-impacted well located 100 m from the study area was applied to all cuttings via hand irrigation for an establishment period of 14 d. Following establishment, trees were hand irrigated with either fertilized water or municipal solid waste landfill leachate, using a low-flow distribution nozzle connected to a garden hose. Fertilizer (N, P, and K) was added to the control treatment during each irrigation application at a rate equal to that of the leachate to eliminate fertilization effects of these macronutrients. The 2005 weekly application rate was 3.8 L tree\(^{-1}\) (23.1 mm ha\(^{-1}\) assuming an irrigated soil surface area of 0.16 m\(^2\) per tree). Given eight applications, a total of 1.9 kL of each treatment was applied across the growing season. Drip irrigation was used to apply treatments during 2006. The treatment application rate for 2006 was increased to 22.7 L tree\(^{-1}\) (34.6 mm ha\(^{-1}\) assuming an irrigated soil surface area of 0.66 m\(^2\) per tree) because of root system development and increased water usage as the trees developed. Given twelve applications, a total of 17.4 kL of each treatment was applied across the growing season. To prevent substantial leaching from the experimental plot, application of treatments was adjusted based on precipitation events. Irrigation was postponed if greater than 0.5 cm of rainfall occurred within 2 d prior to watering or was expected to occur with a 40% chance or greater for 2 d following watering.
2.1. Sampling and measurements

2.1.1. Well water (control) and municipal solid waste landfill leachate

Water and leachate from the same source as the irrigation treatments were sampled from the Oneida County Landfill during April and October of 2005 and 2006. The water and leachate chemistry was analyzed (Northern Lake Service, Inc., Crandon, Wisconsin, USA) using approved United States Environmental Protection Agency methods. The leachate was brown in color and had a putrid odor. The concentrations of the following elements in the water and leachate are given in Table 5.1: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), boron (B), manganese (Mn), iron (Fe), copper (Cu), aluminum (Al), and lead (Pb). The rate per application of these elements, expressed on a kg ha\(^{-1}\) basis, is given in Table 5.2. Zalesny et al. (2007c) provided information about pH, electrical conductivity, biological oxygen demand, and chemical oxygen demand. Heavy metals and volatile organic compounds were not detectable in the leachate analysis, and therefore, not a concern with respect to plant establishment and development.

2.1.2. Soil

Using a 5-cm diameter hand auger, nine soil samples at a depth of 0 to 30 cm were collected from each irrigation treatment plot one day before planting (13 Jun. 2005) and harvesting (17 Aug. 2006). For each date, soil from three sampling points was bulked, and three bulked samples were sent to the University of Wisconsin Soil & Plant Analysis
Laboratory (Madison, Wisconsin, USA) for analysis of total P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Pb concentration using inductively coupled plasma optical emission spectrometry (ICP-OES). Total N concentration of the samples was analyzed at the Forestry Sciences Laboratory (Rhineland, Wisconsin, USA) using a Flash EA1112 N-C analyzer (Thermo Electron, via CE Elantech, Inc., Lakewood, New Jersey, USA) with a model MAS 200 autosampler. The soil concentrations of these elements are given in Table 5.3.

2.1.3. Plant tissues

All trees were destructively harvested in two stages on 18 Aug. 2006. First, the aboveground portion of each tree was cut at 10 cm above the soil surface, and leaf and woody (stems + branches) components were separated and dried at 70 °C to a constant mass. Second, root systems were excavated using a mechanized tree spade that removed a uniform, conical volume of soil (diameter × depth = 0.28 m³) for each tree. Root systems were washed and dry mass was determined identically to shoot components. Leaf, woody, and root samples for each irrigation treatment × clone interaction were sent to A & L Great Lakes Laboratories, Inc. (Fort Wayne, Indiana, USA) for analysis of total P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Pb concentration (ICP-OES), while total N concentration was analyzed at the Forestry Sciences Laboratory as with soil N.
2.2. Data analysis

Soil elemental data were analyzed using analyses of variance (PROC GLM; SAS Institute, Inc. 2004) assuming a completely random design with a fixed main effect for soil sample (preplanting, harvest control, and harvest leachate).

Tissue elemental data were analyzed using analyses of variance (PROC GLM; SAS Institute, Inc. 2004) assuming a split split plot design with a random block effect and fixed main effects for irrigation treatment (whole plot), clone (sub plot), and plant tissue (sub sub plot). Where appropriate, non-significant ($P > 0.25$) interaction terms that included the block main effect were pooled into a common error term to increase precision of $F$-tests (Zalesny et al. 2005). Given the fixed main effects in both models, means were evaluated rather than variances. Fisher’s protected least significant difference (LSD) was used to compare means of soil and tissue data.

3. Results

3.1. Soil

There were four general trends in the soil concentration of macro- and micro-nutrients before planting and at the time of harvest for fertilized well water (control) and leachate irrigation treatments (Table 5.3): 1) the soil N concentration was greatest for leachate irrigation, while preplanting and control levels did not differ from one another; 2) the soil P,
K, S, Zn, and Pb concentration was greatest before planting, while control and leachate levels did not differ from one another; 3) the soil Ca, Mg, B, Fe, and Al concentration was greatest before planting and least for the control irrigation; 4) the soil Mn and Cu concentration was greatest and similar before planting and after leachate irrigation.

3.2. Macronutrients

Clone main effects were significant for N, P, K, Ca, and S (Table 5.4). The irrigation × clone interaction was significant for N, Ca, and S. Likewise, the tissue main effect was significant for all macronutrients. The irrigation × tissue interaction was significant for the following macronutrients: P, Mg, and S. The P concentration was significantly greatest for leaf tissue and least in the woody tissue (Fig. 5.1A). The leaf and woody P concentration was greater with water irrigation than leachate, while trees of the leachate treatment exhibited greater P in the roots. The Mg concentration was significantly greatest for the combination of leachate irrigation and leaf tissue (Fig. 5.1B). The control × leaf and leachate × root interactions were similar to one another yet greater than the remaining irrigation × tissue combinations.

The clone × tissue interaction was significant for the following macronutrients: N, P, Ca, Mg, and S (Table 5.4). The concentration of N, P, Ca, and Mg was greatest in the leaves, with the least amount allocated to the woody tissue (Fig. 5.2). There was broad variation among and within genomic groups for N, P, Ca, and Mg concentration in the tissues. The BC₁ clones [(P. trichocarpa × P. deltoides) × P. deltoides] and clone DN5 (P. deltoides × P.
*P. nigra* exhibited greater concentrations of N and P in the leaves than those of the DM (*P. deltoides × P. maximowiczii*) and NM (*P. nigra × P. maximowiczii*) genomic groups (Fig. 5.2A; Fig. 5.2B). Clone NC14018 had a significantly greater amount of N and P in the leaves than NC13460. The woody N and P concentration was similar among genomic groups. There was more P in the woody tissue of NM2 versus NM6. Root N concentration was similar among genomic groups and clones, with the exception of NC14106 and DN5 that had less N than the other genotypes. The BC₁ genomic group and DN5 had greater root P concentration than the DM and NM genotypes, which resulted from significantly less P in the roots of NC14106 and NM6. Furthermore, the DM and NM clones, along with DN5, exhibited similar leaf Ca concentration to one another, while the BC₁ clones varied (Fig. 5.2C). Clone NC14018 exhibited significantly greater Ca in the leaves than NC13460. Differences for woody Ca concentration were negligible for genomic groups and clones. Root Ca concentration was uniform across genomic groups, despite variation among the DM genotypes. Clone NC14104 exhibited the greatest root Ca concentration, while NC14106 had the least amount of Ca in the roots. Moreover, the NM genomic group exhibited the greatest leaf Mg concentration, while the other genomic groups exhibited similar Mg levels in the leaves (Fig. 5.2D). Clone NC14018 had significantly greater leaf Mg concentration than NC13460. The BC₁ and DM clones, along with DN5, exhibited greater woody Mg concentration than the NM genotypes. No differences existed among clones within genomic groups. The DM clones exhibited greater root Mg concentration than those of the other genomic groups, while also differing among one other. Clone NC14104 had the greatest root Mg concentration and NC14106 the least.
The irrigation × clone × tissue interaction influenced the distribution of S in leaf, woody, and root tissue (Table 5.4). Sulfur levels were greatest in the trees irrigated with water, along with being most concentrated in the leaf tissue and least concentrated in the woody tissue (Fig. 5.3). Leaf S concentration was dissimilar for genomic groups, with the following ranking from greatest to least S concentration: BC₁, DM, clone DN5, and NM. All clones exhibited greater leaf S concentration with water versus leachate, except for clone DN5 and NM6 that did not differ. The S concentration in woody tissue was not different among genomic groups and clones. Similarly, except for clone NC14018 that had greater root S concentration with leachate irrigation versus water, differences among irrigation × clone combinations were negligible.

