Productivity and biometry of hybrid poplars with respect to establishment, regeneration, regional modeling, and utilization of bio-energy byproducts

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Productivity and biometry of hybrid poplars with respect to establishment, regeneration, regional modeling, and utilization of bio-energy byproducts

by

William Landon Headlee

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Forestry

Program of Study Committee:
Richard B. Hall (Co-Major Professor)
Ronald S. Zalesny, Jr. (Co-Major Professor)
Philip M. Dixon
Steven E. Jungst
Kenneth J. Moore

Iowa State University
Ames, Iowa
2012

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DEDICATION

This dissertation is dedicated to my wife, Jen, for reminding me to enjoy life, and to my sons Teddy and Sawyer for showing me how; to my sisters Anora, Corale, Monica, and Donella for instructing me in the art of irreverence; and to my parents, Bill and Norma, for teaching me the importance of knowledge, hard work, and self-reliance.
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INTRODUCTION

Trees of the genus *Populus* (cottonwoods, aspens, and hybrids thereof; hereafter referred to as “poplars”) are among the most widely studied trees on the planet (Dickmann et al., 2001). Poplars are fast-growing and widely distributed, making them of particular interest for meeting society’s ever-growing demand for fiber and energy (Stettler et al., 1996). To this end, considerable time and effort have been invested in making genetic improvements to poplars in order to maximize their productivity (Riemenschneider et al., 2001; Bisoffi and Gullberg, 1996), and regional networks of trials have been established to test promising hybrids in an array of environments (Netzer et al., 2002; Zalesny et al., 2009). Despite this massive knowledge-base, the growing demand for fiber and energy, and the investments made in genetic improvements and testing, barriers remain for the widespread deployment of poplars.

Some of the barriers relate to landowner preferences; such as, many landowners are accustomed to growing annual crops and are not willing to wait for the length of a poplar rotation (typically 10 years in the North Central United States) to harvest a crop, even though in some cases the total amount of biomass produced by poplars may exceed that of row crops (Thelemann et al., 2010). Other barriers stem from gaps in the current knowledge: for example, little has been published about how best to manage the abundant root sprouts that follow the harvest of mature hybrid aspen, and researchers are limited in their ability to quantitatively predict the productivity of lands not currently growing poplars. Still other barriers relate to industrial-scale economics: value-added uses for the byproducts of bio-
energy production are needed to improve the economic feasibility of the industry, similar to the benefits realized by the ethanol industry from marketing distillers’ grains as livestock feed.

Though much is already known about poplars, clearly there is much more work to be done. The challenges described above, which are the subject of the remainder of this dissertation, are but a small sample. Poplars offer many benefits to society in addition to their sheer productivity, such as improving water quality when used in riparian buffer strips (Schultz et al., 2004), providing habitat for certain wildlife species (Moser and Hilpp, 2003; Giordana and Meriggi, 2009), mitigating climate change via sequestration of carbon in the soil (Marquez et al., 1999), and providing remediation of contaminated lands (James et al., 2009; Zalesny and Bauer, 2007). Further study is warranted for each of these subjects, as well as the subject of assigning economic values to the many environmental and social benefits that poplars can provide for society (Updegraff et al., 2004).

OBJECTIVES

Five studies were conducted to address some of the gaps in our current knowledge of poplar production described in the preceding section. The first was a study of an alleycropping system in which winter triticale was grown as a source of early income between rows of establishing hybrid aspen trees, with the objective of evaluating whether the productivity of the system would be affected by topographic position and fertilizer rate. The objectives of the second study were to quantify the regeneration of hybrid aspen arising from roots sprouts following the harvest of a mature plantation, and to devise an equation to aid in the thinning of root sprouts to a density suitable for another short rotation. The objectives of
the third study were to use previously-published productivity data to calibrate and validate a tree growth model for hybrid poplars in the North Central U.S., and to use the validated model to map predicted yields for the region. The objective of the fourth study was to evaluate the feasibility of using fly ash (produced by a biomass boiler at an ethanol facility) as a foliar fertilizer for hybrid aspen in both greenhouse and field settings. The objective of the fifth study was to evaluate the feasibility of using biochar (produced by a fast-pyrolysis bio-oil reactor) as a substitute for vermiculite to grow hybrid poplar in the greenhouse.

**DISSERTATION ORGANIZATION**

This dissertation consists of five research chapters organized in journal format, along with an introduction to the research (Chapter 1) and a summary of the results (Chapter 7). One research chapter is devoted to each of the five studies described in the preceding section, and each chapter is formatted for the journal to which it has been (or will be) submitted. Chapter 2 is titled “Early growth of hybrid aspen ‘Crandon’ in an alleycropping system, as influenced by topographic position and fertilizer rate” and will be submitted for publication to *Agroforestry Systems*. Chapter 3 is titled “Methods to inventory, estimate biomass, and thin in high-density stands of hybrid aspen root sprouts” and will be submitted to *Northern Journal of Applied Forestry*. Chapter 4 is titled “Using a process-based model (3-PG) to predict and map hybrid poplar biomass productivity in Minnesota and Wisconsin, USA” and has been submitted to *BioEnergy Research*. Chapter 5 is titled “Biomass fly ash as foliar fertilizer for hybrid aspen trees: nutrient uptake, growth response, and compatibility with nitrogen fertilizer” and has been submitted to *Journal of Plant Nutrition*. Chapter 6 is titled
“Biochar as a substitute for vermiculite in potting mix for hybrid poplar ‘NM6’” and will be submitted to *Plant and Soil*.

Authors listed on the research chapters include William L. Headlee, Richard B. Hall, Ronald S. Zalesny Jr., Deahn M. Donner, and Catherine E. Brewer. Mr. Headlee designed and implemented the experiments, collected and analyzed the data, and wrote the chapters. Drs. Hall and Zalesny provided oversight and input for the design, implementation, analysis, and writing of the research. Dr. Donner provided oversight and input for the mapping efforts described in Chapter 4, and Dr. Brewer provided experimental materials and input for the writing of Chapter 6.

**REFERENCES**


CHAPTER 2. EARLY GROWTH OF HYBRID ASPEN ‘CRANDON’ IN AN ALLEYCROPPING SYSTEM, AS INFLUENCED BY TOPOGRAPHIC POSITION AND FERTILIZER RATE

A paper to be submitted to Agroforestry Systems

William L. Headlee, Ronald S. Zalesny Jr., and Richard B. Hall

ABSTRACT

Hybrid poplars have demonstrated high productivity as short rotation woody crops (SRWCs) in the U.S. Midwest, and the hybrid aspen ‘Crandon’ (Populus alba L. × P. grandidenta Michx.) has exhibited particularly promising yields across topographic positions (including sloping marginal lands) in Iowa. However, a key obstacle for landowner acceptance of SRWCs is the lack of economic returns early in the rotation. Planting annual crops between the tree rows (alleycropping) has the potential to address this issue, especially with the use of winter triticale which completes its growth cycle early in the summer and therefore is expected to have minimal competitive interaction with the establishing trees. In addition, well-placed fertilizer in low rates at planting has the potential to improve tree establishment and shorten the rotation, which is also economically desirable. To test the potential productivity of ‘Crandon’ in this alleycropping system under a variety of conditions, plots were established on five different topographic positions (floodplain, toe slope, back slope, shoulder slope, and summit) with four different rates (0, 10, 20, and 40 g tree⁻¹) of 20-10-5 NPK fertilizer tablets placed in the planting hole. Trees were then
harvested from the plots after each of the first three growing seasons. Analysis of total aboveground dry biomass productivity showed topographic position to be significant only for the floodplain in the first year, whereas fertilization resulted in significant increases in biomass across all three years. The highest fertilizer rate produced approximately twice as much biomass as the lowest (no fertilizer) rate.

INTRODUCTION

Using baseline scenarios, Perlack et al. (2011) estimated that forestlands in the contiguous United States have the capability to produce 298 million dry Mg of biomass annually by the year 2030. Likewise, their baseline estimate for perennial crops (woody and herbaceous) on agricultural lands was 346 million dry Mg of biomass annually, with estimates for high-yield scenarios reaching 705 million dry Mg annually. Production from both land cover types will be vital to meet the nation’s demands for biofuels, bioenergy, and bioproducts. For example, adequate woody feedstock supply is necessary for achieving our national goal of 16 billion gallons of cellulosic biofuels by 2022, established under the U.S. Energy Independence and Security Act of 2007. Short rotation woody crops (SRWCs) are purpose-grown trees that are a vital component of this potential woody biomass supply. These trees are environmentally and economically sustainable and highly productive. In fact, biomass yields of up to 10 Mg ha\(^{-1}\) yr\(^{-1}\) are common in the Midwest and those approaching 20 Mg ha\(^{-1}\) yr\(^{-1}\) are attainable when growing adapted genotypes at sites with optimal climatic and environmental conditions (Netzer et al. 2002, Zalesny et al. 2009). The hybrid aspen ‘Crandon’ (\textit{Populus alba} L. \(\times\) \textit{P. grandidenta} Michx.) appears particularly promising, both for its high productivity and its adaptability to marginal lands (Goerndt and Mize 2008).
In addition, SRWCs can be grown in conjunction with row crops in the form of alleycropping systems. These systems of alternating strips of trees and annual crops provide income early in the rotation as the trees establish; the lack of such early returns is considered a major obstacle to adoption of SRWC in the region. In addition, alleycropping systems have been shown to provide numerous benefits in temperate regions including diversification of crops, erosion control, more efficient nutrient cycling, improved water quality, greater soil carbon sequestration, and higher productivity when tree and row crops are properly matched to minimize competition with one another (Tsonkova et al. 2012). Hybrid poplars have been used in alleycropping systems with corn (Reynolds et al. 2007), soybeans (Manceur et al. 2009, Reynolds et al. 2007, Rivest et al. 2009), canola (Beaudette et al. 2010), and various other crops (Delate et al. 2005, Rivest et al. 2009) with mixed success. For hybrid poplars, which experience peak growth during mid- to late-growing-season (Devine et al. 2010), it is logical that winter triticale (Triticum spp. × Secale spp.) which completes its growth cycle by mid-season would be a better match in alleycropping systems than crops with similar peak-growth periods as hybrid poplars (such as corn). Winter triticale has proven to be productive in double-cropping systems with corn (Hegenstaller et al. 2008, Jemison et al. 2012) and sorghum (Goff et al. 2010), but the literature appears to lack any studies of winter triticale alleycropped with hybrid poplars.

Shortening rotations via fertilization may also improve the economics of SRWCs. Broadcast fertilization at agronomic rates around the time of canopy closure (typically at mid-rotation) may substantially increase biomass growth (Coleman et al. 2006); however, such fertilizer applications are not considered to be practical early in the rotation as the trees have not yet fully occupied the site and therefore take up little of the applied nutrients.
Alternatively, much lower rates of well-placed fertilizer have been shown to increase the early growth of hybrid poplars (van den Driessche 1999, Guillemette and DesRochers 2008).