3.3. Micronutrients

Irrigation treatments were significant for B, Mn, Fe, and Al, while clone main effects were significant for Mn and Cu (Table 5.4). The irrigation × clone interaction was significant for Cu. Likewise, the tissue main effect was significant for all micronutrients, except Zn and Pb. The irrigation × tissue interaction was significant for the following micronutrients: B, Mn, Fe, Cu, and Al (Table 5.4). The B concentration was significantly greatest for leaf tissue with leachate irrigation (Fig. 5.4A). Additionally, the leachate treatment increased root B concentration relative to the water irrigation. The Mn concentration was greatest in the leaves and least in the woody tissue (Fig. 5.4B). The leaf Mn concentration was significantly greater for water irrigation versus leachate. The root Fe concentration was significantly
greater than in the leaf and woody tissue, and the leachate irrigation increased the root Fe concentration over the control (Fig. 5.4C). The concentration of Cu and Al in leaf, woody, and root tissue showed similar trends as that of Fe (Fig. 5.4D; Fig. 5.4E).

The clone × tissue interaction was significant for the following micronutrients: Mn and Cu (Table 5.4). There was broad variation among and within genomic groups for Mn and Cu concentration in the tissues (Fig. 5.5). The concentration of Mn was greatest in the leaves, with the least amount allocated to the woody tissue (Fig. 5.5A). Clones within the BC1, DM, and NM genomic groups exhibited broad variation in leaf Mn concentration. Of the BC1 genotypes, clone NC14018 had greater leaf Mn levels than NC13460, while NC14104 and NC14106 had the greatest and least leaf Mn concentration, respectively, of the DM clones. Likewise, NM6 had significantly greater leaf Mn levels than NM2. Genomic group differences for woody and root Mn concentration were negligible. Clone NC14018 had greater root Mn concentration than NC13460. Furthermore, the concentration of Cu was greatest in the roots, with the least amount allocated to the leaves (Fig. 5.5B). Genomic group differences for leaf and woody Cu concentration were negligible. However, the leaf Cu concentration of NC13460 was greater than NC14018, while the woody Cu concentration of NC14106 was greater than NC14104. The BC1 genomic group exhibited significantly greater root Cu concentration than all other genomic groups. Clone DM115 had more Cu in the roots than NC14106.
4. Discussion

There was successful macro- and micro-nutrient accumulation and distribution in leaf, woody, and root tissue of *Populus* when irrigated with municipal solid waste landfill leachate during two growing seasons in the field. These results are important for maximizing biomass production during a growing season, as well as, understanding negative impacts of phytotoxic amounts of any nutrient to tree health, soil health, and groundwater quality. In this study, N, P, and K were equalized across treatments to reduce fertilization effects and thereby isolate the effects of the other leachate constituents. Overall, there was successful phytoaccumulation of macro- and micro-nutrients without detrimental impact to tree health, which validated the use of landfill leachate as an irrigation and fertilization source for the trees.

4.1. Macronutrients

Urea [(NH$_2$)$_2$CO] was used as the N source for the water irrigation treatment in the current study, while the leachate analyses showed N came from NH$_4^+$ and NO$_3^-$ sources. *Populus* trees have utilized both NH$_4^+$ and NO$_3^-$ forms of N, but have shown a preference for NH$_4^+$ (Dickmann et al. 2001). Similarly, *P. tremuloides* Michx. seedlings have utilized both NH$_4^+$ and NO$_3^-$ sources of fertilizer; however, there were interactions between pH and fertilizer source. DesRochers et al. (2003) reported that NH$_4^+$ was more available at high pH and NO$_3^-$ was more available at low pH. They speculated that the broad ecological range of
P. tremuloides may be partly attributed to its successful use of both sources of N fertilization (DesRochers et al. 2003). The four parental Populus species (P. trichocarpa, P. deltoides, P. nigra, and P. maximowiczii) of the clones tested in the current study have broad geographic ranges that likely contributed to the ability of the genotypes to utilize different N sources.

At harvest (mid August), the N concentration across tissues and clones in the control treatment ranged from 9.34 to 38.61 g kg\(^{-1}\), with a mean of 21.58 ± 2.09 g kg\(^{-1}\) (2.16%), while the leachate treatment ranged from 10.70 to 36.87 g kg\(^{-1}\), with a mean of 20.25 ± 1.82 g kg\(^{-1}\) (2.03%). The foliar N concentration of the control (3.5%) and leachate (3.2%) treatments were greater than the optimal amount recommended for poplar clones (3%) in mid July in northern Wisconsin (Hansen et al. 1988), and that of 2.3% to 2.8% N reported for a P. trichocarpa × P. deltoides (TD) hybrid in British Columbia, Canada (van den Driessche 2000). However, our leachate application rate in 2006 (236 kg N ha\(^{-1}\)) exceeded the range of recommended optimal N fertilization rates (85 to 185 kg N ha\(^{-1}\)) for the North Central United States (Hansen et al. 1988; Hansen 1994; Stanturf et al. 2001). More specifically, Coleman et al. (2004) reported two-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. The excess N applied in the current study likely contributed to luxury consumption of N into leaves. Similarly, DesRochers et al. (2006) reported 3.2% N in the leaves of one P. balsamifera L. (B) × P. simonii Carr. (S) hybrid ‘33 cv. P38P38’ and two P. deltoides (D) × P. × petrowskyana (P) hybrids ‘24 cv. Walker’ ‘794 cv. Brooks6’ receiving 16 g N tree\(^{-1}\), which was similar to that applied in 2006 in the current study (15.6 g N tree\(^{-1}\)). Likewise, leaf N
concentration after one growing season of two TD clones (49-177, 15-29), one DT clone (*P. deltoides* × *P. trichocarpa* ‘DTAC-7’), and one TM clone (*P. trichocarpa* × *P. maximowiczii* ‘286-43’) receiving 250 kg N ha⁻¹ ranged from 2.6% to 4.1% (Brown and van den Driessche 2005).

Our mid-August measurement (taken prior to leaf fall) of 3.2 to 3.5% N in leaf tissue indicated substantial late season N availability for plant processes. Leaf nutrient cycling is an important mechanism for deciduous trees, with more than 50% of N exported to woody and root tissues prior to leaf senescence (Dickmann et al. 2001). Baker and Blackmon (1977) reported seasonal changes in foliar, woody, and root N content for *P. deltoides*, with the greatest decrease in leaf N occurring prior to leaf fall. In late May, they measured 92% of tissue N in the leaves and 8% in the woody tissues (roots not reported), while tissue N allocation in late September was 53% (leaves), 15% (woody), and 32% (roots). The N distribution in November was 15% (leaves), 35% (woody), and 50% (roots). Overall, the leaf nutrient distributional changes were attributed to internal cycling processes and not shifts in biomass allocation (Baker and Blackmon 1977). Additionally, foliar N concentrations peaked in July (2.9%) and declined (1.5%) at leaf abscission, given N export to woody and root tissue (Baker and Blackmon 1977).

Furthermore, in our study the soil N concentration at harvest was 2.5 times greater than preplanting levels, indicating quantities were applied that exceeded tree uptake. Given the possibility of N leaching into the groundwater, excess N and other nutrients in the leachate of future studies could be managed through dilution with water to reduce the concentration of elements that may have harmful effects on the soil and water.
Although the P application rate was equalized in the water and leachate treatments, differences existed within the irrigation treatment × tissue interaction. There was more P in the leaf and woody tissue of the water treatment, while the greatest root P concentration was with leachate irrigation. The optimal range of plant P is from 0.1% to 0.5%; however, levels of 0.15% P have been deficient for *Populus* (van den Driessche 1999; Brown and van den Driessche 2005). Baker and Blackmon (1977) reported decreasing leaf P concentrations from 0.23% in May to 0.12% in November. In late September, total tree P allocated to tissues was 32% (leaves), 21% (woody), and 47% (roots), while such allocations in November were 11% (leaves), 33% (woody), and 56% (root). These decreases in leaf P have been attributed to internal cycling processes that redistributed nearly 30% of P for future plant growth (Dickmann et al. 2001). DesRochers et al. (2006) reported differences in leaf P allocations among three N fertilization treatments (0, 8, and 16 g N tree\(^{-1}\)) for *Populus* clones 33 cv. P38P38, 24 cv. Walker, and 794 cv. Brooks6, with 0 g N tree\(^{-1}\) (0.20%) being greater than with 16 g N tree\(^{-1}\) (0.18%). The irrigation in the current study was most similar to their 16 g N tree\(^{-1}\) treatment; however, our leaf P levels were greater in both water (0.25%) and leachate (0.22%) treatments. Likewise, the stem P concentration for water (0.15%) and leachate (0.12%) irrigation in the current study substantiated that of poplar clones Beauprê and Trichobel (0.15%) that were irrigated with effluent and sewage sludge for three growing seasons (Moffat et al. 2001). Furthermore, the soil P concentration before planting was 12 times greater than the harvest control plot and 10 times greater than the harvest leachate plot, which likely resulted in the soil providing additional P for plant uptake that was deficient in
the irrigation treatments. Overall, the reduction of soil P is ecologically important, especially for the reduction of lake and river enrichment.