In this study, an alleycropping system consisting of winter triticale between rows of the hybrid aspen ‘Crandon’ was established at multiple topographic positions with various low rates of fertilizer placed in the planting hole. The trees were then harvested after each of the first three growing seasons to determine the effects of topographic position, fertilizer rate, and age on total (stem + branch) aboveground dry biomass, as well as the fraction of biomass allocated to branches (branch / total dry biomass). Biomass allocation is of interest because more and/or larger branches may reduce the value of the trees for non-bioenergy (e.g. lumber) markets.

MATERIALS AND METHODS

Tree Materials

The trees used in this study were established in the greenhouse during spring 2009 using 10 cm long dormant cuttings grown in 236 cm³ Accelerator® containers (Nursery Supplies Inc., Chambersburg, PA). They were continuously sub-irrigated until reaching a height of approximately 10 cm, after which they were watered twice daily with an automated overhead sprinkler system. They were trimmed to a height of approximately 20 cm before being placed outdoors to harden off for two weeks prior to planting.

Study Site and Experimental Design

The study site is located approximately 20 km southwest of Ames, IA, on an east-facing hillside (ranging in elevation from 305 to 325 m above sea level) adjacent to Big
Creek. Soil surveys indicate that the floodplain (previously in mixed grass) consists of Coland clay loam, whereas the rest of the study area (previously in row crops) consists primarily of Clarion loam. Plots were established at each of five topographic positions (floodplain, toe slope, back slope, shoulder slope, and summit), with one plot in each of three blocks, for a total of 15 plots. Within each plot, two sets of trees were planted: 48 trees spaced at 3.0 × 3.7 m for long-term evaluation of growth and environmental impacts relative to other perennial and annual biomass cropping systems, and 24 trees placed at half-spacings (1.5 m × 3.7 m; see Figure 1) which were harvested over the first three years and are the subject of this paper. These short-term trees were randomly assigned to one of the three harvest years and one of four fertilizer rates (0, 10, 20, or 40 g tree⁻¹ of 20-10-5 NPK tablets; Henry Field’s Seed and Nursery Co., Aurora, IN), with two trees planted for each combination of fertilizer rate and harvest year. Thus, the total number of trees in the short-term study was 360 (5 topographic positions × 3 plots × 4 fertilizer rates × 3 years × 2 trees yr⁻¹). For a description of the long-term study, and the additional biomass cropping systems evaluated therein, see Welsh (2012).

Site Preparation and Planting

Plots were tilled and planted to winter triticale in fall of 2008, and tree rows were prepared by spraying glyphosate herbicide in 1 m wide swaths prior to planting in spring of 2009. Trees were planted into the strip-killed triticale starting in late May, using a tractor-mounted auger (20 cm diameter) to dig the planting holes. Fertilizer tablets were placed at a distance of approximately 10 cm from the trees, and at depth of approximately 10 cm below the ground. The triticale was harvested from the plots in early July, and was similarly grown
in the alleys between the tree rows in the following two years (planted with a no-till drill in the fall and harvested in July). Fertilizer (30 kg ha\textsuperscript{-1} N as urea) was broadcast in the alleys of triticale each spring. Glyphosate was applied to the plots twice during the first growing season; once in mid-summer using wick applicators in the immediate vicinity (~0.5 m radius) of the trees, and once in early-fall using a shielded backpack sprayer for spot-treatment (primarily on the floodplain where weed pressure was heavy). Trees were harvested during the dormant season following each of the first three growing seasons. Harvestable biomass (aboveground biomass excluding 10 cm tall stump) was separated into components (stem and branch) and oven-dried at 100°C until stable, at which time dry weights were recorded.

**Data Analyses**

The experiment was analyzed as a split-plot design. Topographic position was the main plot effect, with blocks as fixed effects (randomized complete block design). The split-plot effects included the two-way factorial of age and fertilizer (completely randomized design). Analyses of variance (ANOVA) were conducted using PROC MIXED (method=type3) in SAS (SAS Institute Inc., Cary, NC) for total aboveground (stem + branch) biomass and for branch fraction of biomass (branch / total biomass). Total aboveground dry biomass was log-transformed prior to analysis due to the variance being proportional to the mean, which increased substantially (almost two orders of magnitude) from the first year to the third year. Denominator degrees of freedom were determined via the Kenwood-Rogers method, and significant treatment effects were further evaluated using multiple comparisons analysis with Tukey adjustment (SAS Institute Inc. 2004). In addition, linear regression was
conducted using PROC GLM in SAS, with a focus on using the treatment factors identified as significant by ANOVA to predict total aboveground dry biomass and branch fraction.

RESULTS

The ANOVA results indicated there were significant (P < 0.05) age and fertilizer effects both for total aboveground dry biomass and for branch fraction of biomass (Table 1). In addition, there were significant block and position × age effects for total aboveground dry biomass. There was no evidence of significant effects for any of the remaining interactions.

Multiple comparisons analysis showed that block 3 had significantly lower total aboveground dry biomass than the other two blocks (Figure 2). Both total aboveground dry biomass and branch fraction of biomass increased significantly with age (Figure 3). For the fertilizer effects, both total aboveground dry biomass and branch fraction of biomass were significantly higher at 20 and 40 g tree⁻¹ than at 0 g tree⁻¹, with 10 g tree⁻¹ being intermediate (Figure 4). To evaluate the position × age interaction (Figure 5) the data was sliced by age, which revealed that topographic position had a significant (P = 0.0034) effect on aboveground biomass at age 1 but not at age 2 (P = 0.26) or age 3 (P = 0.27). Multiple comparisons for topographic position at age 1 showed that the floodplain had less aboveground biomass than the other four positions (P = 0.04 to 0.09), whereas the other four positions did not differ significantly from one another (P > 0.99).

Because the effects of the position × age interaction on aboveground biomass were limited to the floodplain, and this position experienced unusually high weed pressure, it was dropped from the linear regression analysis to allow for a simpler model for the remaining
positions based on fertilizer rate and age. Using this model, a somewhat strong relationship 
\( R^2 = 0.71 \) was observed, with the resulting equation of:

\[
\ln(DW) = 1.37 + 0.0118F + 1.72A 
\] (1a)

where \( DW \) is total aboveground dry biomass (g tree\(^{-1}\)), \( F \) is fertilizer rate (g tree\(^{-1}\)), and \( A \) is tree age (yrs). For practicality this can be rewritten as:

\[
DW = e^{1.37+0.0118F+1.72A} 
\] (1b)

For the branch fraction of total biomass, linear regression demonstrated a weak relationship with age and fertilizer rate \( R^2 = 0.21 \). A slightly stronger relationship \( R^2 = 0.36 \) was found with total aboveground dry biomass, with the resulting equation of:

\[
B_f = 0.183 + 0.0284 \ln(DW) \] (2)

where \( B_f \) is branch fraction (unitless) and \( DW \) is total aboveground dry biomass (g tree\(^{-1}\)).

**DISCUSSION**

The results demonstrate that the hybrid aspen ‘Crandon’ produced similar amounts of aboveground dry biomass across topographic positions, with the exception of the floodplain in the first year, where weed pressure was observed to be high. While the use of fixed blocks precludes any inference beyond this site, previous research in Iowa similarly found that
‘Crandon’ produced more consistent (and often higher) yields on upland, sloping, and bottomland sites relative to hybrid cottonwood ‘Eugenei’ (*P. deltoides × P. nigra*) and silver maple (*Acer saccharinum*) (Goerndt and Mize 2008). Thus, the virtual lack of significant differences among topographic positions is likely due in part to the versatility of ‘Crandon’; however, the reduced statistical power for detecting main plot effects in split-plot designs also may have been a contributing factor.

The trees growing on the floodplain had twice as much individual biomass by the third year as those growing on the summit and shoulder slope; however, the trees’ survival rate was not as high on the floodplain (Table 2). Thus, the productivity advantage of the floodplain was slightly reduced at the stand scale. The lower survival rate (as well as the low initial biomass productivity) on the floodplain appeared to be attributable to greater weed competition, which in turn was likely due to greater water (and possibly nutrient) availability and a large seed bank built up under the previous land cover of mixed grasses. The yields in Table 2 are low compared to Goerndt and Mize (2008) who reported productivity for three-year-old ‘Crandon’ of 1.8, 1.3, and 5.9 Mg ha⁻¹ yr⁻¹ on upland, sloping, and bottomland topography, respectively; however, it is unclear whether this reduction in productivity was due to the use of smaller planting stock in this study, poor growing conditions, negative interactions with the triticale, or a combination thereof.

The low biomass productivity associated with block 3 (see Figure 2) is likely attributable to deer damage (i.e. browsing of growing points, and rubbing of stems during the rutting season). This block was located at the north end of the study site where deer and their tracks were frequently observed. The physical condition of the trees was surveyed after the first growing season, and it was found that approximately 13% of the trees in block 3 had
been damaged by deer, as compared to rates of 7% and 8% for blocks 1 and 2, respectively. By the end of the second growing season, the trees had grown sufficiently tall (mean height = 2.2 m) that the tops were effectively out of reach of browsing. However, damage from deer rub continued throughout the three years in all plots and resulted in girdling of stems, which undoubtedly reduced biomass productivity through stem dieback and breakage (following which the trees re-sprouted from the lower stems).

The results also suggest that small amounts of well-placed fertilizer at planting can significantly improve early productivity (see Figure 4a). Total aboveground dry biomass at age 3 for the trees receiving the highest fertilizer rate was almost twice that of the trees receiving no fertilizer (Table 3). This fertilizer response was similar to Guillemette and DesRochers (2008), who reported first- and second-year increases in stem volume of approximately 20 to 70% for hybrid poplars supplied with 20 to 25 g tree\(^{-1}\) of fertilizer at planting (placed underground approximately 15 cm from the trees) at former farmland sites. Furthermore, fertilizer rate and age were found to be relatively strong predictors of total aboveground dry biomass (R\(^2\) = 0.71; Equation 1a).

The results also show that the allocation of biomass to the branches was significantly higher when fertilizer was used than when no fertilizer was used (0 g tree\(^{-1}\)), though the various rates of fertilizer used (10, 20, or 40 g tree\(^{-1}\)) did not differ significantly from one another in this regard (see Figure 4b). Similarly, branch fraction increased significantly with age (see Figure 3b); however, total aboveground dry biomass was found to be a better predictor of branch fraction for the development of Equation 2. This is consistent with Coyle and Coleman (2005), who demonstrated that differences in biomass allocation for two P.
 deltoides clones were the result of developmental (i.e. size) differences rather than environmental differences (i.e. nutrient or water availability).