Trees require the secondary macronutrients (Mg, Ca, and S) for growth and development at quantities that are similar to P. The Mg application rate in the current study was not equalized in the water and leachate irrigation treatments. Thus, there were differences for irrigation × clone and irrigation × tissue interactions. The leachate Mg concentration was 33 times greater than the water concentration. Greater leaf and root Mg levels were exhibited with leachate, while the stem Mg concentration was greatest with water. However, when irrigated with either treatment, allocation of Mg was greatest in the leaves and least in the woody tissue. Similarly, Baker and Blackmon (1977) reported September Mg allocations in *P. deltoides* of 58% (leaves), 25% (woody), and 17% (roots), while those in November were 41% (leaves), 35% (woody), and 24% (root). Our stem Mg concentrations with water (0.13%) and leachate (0.12%) treatments were similar to TD *Populus* clones Beauprè (0.11%) and Trichobel (0.09%) that were irrigated with effluent and sewage sludge for three growing seasons (Moffat et al. 2001). Furthermore, the soil Mg concentration at preplanting differed from harvest levels, with the water treatment utilizing the greatest amount of soil Mg. There was a 31% reduction of Mg in the control soils over the two years in the current study.

Additions were not made to the water treatment to equalize Ca; therefore, Ca concentration in the leachate was twice that of the water treatment. The distributional trends of Ca in the current study (i.e. greatest in the leaves and least in the woody tissue) differed from Baker and Blackmon (1977), who measured 50% (leaves), 35% (woody), and 10%
(roots) in September and 39% (leaves), 31% (woody), and 30% (roots) in November.

DesRochers et al. (2006) reported differences in leaf Ca allocations among three N fertilization treatments (0, 8, and 16 g N tree⁻¹) for *Populus* clones 33 cv. P38P38, 24 cv. Walker, and 794 cv. Brooks6, with 16 g N tree⁻¹ (0.19%) exhibiting the greatest leaf Ca concentration. The irrigation in the current study was most similar to their 16 g N tree⁻¹ treatment; however, our leaf Ca levels of 0.13% were equal for water and leachate treatments. Our stem Ca concentrations with water (0.71%) and leachate (0.76%) treatments were greater than TD *Populus* clones Beauprè (0.61%) and Trichobel (0.59%) that were irrigated with effluent and sewage sludge for three growing seasons (Moffat et al. 2001). Furthermore, the soil Ca concentration at preplanting differed from harvest levels, with the water treatment utilizing the greatest amount of soil Ca. There was a 69% reduction of Ca in the control soils and 40% reduction in the leachate soils during the two-year field study.

The S concentrations in the water and leachate were inadequate for optimal plant growth. However, the soil provided additional S and maintained overall plant tissue concentrations in leaf (0.37%), woody (0.16%), and root (0.10%) tissue within the general range of 0.1% to 0.5%. The soil S concentration was reduced by 99% in both treatments versus the preplanting value.

4.2. Micronutrients

Boron concentration differed for all water- and leachate-irrigated tissues, with the greatest levels in the leaf tissue. Although this study did not detect differences among clones
for B tissue concentration, the DM genomic group (*P. deltoides* × *P. maximowiczii*) had the
greatest amount of B in all tissues. Furthermore, clone DM115 had the greatest leaf
concentration (172.33 mg B kg⁻¹), NC14106 had the greatest stem concentration (27.67 mg B
kg⁻¹), and NC14104 had the greatest root concentration (43.33 mg B kg⁻¹). Likewise,
Bañuelos et al. (1999) reported higher concentrations of B in the leaves than stems of eight
*Populus* hybrids belonging to three genomic groups (TD, DN, TN) when irrigated with 5 mg
B L⁻¹ at an electrical conductivity of 7 mS cm⁻¹, which was similar to our findings of the
greatest leaf B concentrations at leachate salinity of 9.4 mS cm⁻¹. The concentration of B
remaining in the soil (water, 1.0 mg B kg⁻¹; leachate, 2.0 mg B kg⁻¹) after two seasons of
irrigation with the water (1.0 mg B L⁻¹) and leachate (12.5 mg B L⁻¹) decreased significantly
relative to preplanting levels (8.0 mg B kg⁻¹).

Manganese had greater accumulation in leaf tissue of trees irrigated with water versus
leachate, despite that the leachate contained 12 times greater Mn in solution. The
distributional pattern for Mn was similar for each treatment, with significantly greater
concentration in leaves versus roots and in roots versus woody tissue. This is a similar
response to three DN clones (DN17, DN182, DN34) and two NM clones (NM2, NM6)
irrigated with leachate, whereby the greatest Mn concentration was partitioned in leaf and
stem tissue (Zalesny and Bauer 2007a). The aboveground concentration of Mn ranged from
100 to 350 mg kg⁻¹, with a mean of 220 mg kg⁻¹ (Zalesny and Bauer 2007a), which was ten
times greater than Beaupré and Trichobel (19.4 mg kg⁻¹ each) (Moffat et al. 2001) but
consistent with that reported in the current study that ranged from 119 to 218 mg Mn kg⁻¹,
with a mean of 166 mg Mn kg⁻¹. Furthermore, both irrigation treatments reduced the
preplanting Mn level in the soil of the respective plot at harvest. The harvest soil concentration for the water treatment had a significant 50% decrease in soil Mn, indicating plants were able to extract and utilize stored Mn, which generally is more available for plant uptake in acidic soils (Foth, 1990). The leachate additions of Mn to the soil, along with tree uptake, resulted in the leachate soil Mn concentration being unchanged.

The root Fe concentration differed between the water (330.48 mg kg\(^{-1}\)) and leachate (838.67 mg kg\(^{-1}\)) treatments, which was intuitive given that there was nearly 8 times greater Fe in the leachate than the water. The stem Fe concentration of the water (82.33 mg kg\(^{-1}\)) and leachate (60.79 mg kg\(^{-1}\)) treatments in the current study was similar to that reported by Moffat et al. (2001) for two Populus clones: Beauprè (83.4 mg Fe kg\(^{-1}\)) and Trichobel (93.3 mg Fe kg\(^{-1}\)). Furthermore, the soil Fe concentration at preplanting differed from harvest levels. There was a 51% reduction of Fe in the control soils and 32% reduction in the leachate soils during the two-year field study, indicating the trees were able to utilize soil Fe.

Irrigation treatments differed for the concentration of Cu in the leaves and roots, with the greatest amount of Cu allocated to the root tissue of leachate-irrigated trees (11.89 mg Cu kg\(^{-1}\)) versus water-irrigated trees (9.59 mg Cu kg\(^{-1}\)). The leaf tissue of the water treatment had greater Cu (7.71 mg kg\(^{-1}\)) than the leachate (6.52 mg kg\(^{-1}\)), which was similar to a leaf Cu concentration of 6.00 mg kg\(^{-1}\) reported for three DN and two NM clones irrigated with landfill leachate (Zalesny and Bauer 2007a), but greater than 1.8 to 3.6 mg Cu kg\(^{-1}\) for a TD clone (van den Driessche 1999). Furthermore, soils for the water treatment at harvest showed a significant 31% decrease in soil Cu concentration, indicating plants were able to extract and utilize stored Cu from the soil, which generally is more available for plant uptake in acidic
soils (Foth, 1990). The leachate additions of Cu to the soil, along with plant removal, left the leachate soil Cu concentration unchanged.