In summary, the results of this study reinforce previous research indicating that ‘Crandon’ is an adaptable clone for a variety of topographic positions. In addition, low rates of fertilizer at planting can nearly double ‘Crandon’ productivity during the first three years after establishment. Further research should be done to determine whether these early gains in aboveground biomass associated with early fertilization can be maintained through a full rotation, as well as to evaluate whether triticale may have any negative (e.g. competitive or allelopathic) effects on tree productivity. The branch fraction of biomass increased significantly with fertilizer rate as well as with age, but was more strongly correlated with tree size (i.e. total aboveground dry biomass). Finally, the reduced productivities observed for block 3 and for the floodplain in the first year highlight the importance of controlling deer damage and weed competition, respectively, in hybrid poplar production systems.

ACKNOWLEDGEMENTS

Support and funding for this project have been provided by ISU College of Agriculture and Life Sciences, Leopold Center for Sustainable Agriculture, USDA Agriculture and Food Research Initiative, and US Forest Service Institute for Applied Ecosystem Studies. A portion of the long-term study trees were provided by ArborGen, Inc. (Ridgeville, SC).
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Coyle, DR, Coleman, MD (2005) Forest production responses to irrigation and fertilization are not explained by shifts in allocation. Forest Ecology and Management 208: 137-152


Table 1. P-values from ANOVA of total aboveground dry biomass (DW) and branch fraction (Bf) for the hybrid aspen ‘Crandon’. Significant differences (P < 0.05) are depicted in bold.

<table>
<thead>
<tr>
<th>Effects</th>
<th>DW</th>
<th>Bf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>0.0039</td>
<td>0.1515</td>
</tr>
<tr>
<td>Position</td>
<td>0.7228</td>
<td>0.3389</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>&lt;0.0001</td>
<td>0.0111</td>
</tr>
<tr>
<td>Age × Fertilizer</td>
<td>0.2508</td>
<td>0.7583</td>
</tr>
<tr>
<td>Position × Age</td>
<td>0.0005</td>
<td>0.1445</td>
</tr>
<tr>
<td>Position × Fertilizer</td>
<td>0.7283</td>
<td>0.9933</td>
</tr>
<tr>
<td>Position × Age × Fertilizer</td>
<td>0.6687</td>
<td>0.9477</td>
</tr>
</tbody>
</table>

Table 2. Biomass productivity of ‘Crandon’ after three years on a per-tree basis (not adjusted for survival) and on a per-hectare basis (adjusted for survival), by topographic position.

<table>
<thead>
<tr>
<th>Topographic Position</th>
<th>Tree Biomass (kg tree⁻¹)</th>
<th>Tree Survival (%)</th>
<th>Stand Biomass (Mg ha⁻¹)</th>
<th>Annual Biomass (Mg ha⁻¹ yr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floodplain</td>
<td>1.78</td>
<td>86</td>
<td>2.76</td>
<td>0.92</td>
</tr>
<tr>
<td>Toe Slope</td>
<td>1.32</td>
<td>94</td>
<td>2.22</td>
<td>0.74</td>
</tr>
<tr>
<td>Back Slope</td>
<td>1.02</td>
<td>97</td>
<td>1.78</td>
<td>0.59</td>
</tr>
<tr>
<td>Shoulder Slope</td>
<td>0.89</td>
<td>91</td>
<td>1.45</td>
<td>0.48</td>
</tr>
<tr>
<td>Summit</td>
<td>0.87</td>
<td>93</td>
<td>1.46</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Table 3. Biomass productivity of ‘Crandon’ after three years on a per-tree basis and on a per-hectare basis, by fertilizer rate.

<table>
<thead>
<tr>
<th>Fertilizer Rate (g tree⁻¹)</th>
<th>Tree Biomass (kg tree⁻¹)</th>
<th>Stand Biomass (Mg ha⁻¹)</th>
<th>Annual Biomass (Mg ha⁻¹ yr⁻¹)</th>
</tr>
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<td>1.55</td>
<td>0.52</td>
</tr>
<tr>
<td>10</td>
<td>1.03</td>
<td>1.86</td>
<td>0.62</td>
</tr>
<tr>
<td>20</td>
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<td>2.26</td>
<td>0.75</td>
</tr>
<tr>
<td>40</td>
<td>1.55</td>
<td>2.79</td>
<td>0.93</td>
</tr>
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</table>
Figure 1. Plot layout of long-term study trees, short-term study trees, and winter triticale as viewed from above.
Figure 2. Least squares means of log-transformed total aboveground dry biomass by block. Error bars represent +/- 1 standard error of the mean. Statistically significant differences are denoted by different letters above the bars.
Figure 3. Least squares means by age for (a) log-transformed total aboveground dry biomass and (b) branch fraction of biomass. Error bars represent +/- 1 standard error of the mean. Statistically significant differences are denoted by different letters above the bars.
Figure 4. Least squares means by fertilizer rate for (a) log-transformed total aboveground dry biomass and (b) branch fraction of biomass. Error bars represent +/- 1 standard error of the mean. Statistically significant differences are denoted by different letters above the bars.
Figure 5. Least squares means of log-transformed total aboveground dry biomass, by age for each topographic position: floodplain (+), toe slope (◊), back slope (Δ), shoulder slope (○), and summit (□).
CHAPTER 3. METHODS TO INVENTORY, ESTIMATE BIOMASS, AND THIN IN HIGH-DENSITY STANDS OF HYBRID ASPEN ROOT SPROUTS

A paper to be submitted to *Northern Journal of Applied Forestry*

William L. Headlee and Richard B. Hall

**ABSTRACT**

Hybrid aspen are highly productive trees across a wide range of conditions, and are distinct among hybrid poplars in their ability to produce copious vegetative regeneration from root sprouts following the harvest of older trees. This makes hybrid aspen desirable for bio-energy plantations, as re-planting costs may be avoided and early returns are possible from harvesting or thinning the densely regenerating stands. Row thinning has been proposed as a method for capturing root sprout biomass that would be lost to mortality early in the rotation. Using large machinery to row thin is a fast and efficient approach, but determining an appropriate width for the unharvested rows (as well as expected yields) is problematic. This is due in part to the uneven spacing of the root sprouts, as well as the difficulty in conducting inventories with traditional fixed-area sampling methods in densely-regenerating stands. In this study, the use of variable-radius plot sampling was evaluated for 1-year-old hybrid aspen root sprouts. From this inventory data, stand density (sprouts ha\(^{-1}\)) and harvestable dry biomass (Mg ha\(^{-1}\)) were estimated. Finally, an equation was developed to facilitate row thinning by estimating the appropriate width for the unharvested rows of root sprouts, based on the desired size of the largest gap in the row.
INTRODUCTION

Hybrid aspen are highly productive trees across a wide range of conditions (Goerndt and Mize 2008; Zalesny et al. 2009), and are distinct among hybrid poplars in their ability to produce copious vegetative regeneration from root sprouts following the harvest of mature trees. While most hybrid poplars (including hybrid aspen) produce sprouts from the cut stumps, these large wounds are easily infected by pathogens. This often results in significant stump sprout mortality within a few years after harvest of the mature trees, either directly from infection or indirectly from the sprouts breaking off of the rotting stumps. As a result, root sprouts are considered a more viable source of regeneration than stump sprouts for typical short-rotation production. It is this ability to provide a reliable source of regeneration, which in turn offers the opportunity to avoid re-planting costs and to derive early returns from thinning the sprouts, which makes hybrid aspen highly desirable for bio-energy plantations.

Row thinning provides a method for capturing biomass that would otherwise be lost to mortality early in the rotation (Rytter 2006), and can also improve the individual growth of the residual trees (Gilmore 2003). Using large machinery to row thin is a fast and efficient approach (Christian et al. 1996), but determining an appropriate width for the unharvested rows is problematic. Unlike the original plantation, the root sprouts are not evenly spaced, resulting in potentially large variation in the distances between sprouts within the unharvested row. However, based on the desired size of the root sprouts at the end of their rotation, in combination with the self-thinning rule which dictates the maximum stand density possible for a given tree size (Reineke 1933), it follows that the largest gap in the
unharvested rows should be maintained equal to the spacing suggested by the self-thinning rule for the desired tree size. Such an approach would avoid understocking (i.e. unoccupied growing space) at the end of the rotation, and optimize row thinning yields by avoiding overstocking (i.e. wider-than-necessary rows of unharvested sprouts).

Thus, an equation which predicts the appropriate width of the unharvested row based on the desired maximum gap size within the row is desirable. The utility of such an equation is, however, dependent upon a reliable method to inventory root sprouts density and to estimate the yields which can be expected from thinning operations. Due to high stand density (>100,000 sprouts ha\(^{-1}\)) and potentially large size (up to 3 m tall) of 1-year-old root sprouts, traditional fixed-area sampling methods developed for seedling regeneration are cumbersome and time-consuming. Variable-radius sampling (also known as the Bitterlich method), in which measurement trees are selected based on their size relative to their distance from the sample point, is widely used to inventory mature trees and produces similar estimates of stocking in considerably less time than fixed-area sampling (Avery and Burkhart 2002). In conjunction with limited destructive sampling, the inventory data may also be useful in estimating harvestable biomass, which in turn can better inform row thinning operations.

In this study, the practical application of variable-radius sampling to inventory hybrid aspen root sprouts was tested. Root sprouts were also harvested to determine the relationship between sprout diameter and biomass, so that yields could be predicted from diameter measurements collected during the inventory. Finally, a row thinning equation was developed and tested for the purposes of estimating the appropriate row width for
unharvested root sprouts, based on the maximum desired gap in the row and the density of root sprouts as determined by inventory.

MATERIALS AND METHODS

Inventory Methods

Hybrid aspen root sprouts were inventoried while dormant in spring 2009 using a modified version of the Bitterlich method of variable-radius plot sampling. Whereas the Bitterlich method typically selects trees for measurement based on distance from the sample point and diameter at breast height (dbh; breast height = 1.37 m), many of the root sprouts were shorter than breast height; therefore, diameter at harvest height (the height above the ground which is considered harvestable by large machinery [10 cm]; hereafter referred to as dhh) was used instead. The basal area factor (BAF) used for sampling was 1.56 m² ha⁻¹, as this BAF conveniently dictates that trees selected for measurement must fall within a distance (cm) equal to 4 times the dhh (mm) of the tree. This is equivalent to a 40:1 ratio when the distance and diameter units are the same.

The study site consisted of a stand of 1-year-old root sprouts near Ames, IA. To randomly assign sample points to the study area, hypothetical grids were drawn centered around 27 randomly selected stumps (staggered rows spaced at 1.5 × 1.7 m; for more information, see Green 1998) which remained from the harvest of the mature plantation conducted during the previous dormant season. Sample point coordinates (x,y) were then randomly assigned to the 1.7 m east-west (x) axis and the 1.5 m north-south (y) axis. From these coordinates, the azimuth and distance from the center of the stump were calculated for use in the field.
In the field, a compass and measuring tape were used to locate each sample point based on the previously-determined azimuth and distance from the center of the stump. The sample point was temporarily marked using a steel rod with a measuring tape affixed at 10 cm above the ground. This measuring tape was used to determine the distance from the plot center to potential measurement trees, and a hand-held digital caliper was used to determine which of these trees were of appropriate dhh to be included as measurement trees. For each of the measurement trees (n = 165), the distance from plot center and dhh were recorded. In addition, approximately one-third of the measurement trees were harvested for determination of biomass (n = 54). The harvested trees were cut at dhh and oven-dried at 100°C until stable, at which time dry weights were recorded.

Stand Density Estimates

After returning from the field, estimates of stand density were generated for each sample point (n=27) using the equation:

\[ D_p = \sum \left( \frac{BAF}{BA_i} \right) \]  

where \( D_p \) is the estimate of stand density (sprouts ha\(^{-1}\)) for a given sample point, BAF is the basal area factor (m\(^2\) ha\(^{-1}\)), and BA is the basal area (m\(^2\)) for the measurement tree \( i \).

To gauge whether other BAFs might produce different stand density estimates and/or be more efficient (i.e. produce similar estimates with fewer measurement trees), the distance and dhh of the measurement trees were also used to estimate stand density with BAFs of 2.78
(distance [cm]: dhh [mm] ≤ 3) and 6.22 (distance [cm]: dhh [mm] ≤ 2) for each sample point. Analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute Inc., Cary, NC) was used to test for differences among BAFs in estimated stand density. Sampling efficiency was evaluated by comparing the estimated mean stand density, 95% confidence interval, and number of measurement trees for each of the BAFs.

**Biomass Estimates**

The dry weight data were used to determine the relationship between dhh and dry biomass of the sprouts via linear regression. A strong ($R^2 = 0.986$) log-linear relationship was observed (Figure 1), and is described by the equation:

$$DW = 0.065(dhh)^{2.60}$$

where $DW$ is dry biomass (g sprout$^{-1}$) and dhh is diameter at harvestable height (mm). This equation was then used to produce biomass estimates for each sample point as follows:

$$DW_p = \sum(DW_i \times D_i)$$

where $DW_p$ is the estimate of biomass (Mg ha$^{-1}$) for a given sample point, $DW_i$ is the predicted biomass (Mg sprout$^{-1}$) for the measurement tree $i$, and $D_i$ is the estimate of stand density (sprouts ha$^{-1}$) for measurement tree $i$.

As with stand density, alternative BAFs of 2.78 and 6.22 were evaluated to test for differences in dry biomass estimates and sampling efficiency. Analysis of variance
(ANOVA) was used to test for differences among BAFs in estimated dry biomass. Sampling efficiency was evaluated by comparing the estimated mean dry biomass, 95% confidence interval, and number of measurement trees for each of the BAFs.

Row Thinning Equation

Two main assumptions were made in developing the row thinning equation. First, it was assumed that the spatial distribution of mature aspen roots (and thereby the sprouts that arise from them) can reasonably be described as random. Second, it was assumed that the width of an unharvested row of root sprouts would be small relative to the length of the row. Under these two assumptions, the distances between sprouts in the unharvested row are analogous to the distances between randomly distributed points on a line. Thus, the first step in developing a row thinning equation was to establish the mathematical relationship between the number of randomly distributed points on a line and the maximum distance between those points.

To do this, a dataset simulating 6 lines having 4, 8, 16, 32, 64, or 128 points per line (for a total of 252 points per dataset) was created. This was replicated by creating 15 such datasets (for a total of 90 lines and 3,780 points). Using a random number generator, each point was assigned a value between 0 and 1, which was treated as the location of that point along a line having a length of 1 unit. Next the points on each line were sorted from least to greatest, and the distances (or gap size) between adjacent points were determined. The largest gap in each line was then identified (90 total gaps) and used to evaluate the relationship between maximum gap size and the number of points on the line. A strong ($R^2 = 0.91$) log-linear relationship (Figure 2) was observed for the relationship between the proportion of the
line occupied by the largest gap ($G_{prop}$) and the number of points ($N$) on the line, as described by the equation:

$$G_{prop} = 1.18N^{-0.67}$$  \hspace{1cm} (4)

To test the validity of this relationship, two rows having lengths of 50 m and widths of 0.3 m were established at the study site. The dhh and distance of each root sprout from the start of the row was measured, from which the size of the gaps between sprouts was calculated. In order to test across a wide range of maximum gap sizes, sprouts were randomly dropped from each row, the gaps were recalculated after each dropped sprout, and the largest gaps were compared to the values predicted by Equation 4. For each of the two rows, the randomization and subsequent dropping of sprouts was replicated three times, resulting in six total runs of the simulation.

**RESULTS**

**Stand Density Estimates**

The ANOVA results showed no significant effects of BAF on estimated stand density ($P = 0.45$; not shown). The differences among BAFs may nonetheless have important impacts on factors related to sampling efficiency (Table 1). With a BAF of 1.56, the density of the overall stand was estimated to be approximately 193,000 sprouts ha$^{-1}$, with 95% confidence interval (CI) of 134,000 to 252,000 sprouts ha$^{-1}$. A BAF of 2.78 produced a similar estimate of stand density (207,000 sprouts ha$^{-1}$) using 44% fewer measurement trees, but also resulted in a larger 95% CI (118,000 to 296,000 sprouts ha$^{-1}$). A BAF of 6.22
resulted in 77% fewer measurement trees and produced a lower estimate of stand density (141,000 sprouts ha\(^{-1}\)), as well as a relatively large 95% CI (57,000 to 226,000 sprouts ha\(^{-1}\)).

**Biomass Estimates**

The ANOVA results showed no significant effects of BAF on estimated dry biomass (\(P = 0.94\); not shown). However, as with stand density, factors important to sampling efficiency were affected by the BAFs. With a BAF of 1.56, the dry biomass of the overall stand was estimated to be approximately 3.35 Mg ha\(^{-1}\), with 95% CI of 2.70 to 4.00 Mg ha\(^{-1}\) (Table 2). Applying a BAF of 2.78 resulted in a similar biomass estimate (3.25 Mg ha\(^{-1}\)) using 44% fewer measurement trees, but a larger 95% CI (2.36 to 4.13 Mg ha\(^{-1}\)). Likewise, a BAF of 6.22 resulted in a similar biomass estimate (3.14 Mg ha\(^{-1}\)) using 77% fewer measurement trees, but with an even larger 95% CI (1.96 to 4.32 Mg ha\(^{-1}\)).

**Row Thinning Equation**

The results of the row thinning test (Figure 3) show a strong correlation (\(R^2 = 0.84\)) between actual maximum gap sizes and those predicted by Equation 4. To facilitate its use in the field, however, Equation 4 requires translation into more practical terms. Specifically, in practice \(G_{\text{prop}}\) is equivalent to the length of the maximum desired gap divided by the length of the unharvested row, and \(N\) is the product of the row width, length, and root sprout density. Substituting these terms in Equation 4 and solving for row width therefore gives:

\[
W = \frac{L^{0.5}}{0.78 \times D \times G_{\text{max}}^{1.5}}
\]
where \( W \) is the row width (m), \( L \) is the row length (m), \( G_{\text{max}} \) is the length of the maximum desired gap (m), and \( D \) is the stand density (sprouts \( \text{m}^{-2} \)).

**DISCUSSION**

The results of this study suggest that variable-radius plot sampling is a feasible approach for inventorying dense stands of hybrid aspen root sprouts. Sampling with a BAF of 1.56 \( \text{m}^2 \text{ ha}^{-1} \) required less time (\( \leq 5 \) minutes per plot) and provided similar estimates of stand density compared to fixed-plot sampling efforts in the same stand (Ruigu and Hall, unpublished data). Applying a BAF of 2.78 \( \text{m}^2 \text{ ha}^{-1} \) produced similar estimates of root sprout density from roughly half as many measurement trees (see Table 1); however, it also resulted in a larger confidence interval. Thus, the time potentially saved with this BAF must be weighed against the reduction in precision for stand density estimates. Applying a BAF of 6.22 \( \text{m}^2 \text{ ha}^{-1} \) further reduced the number of measurement trees, but resulted in both a larger confidence interval and a lower (though not statistically different) estimate of stand density. The lower estimate of stand density appears to be linked to a failure to detect small trees; only about 13% of the measurement trees had \( \text{dbh} < 5 \text{ mm} \) for this BAF, whereas about 26% of the measurement trees had \( \text{dbh} < 5 \text{ mm} \) for both of the other BAFs. Theoretically, this failure to detect small sprouts could be offset by adding more sample points, but doing so constitutes a reduction in efficiency and therefore is not desirable.

The results also suggest that \( \text{dbh} \) is a highly reliable predictor of root sprout biomass (see Figure 1). As such, harvesting a relatively small number of measurement trees (in conjunction with the inventory methods described here) can be considered an easy method to
estimate stand biomass. Furthermore, it appears that the inaccuracies in stand density
encountered with a BAF of 6.22 may not significantly affect stand biomass estimates (see
Table 2). However, the larger 95% CI encountered with this BAF demonstrates that a trade-
off exists between the number of measurement trees and the precision of the biomass
estimates, similar to that described for stand density.

Finally, our results suggest that the relationship observed between the number of
randomly distributed points on a line and the maximum gap size between the points (see
Figure 2) can be reasonably applied to row thinning of hybrid aspen roots sprouts. When
Equation 4 was tested against a wide range of functional stand densities, as simulated by
randomly dropping sprouts from the dataset, there was a strong correlation ($R^2 = 0.84$)
between actual and predicted values (see Figure 3). Thus, the relationship (as stated in its
practical form; see Equation 5) should be useful for determining the appropriate width of the
unharvested rows when planning row thinning operations. To this end, additional testing
under a wider variety of environmental, age, and stocking conditions is recommended.

ACKNOWLEDGEMENTS

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project.
REFERENCES


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Table 1. Estimates of stand density (sprouts ha⁻¹) using BAFs of 1.56, 2.78, and 6.22 m² ha⁻¹, along with the number of measurement trees (n), and the upper and lower limits of the 95% confidence interval. Based on 27 sample points.

<table>
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<th>Upper 95%</th>
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<td>165</td>
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<td>252,000</td>
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<td>2.78</td>
<td>93</td>
<td>207,000</td>
<td>118,000</td>
<td>296,000</td>
</tr>
<tr>
<td>6.22</td>
<td>38</td>
<td>141,000</td>
<td>57,000</td>
<td>226,000</td>
</tr>
</tbody>
</table>

Table 2. Estimates of dry biomass (Mg ha⁻¹) using BAFs of 1.56, 2.78, and 6.22 m² ha⁻¹, along with the number of measurement trees (n), and the upper and lower limits of the 95% confidence interval. Based on 27 sample points.