Aluminum concentrations were significantly different for root tissue, with the mean for the leachate irrigation (1069.05 mg Al kg\(^{-1}\)) being 191% of the root concentration of the water treatment (559.23 mg Al kg\(^{-1}\)). Aluminum availability from irrigation was limited. Laboratory analyses detected a very small quantity in the leachate and nothing in the well water. However, the preplanting soil had 16.61 mg Al kg\(^{-1}\) across both treatment plots. Therefore, Al was available to all trees, especially given the low pH of the soil that increased the availability of Al for plant uptake. The preplanting and harvest soil analyses for water and leachate differed for Al concentration. The water treatment had a 63% decrease and the leachate treatment a 39% decrease in soil Al, indicating soil Al was available for uptake and the plants were able to utilize it for growth and development.

5. Conclusion

Biomass production of *Populus* is generally increased with irrigation and fertilization (Brown and van den Driessche 2002; Coyle and Coleman 2005; DesRochers et al. 2006), with adequate water supply necessary for overall productivity (Dickmann et al. 1996). Landfill leachate offers an opportunity to supply water and plant nutritional benefits at a lower cost than traditional sources. However, routine evaluation of leachate throughout the rotation is necessary to correct for any relevant changes in leachate chemistry that might affect plant health (Shrive et al. 1994; Kjeldsen et al. 2002). Such evaluation may elucidate
the need for the addition of nutrients that are deficient, such as P, or for dilution to compensate for toxicity of specific elements. This study was conducted at a landfill site that was highly disturbed and that exhibited elevated concentrations of many macro- and micro-nutrients in the soil before planting. However, leachate irrigation did not increase the concentration of any element over that found in the plot prior to leachate treatment, with the exception of N that did accumulate in the soil over preplanting values. Thus, there was effective uptake of inorganic elements required for plant growth without detrimental impact to tree health, which validated the use of landfill leachate as an irrigation and fertilization source for the trees.

Acknowledgements

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Table 5.1. Elements in well water (control) and leachate from the Oneida County Landfill (Rhinelander, Wisconsin, USA) during the 2005 and 2006 growing seasons.

<table>
<thead>
<tr>
<th>Element</th>
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<th>Leachate</th>
<th>Control</th>
<th>Leachate</th>
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</thead>
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<tr>
<td>N</td>
<td>480.0</td>
<td>597.5 ± 86.3</td>
<td>660.0</td>
<td>685.0 ± 25.0</td>
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<tr>
<td>P</td>
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<td>3.0 ± 0.7</td>
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<tr>
<td>K</td>
<td>400</td>
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<td>420.0</td>
<td>450.0 ± 30.0</td>
</tr>
<tr>
<td>Ca</td>
<td>na</td>
<td>na</td>
<td>11.00</td>
<td>25.0</td>
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<tr>
<td>Mg</td>
<td>na</td>
<td>na</td>
<td>4.50</td>
<td>150.0</td>
</tr>
<tr>
<td>S</td>
<td>nd&lt;sup&gt;c&lt;/sup&gt;</td>
<td>nd</td>
<td>nd</td>
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<td>Zn</td>
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<td>Mn</td>
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<td>Fe</td>
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<td>Cu</td>
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<td>Al</td>
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<td>na</td>
<td>nd</td>
<td>0.1</td>
</tr>
<tr>
<td>Pb</td>
<td>na</td>
<td>0.3 ± 0.3</td>
<td>0.01</td>
<td>nd</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are means ± one standard error (n = 2), except for N, P, and K in the control treatment both years, that were based on April leachate analyses, and additional values in 2006 (n = 1).

<sup>b</sup>Not available.

<sup>c</sup>Not detectable.
Table 5.2. Rate per application of elements in well water (control) and leachate from the Oneida County Landfill (Rhineland, Wisconsin, USA) during the 2005 and 2006 growing seasons.

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Leachate</th>
<th>Control</th>
<th>Leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>114.00</td>
<td>141.91</td>
<td>227.00</td>
<td>235.60</td>
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<tr>
<td>P</td>
<td>0.36</td>
<td>0.45</td>
<td>1.27</td>
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</tr>
<tr>
<td>K</td>
<td>95.00</td>
<td>106.88</td>
<td>144.45</td>
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<tr>
<td>Ca</td>
<td>na</td>
<td>na</td>
<td>3.78</td>
<td>8.60</td>
</tr>
<tr>
<td>Mg</td>
<td>na</td>
<td>na</td>
<td>1.55</td>
<td>51.59</td>
</tr>
<tr>
<td>S</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.88</td>
</tr>
<tr>
<td>Zn</td>
<td>na</td>
<td>na</td>
<td>0.41</td>
<td>0.03</td>
</tr>
<tr>
<td>B</td>
<td>na</td>
<td>1.21</td>
<td>0.02</td>
<td>4.30</td>
</tr>
<tr>
<td>Mn</td>
<td>na</td>
<td>0.12</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Fe</td>
<td>na</td>
<td>1.83</td>
<td>0.22</td>
<td>1.70</td>
</tr>
<tr>
<td>Cu</td>
<td>na</td>
<td>na</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Al</td>
<td>na</td>
<td>na</td>
<td>nd</td>
<td>0.03</td>
</tr>
<tr>
<td>Pb</td>
<td>na</td>
<td>0.07</td>
<td>0.00</td>
<td>nd</td>
</tr>
</tbody>
</table>

*Eight applications total. Rate based on an application volume of 3.8 L tree\(^{-1}\) and an irrigated soil surface area of 0.16 m\(^2\) tree\(^{-1}\).*

*Twelve applications total. Rate based on an application volume of 22.7 L tree\(^{-1}\) and an irrigated soil surface area of 0.66 m\(^2\) tree\(^{-1}\).*

*Nitrogen, P, and K fertilizer additions to the control treatment both years were based on April leachate analyses.*

*Not available.*

*Not detectable.*
Table 5.3. Elements in the soil before planting and at whole-tree harvest after irrigating for the 2005 and 2006 growing seasons with well water (control) and leachate from the Oneida County Landfill (Rhineland, Wisconsin, USA).

<table>
<thead>
<tr>
<th>Element</th>
<th>Preplanting</th>
<th>Control</th>
<th>Leachate</th>
<th>LSD_{0.05}</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1.44 ± 0.34</td>
<td>b</td>
<td>1.37 ± 0.59</td>
<td>b</td>
</tr>
<tr>
<td>P</td>
<td>3.55 ± 0.23</td>
<td>a</td>
<td>0.30 ± 0.01</td>
<td>b</td>
</tr>
<tr>
<td>K</td>
<td>0.83 ± 0.01</td>
<td>a</td>
<td>0.08 ± 0.00</td>
<td>b</td>
</tr>
<tr>
<td>Ca</td>
<td>4.81 ± 0.24</td>
<td>a</td>
<td>1.49 ± 0.36</td>
<td>c</td>
</tr>
<tr>
<td>Mg</td>
<td>1.99 ± 0.00</td>
<td>a</td>
<td>1.38 ± 0.08</td>
<td>c</td>
</tr>
<tr>
<td>S</td>
<td>1.36 ± 0.09</td>
<td>a</td>
<td>0.01 ± 0.00</td>
<td>b</td>
</tr>
<tr>
<td>Zn</td>
<td>48.00 ± 4.04</td>
<td>a</td>
<td>2.55 ± 0.09</td>
<td>b</td>
</tr>
<tr>
<td>B</td>
<td>8.00 ± 0.00</td>
<td>a</td>
<td>1.00 ± 0.00</td>
<td>c</td>
</tr>
<tr>
<td>Mn</td>
<td>0.20 ± 0.01</td>
<td>a</td>
<td>0.10 ± 0.01</td>
<td>b</td>
</tr>
<tr>
<td>Fe</td>
<td>10.98 ± 0.36</td>
<td>a</td>
<td>5.41 ± 0.41</td>
<td>c</td>
</tr>
<tr>
<td>Cu</td>
<td>16.00 ± 1.15</td>
<td>a</td>
<td>11.03 ± 1.56</td>
<td>b</td>
</tr>
<tr>
<td>Al</td>
<td>16.61 ± 0.70</td>
<td>a</td>
<td>6.12 ± 1.12</td>
<td>c</td>
</tr>
<tr>
<td>Pb</td>
<td>3.66 ± 0.04</td>
<td>a</td>
<td>1.86 ± 0.59</td>
<td>b</td>
</tr>
</tbody>
</table>

aN, P, K, Ca, Mg, S, Mn, Fe, and Al (g kg⁻¹); Zn, B, Cu, and Pb (mg kg⁻¹). Means for each element followed by different letters were different (LSD_{0.05}).
Table 5.4. Probability values from analyses of variance comparing the elemental concentrations across two irrigation treatments [I: well water (control) and landfill leachate], eight *Populus* clones (C; see Materials and Methods for descriptions) and three tissues (T: leaf, woody, and root). Significant values are in bold.