<table>
<thead>
<tr>
<th>BAF</th>
<th>n</th>
<th>Estimate</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
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<td>1.96</td>
<td>4.32</td>
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</tbody>
</table>
Figure 1. Relationship of root sprout dry biomass (g sprout\(^{-1}\)) to dh (mm), based on 54 measurement trees.

\[ y = 0.065x^{2.60} \]

\[ R^2 = 0.986 \]
Figure 2. Relationship between the proportion of the line occupied by the largest gap ($G_{prop}$) and the number of points in the line ($N$), based on random number simulation.

$G_{prop} = 1.18N^{0.67}$

$R^2 = 0.91$
Figure 3. Actual versus predicted values of maximum gap size (m), based on simulations with field data in which sprouts were randomly dropped from the row. Field data were collected from 2 rows, and 3 simulations were conducted for each row, for a total of 6 simulations. Dashed line represents perfect 1:1 relationship.
CHAPTER 4. USING A PROCESS-BASED MODEL (3-PG) TO PREDICT AND MAP HYBRID POPLAR BIOMASS PRODUCTIVITY IN MINNESOTA AND WISCONSIN, USA

A paper submitted to Bioenergy Research

William L. Headlee, Ronald S. Zalesny Jr., Deahn M. Donner, and Richard B. Hall

ABSTRACT

Hybrid poplars have demonstrated high biomass productivity in the North Central United States as short rotation woody crops (SRWCs). However, our ability to quantitatively predict productivity for sites which are not currently in SRWCs is limited. As a result, stakeholders are also limited in their ability to evaluate different areas within the region as potential supply sheds for wood-based bioenergy facilities. A reliable method for predicting productivity across the region is needed; preferably, such a method will also lend itself to generating yield maps that stakeholders can use to inform their decision-making. In this study, the Physiological Processes Predicting Growth (3-PG) model was (i) assigned parameters for hybrid poplars using species-specific physiological data and allometric relationships from previously-published studies, (ii) calibrated for the North Central region using previously-published biomass data from eight plantations along with site-specific climate and soils data, (iii) validated against previously-published biomass data from four other plantations using linear regression of actual versus predicted total aboveground dry biomass ($R^2 = 0.89$, RMSE = 8.1 Mg ha$^{-1}$, mean bias = 5.3 Mg ha$^{-1}$), (iv) evaluated for
sensitivity of the model to manipulation of the parameter for age at full canopy cover (fullCanAge) and the fertility rating (FR) growth modifier, and (v) combined with soil and climate data layers to produce a map of predicted biomass productivity for the states of Minnesota and Wisconsin. Mean annual biomass productivity (total aboveground dry biomass divided by age) ranged from 4.4 to 13.0 Mg ha$^{-1}$ yr$^{-1}$ across the states, with the highest productivity mainly concentrated in the area stretching from south-central Minnesota across southern Wisconsin.

**INTRODUCTION**

Short rotation woody crops (SRWCs) are purpose-grown trees that are an integral component of the United States’ potential woody biomass supply for bioenergy and biofuels; as such, the production of woody crops on agricultural lands (in addition to herbaceous perennials) is expected to expand to 346 million Mg of dry biomass annually by the year 2030 [39]. These trees are environmentally and economically sustainable and very productive. In fact, mean annual biomass productivity of up to 10 Mg ha$^{-1}$ yr$^{-1}$ are attainable on marginal lands [16] and those approaching 20 Mg ha$^{-1}$ yr$^{-1}$ are possible when growing adapted genotypes at sites with optimal climatic and environmental conditions [48, 55].

Trees belonging to four genera comprise the majority of SRWCs grown in the United States: *Populus* (cottonwoods, poplars, aspens, and hybrids thereof; hereafter referred to as hybrid poplars), *Salix* (willows), *Pinus* (pines), and *Eucalyptus* (eucalypts) [24, 56]. Among these options, intensively-grown hybrid poplars have gained substantial attention in the North Central region. Hybrid poplars are one of the most sustainable sources of biomass, and decades of research and development have resulted in production management systems that
support conservation of soil and water, recycling of soil nutrients, and preservation of genetic diversity [19]. Despite these benefits, deployment of hybrid poplars has been hindered in part by our limited ability to predict the potential yields of sites not currently producing SRWCs.

Biomass yields are largely determined by (i) the combination of genetically-controlled, physiological processes which regulate tree growth, and (ii) the quality of the site, which is in turn influenced by climatological and soil factors. As such, a model that accounts for differences in these genotype- and location-specific characteristics is desirable.

Physiological Processes Predicting Growth (3-PG) is a process-based model that uses species-specific physiological parameters, along with site-level climate and soil factors, to predict tree growth [26, 45-46]. More specifically, 3-PG uses solar radiation and temperature data along with species-specific photosynthetic parameters to establish maximum potential productivity, from which actual productivity is estimated based on limiting factors such as site fertility and water availability (as influenced by precipitation, soil water holding capacity, water table access, etc.) and allocated among tree components (stems, foliage, and roots) based on allometric relationships. Thus, productivity is estimated based on the site-specific availability of key resources and the species-specific physiological processes which govern the conversion of these resources into biomass.

While 3-PG has been used both to model growth and to estimate site productivity for eucalypt and pine species [27], and the model has been tested in Canada for hybrid poplar [2] and willow [3], similar reports for hybrid poplars in the U.S. are lacking. Therefore, given the heightened interest in using these purpose-grown trees for energy, fiber, and environmental benefits, our objective was to parameterize, calibrate, and validate the 3-PG model for hybrid poplars in the region, and use the validated model to map potential biomass yields for
Minnesota and Wisconsin. This type of map-based approach is already being utilized with statistical models for poplars and willows in the United Kingdom [4], and is important for providing industry leaders, policymakers, and resource managers with much-needed information in areas where limited yield data are currently available.

**PARAMETER VALUE ASSIGNMENT**

The spreadsheet-based version of 3-PG (known as 3-PGpjs) was obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO) headquartered in Canberra, Australia. Users can enter species-specific values for up to 60 parameters that describe tree physiology and allometric growth relationships. In this study, we estimated the majority of these parameter values from previously published research on hybrid poplars, assumed values based on expert knowledge or best-fit of the model for a limited selection of the parameters, and used default 3-PG values for the remaining parameters (Table 1).

**Literature-Derived Values**

Previous research has explored the sensitivity (i.e. change in model output relative to change in model parameter) for the 3-PG model [14], and based on those results the parameters have been placed in sensitivity classes (Low, Medium, or High) to aid in adapting the model to new species [46]. A review of previously published poplar research was conducted to determine values for the model parameters, with a particular focus on those in the High sensitivity class. When available in the literature, parameter values for the specific clones modeled in the calibration and validation phases (*Populus deltoides × P. nigra* hybrids DN17, DN34, and DN182) were used; otherwise, parameter values derived from the parent
species (pure or crossed with other species) were used. For some of the values reported in the
literature, conversions were necessary to match the input units of the model. For others
(particularly several allometric relationships), the parameters were estimated (algebraically,
graphically, or via linear regression) based on values and/or equations reported in the
literature; for more information on these procedures, see the Appendix.

Assumed Values

For several parameters, values were assumed based on the knowledge and experience
of the authors and their collaborators. Age at median litterfall rate (\(t_{\text{gamma}F}\)) was set at 18
months so that the plateau for mature litterfall rate would be reached at approximately the
time of canopy closure. Seedling mortality rate (\(\gamma_{N0}\)), large tree mortality rate
(\(\gamma_{NX}\)), age at median mortality rate (\(t_{\text{gamma}N}\)), and shape of the mortality curve (\(n_{\text{gamma}N}\))
were assigned values which simulate a 5 percent mortality rate concentrated early in the
rotation; this is considered typical for hybrid poplar plantations in the region (Dan Langseth,
Verso Paper Corp., personal communication). Age at average specific leaf area (\(t_{\text{SLA}}\)) was
assigned based on the relationship between specific leaf area (SLA) and height reported by
Smith et al. [47], in which they showed the average SLA for P. tremuloides occurred at
heights of approximately 7.5 to 10 meters; similar heights are frequently achieved around age
5 for the hybrid poplars considered in this study. One parameter (age at full canopy cover;
\(\text{fullCanAge}\)) was assigned its value using an iterative approach for maximizing model fit; this
is described further in the calibration section.
Default Values

For the remaining parameters, default values were used. Several are conversion factors, and all are identified by Sands [46] as parameters which may be assigned generic values.

MODEL CALIBRATION

Regional calibration of 3-PG requires (at minimum) growth data from multiple sites, along with monthly climate data and soil data for each of the sites. Calibration also typically involves manipulation of parameters with unknown values as well as growth modifiers to optimize the fit of the model to the dataset [46]. The following sections describe the biomass plantation data, monthly climate data, and soils data used in this study (summarized in Table 2), as well as the procedures used for manipulating our parameter of unknown value (fullCanAge) and the fertility rating (FR) growth modifier.

Aboveground Biomass Productivity Data

Netzer et al. [35] reported hybrid poplar biomass productivity for a number of sites planted on former agricultural fields in the North Central region in 1987 and 1988. In that study, aboveground dry biomass productivity (averaged across 25-tree blocks of each of the three hybrids DN17, DN34, and DN182) was reported for 12 plantations in Wisconsin, Minnesota, and the eastern Dakotas planted at 2.4 × 2.4-meter spacing and measured at multiple ages (ranging from 3 to 11 years). This dataset (81 total datapoints) was used for calibration (56 datapoints from 8 plantations) and validation (25 datapoints from 4 plantations) of 3-PG for total stem biomass (output variable Wₜ; Mg ha⁻¹). Clone-specific
data were also reported for ages 8 to 11 for the same sites; however, analysis of variance showed no significant difference in biomass across sites for the three clones (P = 0.37). As a result, the data averaged across clones was used in this study, based on the wider range of ages for which the data were available.

**Climate and Soils Data**

Climate data (total precipitation, mean daily maximum temperature, mean daily minimum temperature, and mean daily solar radiation) were retrieved from databases [30, 32] for each month of each year that the plantations were grown, using the weather stations nearest each site. Relevant soils data (texture, maximum available soil water, and depth to water table) were determined for each site based on published soil surveys [34].

Because available water in the top meter of soil is typically considered accessible to plants [51], maximum available soil water (ASW$_{\text{max}}$) was set equal to that reported in the soil survey for the top 100 cm. We developed the following equation to estimate minimum available soil water (ASW$_{\text{min}}$) as a proportion of ASW$_{\text{max}}$ based on minimum annual depth to water table ($D_w$):

$$\text{ASW}_{\text{min}} = \text{ASW}_{\text{max}} \left(1 - \frac{D_w}{100}\right)$$

where any $D_w$ greater than 100 cm is assigned a value of 100.

We evaluated other cutoffs for water table depth (50, 150, and 200 cm); however, their use did not improve the performance of the model relative to using a depth of 100 cm.
(results not shown). Because the plantations were established during what Netzer et al. [35] described as a “historic (100 year) drought”, the initial value of ASW for each site was set equal to $ASW_{\text{min}}$. The soil texture for each site was matched to the most appropriate of the default categories found in 3-PG ($C = \text{clay}$, $CL = \text{clay loam}$, $SL = \text{sandy loam}$, $S = \text{sand}$) based on approximate clay and sand content (Table 3).

**Optimizing Model Fit**

For fitting the model to the calibration sites, the fertility rating (FR) growth modifier and full canopy age (fullCanAge) parameter were systematically manipulated to determine the best-fit values for the calibration dataset; essentially, these best-fit values represent the average values of FR and fullCanAge across all sites. The FR growth modifier has a value between 0 and 1 and acts as a multiplier upon potential growth to account for differences in relative nutrient availability; the fullCanAge parameter represents the year at which canopy closure occurs. The potential values of FR and fullCanAge were evaluated under the assumptions that (i) it is possible all the sites have $FR \approx 1$, based on the agricultural history of the sites resulting in high levels of residual nutrients (particularly at depths below the rooting zone of annual crops but still accessible to tree roots), and (ii) if $FR \neq 1$ for all sites then, given the number of sites, the range of potential values for FR in the region should be reasonably represented and therefore at least one site should have $FR \approx 1$.

Decreasing values of fullCanAge result in higher estimates of biomass, whereas decreasing values of FR result in lower estimates of biomass; thus, in order to maintain predicted values at levels similar to actual values, a decrease in fullCanAge must be met with a decrease in FR. Based on the above assumptions, it is possible to (i) establish the upper
limit for fullCanAge by assuming FR = 1 and reduce fullCanAge in 1-year increments from its highest possible value (11 years) until the best-fit value is found, (ii) establish the lower limit for fullCanAge by further reducing the parameter in 1-year increments (with FR = 1) until the best-fit value is found for the last (most under-predicted) of the calibration sites, (iii) determine the best-fit value of FR for each value of fullCanAge within these upper and lower limits, by iteratively reducing FR from its highest possible value (1, unitless) in increments of 0.05, and (iv) compare the fit statistics ($R^2$ and root mean square error, RMSE) for each resulting combination of FR and fullCanAge, to determine the best-fit average values of FR and fullCanAge for the sites. RMSE reflects the variability between actual and predicted values, and was calculated as the square root of the mean squared differences between actual and predicted values.

Using this approach, the upper limit for average fullCanAge was estimated to be 5 years, and the lower limit was estimated to be 3 years. For each value of fullCanAge within these limits, FR was reduced until the best-fit model was achieved (with the requirement that systemic bias [universal over-prediction or under-prediction] be avoided). The resulting combinations of fullCanAge and FR, along with fit statistics, are shown in Table 4. Because the combination of FR = 1 and fullCanAge = 5 produced the best fit ($R^2 = 0.88$, RMSE = 8.8 Mg ha$^{-1}$), these values were used for the remainder of the study. However, it should be noted that the fit statistics were relatively similar for FR = 0.95 with fullCanAge = 4, and FR = 0.90 with fullCanAge = 3, and therefore these combinations would likely give similar results. Also of note is that the ranges of values for fullCanAge and FR described above are consistent with previous research; Strong and Hansen [50] suggest that hybrid poplars utilized for 10-year rotations will reach canopy closure around age 4, and previous 3-PG
studies with eucalypts demonstrated FR values ranging from 0.70 to 0.95 [43] and 0.60 to 1.00 [49] across study sites.

**MODEL VALIDATION**

The calibrated model was used to predict total aboveground biomass productivity of the four plantations from Netzer et al. [35] assigned to the validation dataset (described in the preceding section). Soil and climate data were obtained for the validation sites in the same manner as described for the calibration sites. All other model settings (tree spacing, initial ASW, FR, fullCanAge) were the same as for calibration. The fit of the model ($R^2$, RMSE, and mean bias) for the validation dataset, as determined by linear regression of actual biomass on predicted biomass, is shown in Figure 1. Mean bias reflects the tendency of the model to over-predict (bias > 0) or under-predict (bias < 0) actual biomass, and was calculated by summing the differences between actual and predicted annual biomass and then dividing by the number of observations.

In addition to the overall fit of the model, the fit of the model for the individual sites was also evaluated. Linear regression coefficients (slope and intercept) for actual versus predicted biomass were determined for the sites via analysis of covariance using PROC GLM in SAS (SAS Institute Inc., Cary, NC), with actual biomass as the dependent variable, site as the independent variable, and predicted biomass as the covariate. The values ranged from 0.70 to 1.18 for the slopes, and -13.2 to 17.6 for the intercepts (Figure 2). To examine the relationship of individual sites relative to the overall model, a surrogate site (MON87) was selected to represent the overall model (based on similarity of slope and intercept), and statistical contrasts were then used in SAS to compare the slope and intercept of the surrogate
site to those of the remaining sites. The results show evidence of a difference in slope for FRM88 ($P = 0.0055$), and differences in intercepts for FAR87 ($P = 0.0158$), GRF87 ($P = 0.0205$), MON88 ($P = 0.0056$).

Finally, the ability of the model to effectively identify high versus low productivity sites is of interest for siting bioenergy facilities and the hybrid poplar plantations which would supply them. Actual and predicted biomass growth over time is shown for plantations established in 1987 (Figure 3) and 1988 (Figure 4).

**SENSITIVITY ANALYSIS**

The sensitivity of the model, as calibrated for hybrid poplars in the region, was evaluated by separately manipulating `fullCanAge` and `FR`. These parameters were selected due to the uncertainty of their true values; the parameters were estimated via model optimization during model calibration. The sensitivity of the model was evaluated in terms of mean annual biomass productivity (Mg ha$^{-1}$ yr$^{-1}$) rather than overall productivity (Mg ha$^{-1}$), so that these measures of variability would be consistent with the units to be used for mapping productivity (described in the following section). The model was run with `fullCanAge` set at 3, 4, 5, 6, and 7 years (with `FR = 1`) to determine the mean bias and RMSE for the calibration sites, the validation sites, and all sites (calibration + validation). Similarly, the model was also run with `FR` set at 1.00, 0.95, 0.90, 0.85, and 0.80 (with `fullCanAge = 5`) for these datasets. In addition, individual sites were evaluated for RMSE to determine their best-fit values for `fullCanAge` and `FR`.

Biomass productivity was sensitive to manipulation of the `fullCanAge` parameter (Figure 5). A change of 2 years in either direction from age 5 produced a mean bias of -0.4 to
2.0 Mg ha\(^{-1}\) yr\(^{-1}\) for the validation sites and -1.1 to 1.6 Mg ha\(^{-1}\) yr\(^{-1}\) across all sites (Figure 5a). Decreasing FR from 1.00 to 0.80 produced a mean bias of -0.3 Mg ha\(^{-1}\) yr\(^{-1}\) for the validation sites and -0.9 Mg ha\(^{-1}\) yr\(^{-1}\) across all sites (Figure 5b). The calibration sites and the overall dataset achieved best-fit at fullCanAge = 5 and at FR = 1, while the validation sites achieved best-fit at fullCanAge = 6 and at FR = 0.85. Similarly, the best-fit values of fullCanAge and FR varied among individual sites (Figure 6). Most of the individual sites achieved best-fit with values of 4 to 6 for fullCanAge; however, one site (MON88) achieved best-fit at fullCanAge = 3, while two sites (ASH87 and SXF87) achieved best-fit at fullCanAge = 7. The majority of sites achieved best-fit at FR = 1; however, one site (FAR87) achieved best-fit at FR = 0.90 and four others (ASH87, FRM88, SXF87, and SXF88) achieved best-fit at FR = 0.80.

**MAPPING ABOVEGROUND BIOMASS PRODUCTIVITY**

Once calibrated and validated for the region, 3-PG was used to model productivity across Minnesota and Wisconsin within a geographic information system (GIS; ArcGIS, ESRI, Redlands, CA). Temperature, precipitation, and solar radiation climate data (32-km resolution) were retrieved from the North American Regional Reanalysis (NARR) [29] through the NOAA National Operational Model Archive and Distribution System (NOMADS) [31, 44]. The NARR climate data (Lambert conformal grid format, [29]) were attributed to an ArcGIS 32-km base grid using geo-referenced latitude and longitude coordinates. The data consisted of 8 datapoints per month (each one representing a 3-hr period of the day), for each month over a 10-year period (1998-2008), giving a total of 960
observations per climate variable; maps of the study area produced from these climate data have been recently published [57].

To determine whether to use 2-meter temperature or surface temperature from the NARR data, both were compared to weather station data at three locations (Fairmont, Granite Falls, and Milaca, MN) over the period 1987-1998. The results showed that maximum temperature is closely matched by the 2-meter data, while minimum temperature is closely matched by the surface data; as such, this combination of temperature data was used for the remainder of the mapping process. Average monthly values of maximum and minimum temperature were determined by averaging the maximum and minimum 3-hour temperatures, respectively, across the 10-year period. Because the NARR data is produced from separate terrestrial and water models, with cells having 50% or more area in water assigned to the water model, a number of the climate grid cells overlapping the shoreline of the Great Lakes contained temperature data which were representative of conditions over water rather than land. To provide terrestrial-based temperature data for the land area within these 23 cells (or about 5% of the total number of cells), temperature data from the next-closest cell inland were used. For average monthly precipitation, the 3-hr values of mean accumulated precipitation were summed and multiplied by the number of days in the month, and then averaged across the 10-year period. To determine average daily solar radiation for each month, the 3-hr values of mean hourly downward shortwave radiation flux were averaged for the month, then averaged across the 10-year period, and finally multiplied by 24 h d⁻¹.

Soils data were retrieved through the State Soil Geographic (STATSGO2) database from the Natural Resource Conservation Service (NRCS) [33]. Available soil water and depth to water table for each soil map unit were obtained directly from the STATSGO2
“muaggatt” tables for Minnesota and Wisconsin. Soil texture group was determined by calculating the weighted average for clay and sand content in the component soils comprising each soil map unit. Specifically, weighted averages were calculated for clay and sand content in the top 100 cm of each component soil (based on soil horizon thickness in the “chorizon” table), which were then used to calculate weighted averages for clay and sand content in each soil map unit (based on the soil component percentages found in the “component” table). The soil map units were then assigned to soil texture groups according to Table 3.