<table>
<thead>
<tr>
<th>Element</th>
<th>I</th>
<th>C</th>
<th>I × C</th>
<th>T</th>
<th>I × T</th>
<th>C × T</th>
<th>I × C × T</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.0577</td>
<td>0.0133</td>
<td>0.0424</td>
<td>&lt;0.0001</td>
<td>0.2112</td>
<td>&lt;0.0001</td>
<td>0.2158</td>
</tr>
<tr>
<td>P</td>
<td>0.4275</td>
<td>0.0008</td>
<td>0.6780</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td>0.0044</td>
<td>0.3201</td>
</tr>
<tr>
<td>K</td>
<td>0.0560</td>
<td>0.0506</td>
<td>0.2325</td>
<td>&lt;0.0001</td>
<td>0.4040</td>
<td>0.0969</td>
<td>0.0995</td>
</tr>
<tr>
<td>Ca</td>
<td>0.9737</td>
<td>0.0255</td>
<td>0.0477</td>
<td>&lt;0.0001</td>
<td>0.1368</td>
<td>&lt;0.0001</td>
<td>0.1393</td>
</tr>
<tr>
<td>Mg</td>
<td>0.0998</td>
<td>0.0822</td>
<td>0.9289</td>
<td>0.0003</td>
<td>0.0043</td>
<td>&lt;0.0001</td>
<td>0.0730</td>
</tr>
<tr>
<td>S</td>
<td>0.1171</td>
<td>&lt;0.0001</td>
<td>0.0257</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Zn</td>
<td>0.0665</td>
<td>0.1909</td>
<td>0.3906</td>
<td>0.0919</td>
<td>0.5748</td>
<td>0.6067</td>
<td>0.9755</td>
</tr>
<tr>
<td>B</td>
<td>0.0059</td>
<td>0.4481</td>
<td>0.5567</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>0.8133</td>
<td>0.7613</td>
</tr>
<tr>
<td>Mn</td>
<td>0.0320</td>
<td>0.0239</td>
<td>0.2333</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0060</td>
<td>0.7443</td>
</tr>
<tr>
<td>Fe</td>
<td>0.0036</td>
<td>0.5035</td>
<td>0.5972</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.8528</td>
<td>0.6017</td>
</tr>
<tr>
<td>Cu</td>
<td>0.0782</td>
<td>&lt;0.0001</td>
<td>0.0220</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Al</td>
<td>0.0019</td>
<td>0.6829</td>
<td>0.4097</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.8559</td>
<td>0.2032</td>
</tr>
<tr>
<td>Pb</td>
<td>0.6983</td>
<td>0.6535</td>
<td>0.6856</td>
<td>0.0608</td>
<td>0.0641</td>
<td>0.2046</td>
<td>0.0902</td>
</tr>
</tbody>
</table>
Fig. 5.1. Concentration of phosphorus (A) and magnesium (B) in the leaf, woody, and root tissue across eight Populus clones when irrigated with well water (control) or landfill leachate for two growing seasons. Error bars represent one standard error of the mean (n_P = 24; n_Mg = 48). Bars labeled with the same letter were not different, according to Fisher’s protected least significant difference (LSD).
Fig. 5.2. Concentration of nitrogen (A), phosphorus (B), calcium (C), and magnesium (D) in the leaf, woody, and root tissue of eight *Populus* clones across two irrigation treatments [well water (control) and landfill leachate]. Error bars represent one standard error of the mean (n\(_N\) = n\(_P\) = 6; n\(_Ca\) = n\(_Mg\) = 12). Bars labeled with the same letter were not different, according to Fisher’s protected least significant difference (LSD).
Fig. 5.3. Concentration of sulfur for each combination of irrigation treatment [well water (control) and landfill leachate], *Populus* clone, and tree tissue (leaf, woody, and root). Error bars represent one standard error of the mean (n = 3). Asterisks denote treatment differences within a clone, according to Fisher’s protected least significant difference (LSD).
Fig. 5.4. Concentration of boron (A), manganese (B), iron (C), copper (D), and aluminum (E) in the leaf, woody, and root tissue across eight Populus clones when irrigated with well water (control) or landfill leachate for two growing seasons. Error bars represent one standard error of the mean ($n_B = 24$; $n_{Mn} = n_{Fe} = n_{Cu} = n_{Al} = 48$). Bars labeled with the same letter were not different, according to Fisher’s protected least significant difference (LSD).
Fig. 5.5. Concentration of manganese (A) and copper (B) in the leaf, woody, and root tissue of eight Populus clones across two irrigation treatments [well water (control) and landfill leachate]. Error bars represent one standard error of the mean (n = 12). Bars labeled with the same letter were not different, according to Fisher’s protected least significant difference (LSD).
CHAPTER 6. GENERAL CONCLUSIONS

General Discussion

A great deal of attention has been focused on disposal of waste in North America during the past few decades, because land and water resources have become increasingly degraded. The United States is the largest global producer of municipal, commercial, and industrial waste. This fact is aggravated by a society overcome with mass consumption that puts forth little worry over the municipal solid waste they produce. It is well known that to move our society toward a more sustainable waste management system, we must have a united focus based on a reduce, recycle, and reuse approach. Not only is the waste volume continually increasing, but also land availability is decreasing. Unfortunately, negative impacts have occurred as a result of improper and excessive waste disposal. Most devastating is the contamination of the soil, water, and air that we all depend on for sustained life.

Regardless of the source of environmental degradation, there are technologies to reduce the damage of such pollution, but these technologies are often under-utilized. Using plants to remove, destroy, and stabilize contaminated soils is a technology currently gaining attention. Phytoremediation consists of using natural plant processes, along with soil amendments and site management practices, to improve a contaminated site in situ. One group of plants currently under investigation for phytoremediation capability includes species and hybrids of *Populus*. Phytoremediation merges the science of short rotation woody crops
(SRWC) with environmental clean-up methodologies to achieve long-term conservation objectives.

Selected genotypes of poplar are ideal for phytoremediation systems because of their ability to: grow fast, produce large plant biomass, grow on heavily contaminated and marginal soils not suitable for agronomic crops, produce extensive root systems that grow deep, adapt well to riparian sites, grow easily from hardwood cuttings, and transpire large volumes of water. In addition, poplars exhibit broad genetic diversity and easily hybridize, which supports increased gains from selection than with other tree species. Asexual propagation allows for indefinite availability of favorable genotypes once they are identified and selected.

**Phytoremediation Technology and Associated Benefits**

The use of phytoremediation as a technology for leachate disposal has three important environmental rewards. First, phytoremediation utilizes natural plant processes whereby the leachate can be biologically cleansed to remove many of the excessive nutrients and chemicals. Second, phytoremediation plantations can be harvested in 8 to 10 years for fiber or energy, which helps to preserve the plant and animal biodiversity of natural stands. Third, when plants remove and sequester excess nutrients and chemicals found in the leachate, it prevents the unwanted leaching of these harmful elements/compounds into nearby watersheds.
**Plant Processes**

Phytoremediation offers a low input alternative to traditional clean-up methods by eliminating the need to remove soil from contaminated sites. Several plant processes are useful in stabilizing, detoxifying, and sequestering various elements and compounds found in landfill leachate. In the rhizosphere, plants produce many exudates that are critical to the breakdown, stabilization, and detoxification of specific chemical species into less toxic or damaging forms. Another method plants utilize to clean up soil or water contamination is the incorporation of chemicals into the plant tissue (sequestration). The remediation of landfill leachate utilizes these plant processes to remove excess nutrients and chemicals from the variable leachate solution. Leachate application can be modified by precipitation events to control leaching into nearby watersheds. The utilization of landfill leachate is one more way to recycle and reuse a waste product from humans. The leachate often contains several of the 17 essential nutrients required by plants and, therefore, contributes a fertilization effect.

**Plant and Animal Conservation**

The use of SRWC offers an important opportunity to conserve natural forest stands, along with the potential for enhancement of biodiversity. In the near future, fiber and energy demand is expected to exceed supply, especially for species of *Populus* in the North Central United States. Genetically superior, disease resistant poplars used for SRWC systems may offset some of these shortages. Poplars can be successfully grown on marginal land. Utilizing managed poplar stands supports the enhancement of plant conservation and biodiversity because less pressure is placed on natural stands to provide biological and social outputs.
Watershed Improvement

Every organism on the planet needs water for survival. Conserving and sustaining enough clean water for urban, agricultural, and industrial use is a global natural resources issue. Pollution of this critical natural resource is an enormous contributing factor to possible shortages of water for people to use. Large river systems play an important role in maintaining ecological processes and regional populations, which cannot be done if contaminated and degraded. The use of poplars for uptake and sequestration of various chemicals and nutrients found in landfill leachate is an important step in removing these contaminants from possible movement through watersheds.

Summary of Key Research Findings

- There is an essential need for initial genotype screening followed by the establishment and evaluation of test plots to ascertain clonal performance prior to large-scale deployment.
- Although leachate irrigation did not enhance tree growth and biomass for most genotypes in the current study, significant productivity reductions associated with the leachate also were not observed.
- The lack of overall differences in biomass accumulation in response to treatments during phyto-recurrent selection cycle 4 was a result of extensive genotypic screening during cycles 1 to 3 that reduced the variability among the clones deployed, relative to the original 25 genotypes.
Tissue concentrations with leachate irrigation were 17 (Na\(^+\)) and four (Cl\(^-\)) times greater than water, while Na\(^+\) levels were greatest in the roots and Cl\(^-\) levels were greatest in the leaves. Sodium and Cl\(^-\) levels were least in the woody tissue.

With regard to the relationship between tissue Cl\(^-\) concentration and biomass production, clones irrigated with leachate segregated into three response groups: 1) NC14104, NM2, and NM6 had elevated levels of total tree Cl\(^-\) concentration along with increased biomass; 2) NC14018, NC14106, and DM115 exhibited elevated levels of total tree Cl\(^-\) concentration along with decreased biomass; 3) NC13460 and DN5 exhibited mid levels of total tree Cl\(^-\) concentration and biomass.

The enhanced distribution of Na\(^+\), Cl\(^-\), and other nutrients in leaf, woody, and root tissue when irrigated with municipal solid waste landfill leachate versus well water (control) was evidence of successful clone-specific elemental uptake using *Populus*.

The concentration of N, P, K, Ca, Mg, S, B, and Mn was greatest in leaves and least in woody tissue, while that of Fe, Cu, and Al was greatest in roots and least in leaves and woody tissue.

There was successful phytoaccumulation and distribution in leaf, woody, and root tissue of macro- and micro-nutrients without detrimental impact to plant health, which validated the use of landfill leachate as an irrigation and fertilization source for the trees.

The genotypic variation that was observed for growth, biomass accumulation, and nutrient uptake was useful for further selection of clones that could be used in a large-scale system.
• Tree-based phytoremediation technologies can be beneficial for the reduction of environmental damage resulting from such pollution.

Recommendations for Future Research

Future landfill leachate remediation research in regions similar to the North Central United States should evaluate:

• rooted cuttings as well as unrooted cuttings, especially for *P. deltoides* genotypes that do not establish well from unrooted cuttings because of erratic or delayed rooting in the field.

• the effect of landfill leachate on macro- and micro-organisms in the rhizosphere.

• impacts of leachate application on life cycles and processes of foliage and stem boring insects.

• reintroduction of remediated chemicals through abscission and decay of leaves.

• impacts of long-term (rotation age) application of leachate to the trees and soil, as well as, additional biotic and abiotic components of the ecosystem.
APPENDIX A. RESEARCH TIMELINE
Table A.1  Research timeline.

<table>
<thead>
<tr>
<th>Research task</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Leachate analysis</td>
<td>★</td>
<td>★</td>
<td>★</td>
</tr>
<tr>
<td>Cycle 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>★</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle 2</td>
<td>★★★</td>
<td>★</td>
<td></td>
</tr>
<tr>
<td>Cycle 3</td>
<td>★★★</td>
<td>★</td>
<td></td>
</tr>
<tr>
<td>Data analysis (1 to 3)</td>
<td>★</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle 4</td>
<td>★★★★</td>
<td>★★★★</td>
<td>★★★★</td>
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<tr>
<td>Growth measurements</td>
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<td></td>
</tr>
<tr>
<td>Chapter 2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
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<tr>
<td>Soil sampling</td>
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</tr>
<tr>
<td>Cycle 4 data collection</td>
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<td>★★★★</td>
<td>★★★★</td>
</tr>
<tr>
<td>Intl. Conference&lt;sup&gt;c&lt;/sup&gt;</td>
<td>★</td>
<td></td>
<td></td>
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<tr>
<td>Cycle 4 data analysis</td>
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<td>★★★★</td>
<td>★★★★</td>
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<tr>
<td>Chapter 3&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>Finish dissertation</td>
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</tbody>
</table>

<sup>a</sup> Phyto-recurrent selection cycles 1 to 3 were conducted in the greenhouse (*ex situ*), while cycle 4 was conducted in the field (*in situ*).


<sup>f</sup> Zalesny, J.A., Zalesny, R.S. Jr., Wiese, A.H., Sexton, B., and Hall, R.B. Submitted. Sodium and chloride accumulation in leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate. Environmental Pollution

APPENDIX B. MAP OF FIELD SITE IN RELATION TO AMES, IA
Figure B.1 Map of field site (Rhineland, WI, USA) in relation to Iowa State University (Ames, IA, USA).
APPENDIX C. EXPERIMENTAL LAYOUT OF
GREENHOUSE AND FIELD STUDIES
Figure C.1 Experimental layout of phyto-recurrent selection cycle 1, conducted in the greenhouse.
Figure C.2 Experimental layout of phyto-recurrent selection cycle 2, conducted in the greenhouse.
Figure C.3 Experimental layout of phyto-recurrent selection cycle 3, conducted in the greenhouse.
Figure C.4 Experimental layout and map of field study (cycle 4) at the Oneida County Landfill.
APPENDIX D. EXPECTED MEAN SQUARES OF
STATISTICAL MODELS USED IN THE ANALYSES
Table D.1  Degrees of freedom and expected mean squares of statistical models used in the analyses of Chapters 2 and 3, assuming a split-plot design with random blocks, fixed treatments (whole plots) and fixed clones (sub plots).

<table>
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<th>Cycle</th>
<th>Source of variation</th>
<th>df</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
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<td>3</td>
<td>$\sigma^2 + 50\sigma_B^2$</td>
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<td></td>
<td>Treatment</td>
<td>1</td>
<td>$\sigma^2 + 25\sigma_{BT} + 100\Phi_T$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment</td>
<td>3</td>
<td>$\sigma^2 + 25\sigma_{BT}$</td>
</tr>
<tr>
<td></td>
<td>Clone</td>
<td>24</td>
<td>$\sigma^2 + 2\sigma_{BC} + 8\Phi_C$</td>
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<td></td>
<td>Block × Clone</td>
<td>72</td>
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<td></td>
<td>Treatment × Clone</td>
<td>24</td>
<td>$\sigma^2 + \sigma_{BTC} + 4\Phi_{TC}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone</td>
<td>72</td>
<td>$\sigma^2 + \sigma_{BTC}$</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>$\sigma^2 + 24\sigma_B^2$</td>
</tr>
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<td>Treatment</td>
<td>1</td>
<td>$\sigma^2 + 12\sigma_{BT} + 48\Phi_T$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment</td>
<td>3</td>
<td>$\sigma^2 + 12\sigma_{BT}$</td>
</tr>
<tr>
<td></td>
<td>Clone</td>
<td>11</td>
<td>$\sigma^2 + 2\sigma_{BC} + 8\Phi_C$</td>
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<tr>
<td></td>
<td>Block × Clone</td>
<td>33</td>
<td>$\sigma^2 + 2\sigma_{BC}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Clone</td>
<td>11</td>
<td>$\sigma^2 + \sigma_{BTC} + 4\Phi_{TC}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone</td>
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<td>$\sigma^2 + \sigma_{BTC}$</td>
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<td>Treatment</td>
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<td>$\sigma^2 + 12\sigma_{BT} + 72\Phi_T$</td>
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<tr>
<td></td>
<td>Block × Treatment</td>
<td>5</td>
<td>$\sigma^2 + 12\sigma_{BT}$</td>
</tr>
<tr>
<td></td>
<td>Clone</td>
<td>11</td>
<td>$\sigma^2 + 2\sigma_{BC} + 12\Phi_C$</td>
</tr>
<tr>
<td></td>
<td>Block × Clone</td>
<td>55</td>
<td>$\sigma^2 + 2\sigma_{BC}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Clone</td>
<td>11</td>
<td>$\sigma^2 + \sigma_{BTC} + 6\Phi_{TC}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone</td>
<td>55</td>
<td>$\sigma^2 + \sigma_{BTC}$</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Cycle 4 (chapter 3)</td>
<td>Block</td>
<td>7</td>
<td>$\sigma^2 + 16\sigma_B^2$</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>1</td>
<td>$\sigma^2 + 8\sigma_{BT} + 64\Phi_T$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment</td>
<td>7</td>
<td>$\sigma^2 + 8\sigma_{BT}$</td>
</tr>
<tr>
<td></td>
<td>Clone</td>
<td>7</td>
<td>$\sigma^2 + 2\sigma_{BC} + 16\Phi_C$</td>
</tr>
<tr>
<td></td>
<td>Block × Clone</td>
<td>49</td>
<td>$\sigma^2 + 2\sigma_{BC}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Clone</td>
<td>7</td>
<td>$\sigma^2 + \sigma_{BTC} + 8\Phi_{TC}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone</td>
<td>49</td>
<td>$\sigma^2 + \sigma_{BTC}$</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>127</td>
<td></td>
</tr>
</tbody>
</table>
Table D.2 Degrees of freedom and expected mean squares of statistical models used in the analyses of Chapters 4 and 5, assuming a split-split-plot design with random blocks, fixed treatments (whole plots), fixed clones (sub plots), and fixed tree tissues (sub-sub plots).