To match the scale of the soils data (various-sized map units) to that of the climate data (32-km geo-referenced cells), soil variables were averaged (weighted by map unit area) for each soil texture group in each climate cell. Mean annual biomass productivity (Mg ha\(^{-1}\) yr\(^{-1}\)) at age 10 was then estimated with 3-PG for each soil texture group in each cell, from which an overall average (weighted by soil texture group area) was calculated for each cell. Mean annual biomass productivity was used for mapping because these units are commonly reported in the literature and to facilitate comparison with annual crops; it was calculated by dividing the total aboveground dry biomass by age. The age 10 was selected because it is within the range of rotation lengths (7 to 10 years) suggested for the region by Netzer et al. [35], and because it allows for simple conversion to total biomass (Mg ha\(^{-1}\)). The resulting map of predicted biomass for Minnesota and Wisconsin is shown in Figure 7. Annual biomass productivity at age 10 ranged from 4.4 to 13.0 Mg ha\(^{-1}\) yr\(^{-1}\) across the states, with the highest productivity mainly concentrated in the area stretching from south-central Minnesota across southern Wisconsin.
DISCUSSION

As parameterized and calibrated in this study, 3-PG appears well-suited for modeling hybrid poplar aboveground biomass productivity in Minnesota and Wisconsin. Linear regression of actual versus predicted total aboveground biomass for the validation dataset demonstrated a strong fit \( R^2 = 0.89 \), \( \text{RMSE} = 8.1 \text{ Mg ha}^{-1} \), mean bias = 5.3 Mg ha\(^{-1}\) or 14.3\% of mean observed biomass; see Figure 1). These results are similar to other studies with 3-PG, where \( R^2 \) values of 0.63 to 0.99 [27, 36, 38, 54] and mean bias of 4 to 22\% [27, 36] for aboveground biomass have been reported for plantations of various species. Individually, few sites deviated significantly from the overall model with regard to slope and intercept for actual versus predicted biomass (see Figure 2), and the model was able to separate higher productivity sites from lower productivity sites (see Figures 3 and 4).

When used to map productivity across Minnesota and Wisconsin (see Figure 7), mean annual biomass predictions and their spatial trends were consistent with previous research. Specifically, the range of biomass estimates (4.4 to 13.0 Mg ha\(^{-1}\) yr\(^{-1}\)) is consistent with that observed for DN34 (4.80 to 9.01 Mg ha\(^{-1}\) yr\(^{-1}\); ages 7 to 10 years) at sites in Minnesota and Wisconsin reported by Zalesny et al. [55], and the overall spatial trend mirrors that of corn grain productivity for the region as mapped by Prince et al. [41]. Interestingly, biomass productivity is predicted to be highest near the boundary between high and low productivity in southern Minnesota; this pocket of high-productivity is also apparent on the aforementioned corn grain productivity map, and may stem from shallower water tables [34] along with relatively high solar radiation and temperature [32]. The RMSE values associated with mean annual productivity (see Figure 5) suggests that, as currently calibrated, actual productivity will typically vary from mapped values by +/- 1 Mg ha\(^{-1}\) yr\(^{-1}\).
The sensitivity analysis suggests biomass productivity is more sensitive to fullCanAge than to FR within the range of values tested in this study (see Figure 5), albeit with a relatively smaller range of potential FR values being tested compared to fullCanAge. A decrease in fullCanAge of 2 years resulted in a mean bias of 1.6 Mg ha\(^{-1}\) yr\(^{-1}\) across the entire dataset, while a decrease in FR of 0.20 resulted in a mean bias of -0.9 Mg ha\(^{-1}\) yr\(^{-1}\). The validation dataset achieved best-fit at fullCanAge = 6 and at FR = 0.85, which indicates the use of fullCanAge = 5 and FR = 1 may overestimate actual productivity; however, to the extent that FR may be raised with fertilization, use of the latter values may be considered reflective of potential productivity. Differences among individual sites within the calibration and validation datasets are also evident (see Figure 6). The apparent separation of sites by best-fit values of FR may be related to disease: all four sites having best-fit FR = 0.80 (ASH87, FRM87, SXF87, and SXF88) were rated by Netzer et al. [35] as being among the most severely affected by stem canker. Similarly, these four sites also had higher best-fit values of fullCanAge (6 to 7 years) than the other sites (3 to 5 years) with the exception of FAR87 (6 years). Finally, the plantations established in 1987 generally achieved best-fit at higher values of fullCanAge (4 to 7 years) than those established in 1988 (3 to 6 years). This may be due to better establishment conditions (relating to extreme weather events, site preparation, and/or weed control) for the 1988 plantations.

These possible disease and establishment effects suggest that a more complete accounting of damaging agents (i.e. insects, weed competition, and extreme weather events) would likely produce more accurate biomass productivity estimates. Likewise, more specific knowledge of physiological parameters (i.e. clone-specific values rather than those for parent species or related clones) should be the subject of additional research to improve model
performance. It should also be noted that fullCanAge and FR are almost certainly related, to the extent that higher fertility is associated with faster growth and therefore earlier canopy closure. By optimizing fit for the individual sites, we hypothesize that the true values of FR may range from 0.85 to 1, with fullCanAge ranging from 3 to 6 years and negatively correlated with FR (Table 5). These site-specific estimates of FR and fullCanAge improve the overall model fit ($R^2 = 0.95$, RMSE = 5.4 Mg ha$^{-1}$) but require prior knowledge of yields, and therefore we did not attempt to use or validate these site-specific values in the present study. To this end, methods for reliably measuring FR and predicting fullCanAge for individual sites without prior knowledge of yields should also be further investigated.

Even though coefficients for other output variables (i.e. DBH, height, volume, self-thinning) were obtained from the literature, the model was only calibrated and validated for aboveground biomass in this study. Additional work should be done to validate model outputs for these other stand variables. Also, it is important to reiterate that the model was only calibrated and validated for the group of clones (DN17, DN34, and DN182) reported in Netzer et al. [35]. As has been observed for *Eucalyptus* [1], different clones may have different parameter values (e.g. optimum temperature, minimum and maximum fraction of NPP to roots, etc), and therefore more work should be done to parameterize and calibrate the model for a wider selection of clones used in the region. Similarly, further work should be done to adapt the model to other regions. Because different clones are more commonly utilized in other regions, the model should be re-calibrated for these clones (or groups of clones), especially when they are not closely related to the ones considered here. While many of the physiological parameters and allometric relationships likely apply equally well in other regions for these specific clones, other values (or ranges of values) are likely to occur for
variables such as FR. Likewise, the most suitable cutoff for depth to water table in the ASW_{\text{min}} equation may also vary by region.

Though the results of this study are promising, it should not be considered the final word in 3-PG calibration for hybrid poplars in the region. Rather, it is intended as a first approximation which can and should be improved based on additional research, particularly with regard to determining clone-specific values for physiological parameters and site-specific values for FR. Also, due to the coarse scale of the biomass productivity map, it should not be used for siting hybrid poplar plantations at local (e.g. individual landowner) scales. Rather, the map is intended to be useful at the regional scale (e.g. county or multi-county scale) to compare average productivity in different areas where bioenergy facilities may be placed. Within such areas, finer-scale site input data (particularly for soils) may be used to generate local-level biomass estimates, which may vary considerably around the averages depicted in the coarse-scale map. In addition, non-biological factors such as land ownership and current land use [5, 10, 20] place constraints on poplar deployment which are not considered here. Additional work has been done to evaluate the potential of using 3-PG to predict and map biomass yields at finer scales, with consideration for such constraints [57].

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APPENDIX

Fertility Rating Equation

The fertility rating equation in 3-PG is of the form:

\[ f_N = 1 - (1 - f_{N0}) \times (1 - FR)^{nf_N} \]  \hspace{1cm} (2)

which can be re-arranged as:

\[ (1 - f_N) = (1 - f_{N0}) \times (1 - FR)^{nf_N} \]  \hspace{1cm} (3)

where \( f_N \) is the proportion of actual versus potential growth at a given FR, \( f_{N0} \) is the proportion of actual versus potential growth when \( FR = 0 \), FR is a measure of fertility, and \( nf_N \) is a species-specific coefficient.

Possible metrics for fertility include but are not limited to applied fertilizer rates, soil nutrient levels, and/or plant nutrient levels. Here, plant nutrient levels are considered as they reflect realized site fertility, whereas the other metrics reflect potential site fertility and are subject to confounding factors such as fertilizer type and placement, as well as soil conditions which may interfere with nutrient uptake.

Previously published data for stem volume and leaf N concentration from a fertility study of four *Populus trichocarpa × P. deltoides* clones [52] were converted to relative scales such that for stem volume, 0 = no stem volume, and 1 = maximum reported stem volume; and for leaf N concentration, 0 = minimum reported leaf N, and 1 = maximum reported leaf N. Relative stem volume and relative leaf N were then used as measures of \( f_N \) and FR, respectively, to solve for \( f_{N0} \) and \( nf_N \) in the re-arranged equation above, using linear regression in SAS (PROC REG). The resulting model (\( R^2 = 0.89 \)) estimates the values of the parameters as: \( f_{N0} = 0.26; nf_N = 1 \).
**Stem Height Relationship**

The height equation in 3-PG is of the form:

\[ H = a_H \times B^{n_{HB}} \times N^{n_{HN}} \]  \hspace{1cm} (4)

or when log-transformed:

\[ \ln H = \ln a_H + n_{HB}(\ln B) + n_{HN}(\ln N) \]  \hspace{1cm} (5)

where \( H \) is mean tree height, \( B \) is mean tree diameter at breast height (DBH), \( N \) is trees per unit area, and the remaining variables (\( a_H, n_{HB}, \) and \( n_{HN} \)) are species-specific coefficients.