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Source of variation</th>
<th>df</th>
<th>Expected mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 4</td>
<td>Block</td>
<td>2</td>
<td>$\sigma^2 + 48\sigma^2_B$</td>
</tr>
<tr>
<td>(chapter 4)</td>
<td>Treatment</td>
<td>1</td>
<td>$\sigma^2 + 24\sigma^2_{BT} + 72\Phi_T$</td>
</tr>
<tr>
<td>(chapter 5)</td>
<td>Block × Treatment</td>
<td>2</td>
<td>$\sigma^2 + 24\sigma^2_{BT}$</td>
</tr>
<tr>
<td>(3 blocks)</td>
<td>Clone</td>
<td>7</td>
<td>$\sigma^2 + 6\sigma^2_{BC} + 18\Phi_C$</td>
</tr>
<tr>
<td></td>
<td>Block × Clone</td>
<td>14</td>
<td>$\sigma^2 + 6\sigma^2_{BC}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Clone</td>
<td>7</td>
<td>$\sigma^2 + 3\sigma^2_{BTC} + 9\Phi_{TC}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone</td>
<td>14</td>
<td>$\sigma^2 + 3\sigma^2_{BTC}$</td>
</tr>
<tr>
<td></td>
<td>Tissue</td>
<td>2</td>
<td>$\sigma^2 + 16\sigma^2_{BP} + 48\Phi_P$</td>
</tr>
<tr>
<td></td>
<td>Block × Tissue</td>
<td>4</td>
<td>$\sigma^2 + 16\sigma^2_{BP}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Tissue</td>
<td>2</td>
<td>$\sigma^2 + 8\sigma^2_{BTP} + 24\Phi_{TP}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Tissue</td>
<td>4</td>
<td>$\sigma^2 + 8\sigma^2_{BTP}$</td>
</tr>
<tr>
<td></td>
<td>Clone × Tissue</td>
<td>14</td>
<td>$\sigma^2 + 2\sigma^2_{BCP} + 6\Phi_{CP}$</td>
</tr>
<tr>
<td></td>
<td>Block × Clone × Tissue</td>
<td>28</td>
<td>$\sigma^2 + 2\sigma^2_{BCP}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Clone × Tissue</td>
<td>14</td>
<td>$\sigma^2 + \sigma^2_{BTCP} + 3\Phi_{TCP}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone × Tissue</td>
<td>28</td>
<td>$\sigma^2 + \sigma^2_{BTCP}$</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>143</td>
<td></td>
</tr>
<tr>
<td>Cycle 4</td>
<td>Block</td>
<td>5</td>
<td>$\sigma^2 + 48\sigma^2_B$</td>
</tr>
<tr>
<td>(chapter 4)</td>
<td>Treatment</td>
<td>1</td>
<td>$\sigma^2 + 24\sigma^2_{BT} + 144\Phi_T$</td>
</tr>
<tr>
<td>(chapter 5)</td>
<td>Block × Treatment</td>
<td>5</td>
<td>$\sigma^2 + 24\sigma^2_{BT}$</td>
</tr>
<tr>
<td>(6 blocks)</td>
<td>Clone</td>
<td>35</td>
<td>$\sigma^2 + 6\sigma^2_{BC} + 36\Phi_C$</td>
</tr>
<tr>
<td></td>
<td>Block × Clone</td>
<td>35</td>
<td>$\sigma^2 + 3\sigma^2_{BTC} + 18\Phi_{TC}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Clone</td>
<td>7</td>
<td>$\sigma^2 + 3\sigma^2_{BTC}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone</td>
<td>35</td>
<td>$\sigma^2 + 3\sigma^2_{BTC}$</td>
</tr>
<tr>
<td></td>
<td>Tissue</td>
<td>2</td>
<td>$\sigma^2 + 16\sigma^2_{BP} + 96\Phi_P$</td>
</tr>
<tr>
<td></td>
<td>Block × Tissue</td>
<td>10</td>
<td>$\sigma^2 + 16\sigma^2_{BP}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Tissue</td>
<td>2</td>
<td>$\sigma^2 + 8\sigma^2_{BTP} + 48\Phi_{TP}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Tissue</td>
<td>10</td>
<td>$\sigma^2 + 8\sigma^2_{BTP}$</td>
</tr>
<tr>
<td></td>
<td>Clone × Tissue</td>
<td>14</td>
<td>$\sigma^2 + 2\sigma^2_{BCP} + 12\Phi_{CP}$</td>
</tr>
<tr>
<td></td>
<td>Block × Clone × Tissue</td>
<td>70</td>
<td>$\sigma^2 + 2\sigma^2_{BCP}$</td>
</tr>
<tr>
<td></td>
<td>Treatment × Clone × Tissue</td>
<td>14</td>
<td>$\sigma^2 + \sigma^2_{BTCP} + 6\Phi_{TCP}$</td>
</tr>
<tr>
<td></td>
<td>Block × Treatment × Clone × Tissue</td>
<td>70</td>
<td>$\sigma^2 + \sigma^2_{BTCP}$</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>287</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX E. DESIGN OF NOVEL HORIZONTAL RHIZOTRON

USED IN PHYTO-RECURRENT SELECTON CYCLE 2
Notes:

1. Drill pilot holes and use self-tapping screws to secure plexiglass to aluminum channel.
2. Use eye screws to secure dark-colored tarps to the rhizotron framework to eliminate light penetration into the underside of the rhizotron.
3. Secure the rhizotron to its framework using wood screws, and place the rhizotron and its framework on the support framework.

Figure E.1 Sketch of the novel rhizotron design used in phyto-recurrent selection cycle 2, including the rhizotron, its framework, and a support framework. Observations of the root systems are taken on the underside of the rhizotrons. Lowercase letter designations in parentheses correspond to those in Table E.1. Design and schematic adapted from: Wiese, A.H., Riemenschneider, D.E., and Zalesny, R.S. Jr. 2005. An inexpensive rhizotron design for two-dimensional, horizontal root growth measurements. Tree Planters’ Notes 51:40-46.
### Table E.1

List of equipment and materials for construction of the inexpensive rhizotron, along with the rhizotron framework and support framework, used for two-dimensional, horizontal root growth measurements during phyto-recurrent selection cycle 2. The designations correspond to those given in Figure E.1. Table adapted from: Wiese, A.H., Riemenschneider, D.E., and Zalesny, R.S. Jr. 2005. An inexpensive rhizotron design for two-dimensional, horizontal root growth measurements. Tree Planters’ Notes 51:40-46.

#### Equipment

Mitre saw, reciprocating saw with blade for cutting metal, screw gun, wrenches, drill bits, hole saw kit, marker

<table>
<thead>
<tr>
<th>System component</th>
<th>Designation</th>
<th>Quantity</th>
<th>Description of part(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Rhizotron</td>
<td>a</td>
<td>2 sheets</td>
<td>Plexiglass [4 ft × 8 ft × 0.25 in (1.219 m × 2.438 m × 0.64 cm)]</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>6 pieces</td>
<td>Terminal adapter with nut [1.5-in (3.81-cm) diameter]</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>4 pieces</td>
<td>Aluminum channel [8 ft × 0.5 in (2.44 m × 1.27 cm)]</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>1 box</td>
<td>Self-tapping screws</td>
</tr>
<tr>
<td></td>
<td>e</td>
<td>48 in (121.92 cm)</td>
<td>Polyvinyl chloride pipe (PVC) [1.5-in (3.81-cm) diameter]</td>
</tr>
<tr>
<td>B. Rhizotron framework</td>
<td>f</td>
<td>3 pieces</td>
<td>Lumber [2 in × 4 in × 8 ft (5.08 cm × 10.16 cm × 2.44 m)]</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td>3 pieces</td>
<td>Lumber [1 in × 4 in × 8 ft (2.54 cm × 10.16 cm × 2.44 m)]</td>
</tr>
<tr>
<td></td>
<td>h</td>
<td>10 pieces</td>
<td>90-degree angle</td>
</tr>
<tr>
<td></td>
<td>i</td>
<td>1 box</td>
<td>Wood screws</td>
</tr>
<tr>
<td>C. Support framework</td>
<td>j</td>
<td>9 pieces</td>
<td>Galvanized steel pipe [8 ft × 1.25 in (2.438 m × 3.175 cm)]</td>
</tr>
<tr>
<td></td>
<td>k</td>
<td>18 pieces</td>
<td>Pipe adapter [1.25 × 1.25 in (3.175 × 3.175 cm)]</td>
</tr>
<tr>
<td></td>
<td>l</td>
<td>18 pieces</td>
<td>Bolt with nut [3 × 0.25 in (7.62 × 0.635 cm)]</td>
</tr>
<tr>
<td>D. Filling and planting</td>
<td>m</td>
<td>3 tarps</td>
<td>Dark-colored tarp [4 × 8 ft (1.219 × 2.438 m)]</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>1 box</td>
<td>Eye screws</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Planting medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Planting stock</td>
</tr>
</tbody>
</table>
APPENDIX F. MAP OF LANDFILL SOIL SAMPLING POINTS
Figure F.1  Map of soil sampling points at the Oneida County Landfill.
APPENDIX G. SOLID WASTE HISTORY OF ONEIDA COUNTY
<table>
<thead>
<tr>
<th>Year</th>
<th>Activity</th>
<th>Tipping fee ($ ton⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1979</td>
<td>Oneida County had 22 operational dumps</td>
<td>Most charged nothing</td>
</tr>
<tr>
<td>1979</td>
<td>Oneida County Sanitary Landfill opened; constructed under NR 140 with 2’ of compacted sandy loam; no leachate collection</td>
<td>5.75</td>
</tr>
<tr>
<td>1980</td>
<td>Tonnage at half of original projection</td>
<td>11.50</td>
</tr>
<tr>
<td>1982</td>
<td>B cell opened (same liner as A cell)</td>
<td>16.50</td>
</tr>
<tr>
<td>1986</td>
<td>C cell opened; lined with 2’ of compacted sandy loam and leachate collection systems</td>
<td>17.75</td>
</tr>
<tr>
<td>1988</td>
<td>NR 500 regulations were promulgated requiring clay liners</td>
<td>20.75</td>
</tr>
<tr>
<td>1989</td>
<td></td>
<td>35.00</td>
</tr>
<tr>
<td>1990</td>
<td></td>
<td>50.00</td>
</tr>
<tr>
<td>1991</td>
<td></td>
<td>60.00</td>
</tr>
<tr>
<td>1992</td>
<td>Southern half of final cell liner constructed with 5’ of compacted clay</td>
<td>62.00</td>
</tr>
<tr>
<td>1994</td>
<td>Sharps collection began; Oneida County became responsible unit (RU) for recycling; first half of landfill cap upgraded to 40 mil PVC and 2’ compacted clay</td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>County decided against a second sanitary landfill; northern half of final cell liner constructed with HDPE and 4’ compacted clay; source separated and fiber cake composting began; annual mobile hazardous waste collection began; United Waste bought out four local haulers with &gt;65% market share</td>
<td>55.00</td>
</tr>
<tr>
<td>1996</td>
<td>United Waste begins exporting waste out of Oneida County</td>
<td>50.00</td>
</tr>
<tr>
<td>1997</td>
<td>USA Waste bought United Waste</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>USA Waste bought Waste Management</td>
<td>45.00</td>
</tr>
<tr>
<td>1999</td>
<td>Fiber cake asphalt composting pad constructed; Permanent hazardous waste facility constructed/opened</td>
<td>42.00</td>
</tr>
<tr>
<td>2000</td>
<td>Gas system expanded to C/D cell leachate, horizontal piping in D cell</td>
<td>40.00</td>
</tr>
<tr>
<td>2001</td>
<td>Recycling sort building/operations opened</td>
<td>38.00</td>
</tr>
<tr>
<td>2002</td>
<td>Sanitary landfill reached permitted waste grades; waste transferred to Outagamie County Sanitary Landfill (Appleton) and Lincoln County Landfills; final closure (capping) of sanitary landfill; $3 ton⁻¹ state landfill surcharge</td>
<td>50.00</td>
</tr>
<tr>
<td>2003</td>
<td>Ten year contract signed with Waste Management for trucking and disposal; waste transferred to Ontonagon, Michigan; operations began for glass processing and bagging, along with wood chip bagging; haulers given $8 ton⁻¹ rebate</td>
<td>52.00</td>
</tr>
<tr>
<td>2005</td>
<td>Trees planted as part of a multi-institutional effort to test the phytoremediation effectiveness of poplars irrigated with landfill leachate</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>Trees harvested</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>Results of leachate phytoremediation project disseminated</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX G. ABSTRACTS FROM: SEVENTH BIENNIAL CONFERENCE OF
THE SHORT ROTATION WOODY CROPS OPERATIONS WORKING GROUP:
SHORT ROTATION WOODY CROP PRODUCTION SYSTEMS FOR
WOOD PRODUCTS, BIOENERGY AND ENVIRONMENTAL SERVICES:
SEPTEMBER 25-28, 2006; PASCO, WASHINGTON, USA; PAGES 51 & 52
Using phyto-recurrent selection to choose *Populus* genotypes for phytoremediation of landfill leachate

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*Corresponding author: 5985 Highway K, Rhinelander, WI 54501, USA
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Abstract

Information about the response of *Populus* genotypes to landfill leachate irrigation is needed, along with efficient methods for choosing genotypes based on leachate composition. We irrigated poplar clones during three cycles of phyto-recurrent selection to test whether genotypes responded differently to leachate and water, and to test whether our methodology had merit as a tool for plant selection during remediation. Fifteen belowground and aboveground traits were evaluated. Twenty-five clones were tested in cycle 1, while the best 12 genotypes were evaluated in cycles 2 and 3. Eight clones were selected and currently are being tested in an *in situ* landfill study (cycle 4). Overall, clones responded differently to irrigation treatments, with certain genotypes exhibiting better belowground and aboveground growth with water than leachate. However, growth was greater with leachate irrigation for some clones. In addition, differences between treatments within clones decreased with days after planting (DAP). There were no treatment differences for number of leaves, height, and root length at the end of cycle 2 (45 DAP) or cycle 3 (30 DAP). Our results supported the extensive variation in clonal responses to leachate irrigation, along with the need and efficacy of using phyto-recurrent selection to choose superior genotypes.
Phytoremediation of landfill leachate using *Populus*

Jill A. Zalesny\(^1,2,*\), Ronald S. Zalesny Jr.\(^2\), Adam H. Wiese\(^2\), Richard B. Hall\(^1\), and Bart Sexton\(^3\)

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Abstract

Proper genotype selection is required for successful phytoremediation. We selected eight *Populus* clones (NC13460, NC14018, DM115, NC14104, NC14106, DN5, NM2, NM6) of four genomic groups after three cycles of phyto-recurrent selection for a field trial that began June 2005 at the Oneida County Landfill in Rhinelander, WI, USA (45.6 °N, 89.4 °W).

During the 2005 growing season, we irrigated the trees with 3.8 L wk\(^{-1}\) of landfill leachate or water and evaluated survival, height, and diameter. We are irrigating the trees in 2006 with 22.8 L wk\(^{-1}\) of leachate or water and will test for inorganic element concentrations (N, P, K, Ca, Mg, S, Zn, Mn, B, Cu, Fe, Na, Al, Cd, Cr, Co, Mo, Ni, Pb, and Cl\(^{-}\)) in the leaves, stems, and roots. Biomass, height, diameter, leaf area, and root architecture also will be tested.

Given broad clonal variation in survival, height, and diameter in 2005 and similar results from our previous studies, we anticipate similar variation in soil-to-root transfer of elements and subsequent root-to-stem and root-to-leaf translocation. We will present 2006 results at the conference. Nevertheless, our results to date have supported the need for further testing and selecting of specific clones for various phytoremediation needs.
ACKNOWLEDGEMENTS

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