Previously-published data (mean heights in meters, mean DBH in centimeters, and trees per hectare derived from tree spacing) from a previous study with *Populus trichocarpa* \( \times \) *P. deltoides* and *P. trichocarpa* \( \times \) *P. nigra* clones \[11\] were log-transformed and evaluated in SAS with linear regression (PROC REG), solving for log-transformed height. The resulting model \((R^2 = 0.98)\) estimates the values of the coefficients as: \( n_{HB} = 1.335; n_{HN} = 0.354; a_H = 0.036.\)

**Stem Volume Relationship**

The volume equation in 3-PG is of the form:

\[ V_S = a_V \times B^{n_{VB}} \times N^{n_{VN}} \]  \hspace{1cm} (6)

or when log-transformed:

\[ \ln V_S = \ln a_V + n_{VB}(\ln B) + n_{VN}(\ln N) \]  \hspace{1cm} (7)

where \( V_S \) is mean tree stem volume, \( B \) is mean tree diameter at breast height (DBH), \( N \) is trees per unit area, and the remaining variables (\( a_V, n_{VB}, \) and \( n_{VN} \)) are species-specific coefficients.
Previously-published data (trees per hectare, DBH in centimeters, and volume estimated from mean annual mass increment \( \times \) age \( \times \) basic density) from a study on an array of hybrid poplars [22], were log transformed and evaluated in SAS with linear regression (PROC REG) solving for log-transformed volume. Because stocking was reported at the stand level, and the data used to estimate stem volume was derived from individual trees within the stands, only individual trees having diameters within 20% of the mean stand diameter were used, under the assumption that individual trees similar to the stand mean were growing at (or near) average density conditions. The resulting model \( (R^2 = 0.72) \) estimates the values of the coefficients as: \( n_{VB} = 1.96; n_{VN} = -0.30; a_v = 0.0072. \)

**Self-Thinning Relationship**

The self-thinning relationship in 3-PG is described by the equation:

\[
W_{Sx} = W_{Sx1000} \left( \frac{1000}{N} \right)^{n_N}
\]  

(8)

where \( W_{Sx} \) is maximum tree biomass, \( N \) is stand density, and the remaining variables are species-specific coefficients representing maximum tree biomass at 1,000 trees per hectare \( (W_{Sx1000}) \) and the slope of the self-thinning line \( (n_N) \).

Stand density and mean stem biomass values were derived from DeBell et al. [11] and Johannson and Karacic [22]. The former reported these two variables directly; the latter reported stand density and mean stem diameter, which was converted to mean stem biomass using an equation given in that study. The data were then graphed, and the location of the self-thinning line was estimated by iteratively manipulating the slope and intercept (at 1,000
trees per hectare) to visually match the upper boundary of tree biomass across stand
densities. The resulting values of the coefficients are estimated as: \( w_{Sx1000} = 500; n_N = -1.45 \).

**Foliage:Stem Partitioning**

The ratio of foliage:stem biomass in 3-PG is described by the equation:

\[
p_{FS} = a_p \times B^{n_p}
\]

(9)

where \( p_{FS} \) is the foliage:stem ratio, \( B \) is mean stem diameter at breast height (DBH), and the
remaining variables (\( a_p \) and \( n_p \)) are species-specific coefficients.

In 3-PG, these coefficients are estimated from foliage:stem ratios measured at 2 cm
DBH (\( p_{FS2} \)) and 20 cm DBH (\( p_{FS20} \)). Equations from Fortier et al. [15] were used to estimate
stem (main stem + branch) and foliage biomass at DBH = 20 for the \( P. deltoides \times P. nigra \)
clone ‘3570’; these biomass values were then used to calculate the foliage:stem ratio (\( p_{FS20} = 0.12 \)). Fortier’s equations were not used to estimate \( p_{FS2} \) directly, as their equations are based
on trees larger than 2 cm DBH (range = 3.6 to 25.1 cm). Instead, the foliage:stem ratio at
DBH = 3.6 was estimated in the same fashion as \( p_{FS20} \) (\( p_{FS3.6} = 0.45 \)); then, \( p_{FS3.6} \) and \( p_{FS20} \)
were used to algebraically solve for the coefficients \( a_p \) and \( n_p \) in the above equation (\( a_p = 1.206; n_p = -0.771 \)). Finally, \( p_{FS2} \) was calculated from the above equation using these
coefficient values and \( B = 2 \) (\( p_{FS2} = 0.71 \)).
**Table 1** Parameter values derived from hybrid poplar literature, assumed from expert knowledge or best-fit, or based on default 3-PG values. Parameter descriptions, 3-PG names, sensitivity classes, and default values are from Sands [46]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3-PG Name</th>
<th>Sens. Class</th>
<th>Hybrid Poplar Value</th>
<th>Source</th>
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<tr>
<td><strong>Literature-Derived Values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Foliage:stem partitioning ratio @ DBH=2 cm</td>
<td>$p_{FS2}$</td>
<td>H</td>
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<td>[15]&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Foliage:stem partitioning ratio @ DBH=20 cm</td>
<td>$p_{FS20}$</td>
<td>H</td>
<td>0.12</td>
<td>[15]&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Constant in the stem mass v. DBH relationship</td>
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<td>0.081</td>
<td>[22]&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>H</td>
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<td>[22]</td>
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<td>Maximum fraction of NPP to roots</td>
<td>$p_{Rx}$</td>
<td>M</td>
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<td>[9]</td>
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<tr>
<td>Minimum fraction of NPP to roots</td>
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<td>0.17</td>
<td>[13]&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Mature litterfall rate per month</td>
<td>$\gamma_{Fx}$</td>
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<td>0.10</td>
<td>[7]&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Litterfall rate per month at $t = 0$</td>
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<td>[8]&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>[37]</td>
</tr>
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<td>$T_{opt}$</td>
<td>M</td>
<td>30</td>
<td>[12]</td>
</tr>
<tr>
<td>Maximum temperature (°C) for growth</td>
<td>$T_{max}$</td>
<td>L</td>
<td>48</td>
<td>[21]</td>
</tr>
<tr>
<td>Value of 'm' when fertility rating (FR) = 0</td>
<td>$m_0$</td>
<td>L</td>
<td>1</td>
<td>[9]&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Value of 'fNutr' when FR = 0</td>
<td>$fN_0$</td>
<td>M</td>
<td>0.26</td>
<td>[52]&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Power of (1-FR) in 'fNutr'</td>
<td>$fN_h$</td>
<td>L</td>
<td>1</td>
<td>[52]&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Max. stem mass (kg tree&lt;sup&gt;-1&lt;/sup&gt;) @ 1000 trees/ha</td>
<td>$w_{Sx1000}$</td>
<td>L</td>
<td>500</td>
<td>[11, 22]&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Power in self-thinning rule</td>
<td>$\text{thinPower}$</td>
<td>L</td>
<td>-1.45</td>
<td>[11, 22]&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Specific leaf area ($m^2$ kg&lt;sup&gt;-1&lt;/sup&gt;) at age 0</td>
<td>$SLA_0$</td>
<td>L</td>
<td>19</td>
<td>[12]</td>
</tr>
<tr>
<td>Specific leaf area ($m^2$ kg&lt;sup&gt;-1&lt;/sup&gt;) for mature leaves</td>
<td>$SLA_1$</td>
<td>H</td>
<td>10</td>
<td>[22]&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extinction coeff. for absorption of PAR by canopy</td>
<td>$k$</td>
<td>M</td>
<td>0.779</td>
<td>[17]</td>
</tr>
<tr>
<td>Max. proportion of rainfall evaporated from canopy</td>
<td>$\text{MaxIntcptn}$</td>
<td>M</td>
<td>0.24</td>
<td>[18]</td>
</tr>
<tr>
<td>LAI for maximum rainfall interception</td>
<td>$\text{LAI_{maxIntcptn}}$</td>
<td>L</td>
<td>7.3</td>
<td>[18]</td>
</tr>
<tr>
<td>Max. canopy quantum efficiency (mol C mol PAR&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$\alpha$</td>
<td>H</td>
<td>0.08</td>
<td>[6]</td>
</tr>
<tr>
<td>Ratio NPP/GPP</td>
<td>$Y$</td>
<td>H</td>
<td>0.43</td>
<td>[23]</td>
</tr>
<tr>
<td>Maximum canopy conductance (m s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$\text{MaxCond}$</td>
<td>H</td>
<td>0.02</td>
<td>[25]</td>
</tr>
<tr>
<td>LAI for maximum canopy conductance</td>
<td>$\text{LAI_{gcx}}$</td>
<td>L</td>
<td>2.6</td>
<td>[25]</td>
</tr>
<tr>
<td>Stomatal response to VPD (1 mBar&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$\text{CoeffCond}$</td>
<td>L</td>
<td>0.05</td>
<td>[53]&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Canopy boundary layer conductance (m s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$BL\text{cond}$</td>
<td>L</td>
<td>0.05</td>
<td>[42]&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Branch and bark fraction at age 0</td>
<td>$f_{BB0}$</td>
<td>L</td>
<td>0.64</td>
<td>[9]</td>
</tr>
<tr>
<td>Branch and bark fraction for mature stands</td>
<td>$f_{BB1}$</td>
<td>L</td>
<td>0.24</td>
<td>[7]&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age at which $f_{BB} = 0.5(frac_{BB0}+frac_{BB1})$</td>
<td>$t_{BB}$</td>
<td>L</td>
<td>3</td>
<td>[9]</td>
</tr>
<tr>
<td>Basic density (t m&lt;sup&gt;-3&lt;/sup&gt;) for young trees</td>
<td>$\rho_{Min}$</td>
<td>H</td>
<td>0.39</td>
<td>[28]</td>
</tr>
<tr>
<td>Basic density (t m&lt;sup&gt;-3&lt;/sup&gt;) for older trees</td>
<td>$\rho_{Max}$</td>
<td>H</td>
<td>0.35</td>
<td>[28]</td>
</tr>
</tbody>
</table>
Table 1 Parameter values derived from hybrid poplar literature, assumed from expert knowledge or best-fit, or based on default 3-PG values. Parameter descriptions, 3-PG names, sensitivity classes, and default values are from Sands [46]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3-PG Name</th>
<th>Sens. Class</th>
<th>Hybrid Poplar Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at which basic density = 0.5(rhoMin+rhoMax)</td>
<td>tRho</td>
<td>M</td>
<td>2</td>
<td>[28]</td>
</tr>
<tr>
<td>Constant in the stem height relationship</td>
<td>aH</td>
<td>L</td>
<td>0.036</td>
<td>[11]a</td>
</tr>
<tr>
<td>Power of stocking in the stem height relationship</td>
<td>nHN</td>
<td>L</td>
<td>0.354</td>
<td>[11]a</td>
</tr>
<tr>
<td>Constant in the stem volume relationship</td>
<td>aV</td>
<td>L</td>
<td>0.0072</td>
<td>[22]a</td>
</tr>
<tr>
<td>Power of DBH in the stem volume relationship</td>
<td>nVB</td>
<td>L</td>
<td>1.96</td>
<td>[22]a</td>
</tr>
<tr>
<td>Power of stocking in the stem volume relationship</td>
<td>nVN</td>
<td>L</td>
<td>-0.30</td>
<td>[22]a</td>
</tr>
</tbody>
</table>

Assumed Values
- Age in months at which litterfall rate has median value: $t_{\gamma F}$, L, 18
- Mortality rate (% yr⁻¹) for large t: $\gamma N_o$, L, 0
- Seedling mortality rate (% yr⁻¹) at t = 0: $\gamma N_0$, L, 3.5
- Age at which mortality rate has median value: $t_{\gamma N}$, L, 1
- Shape of mortality response: $n_{\gamma N}$, L, 1
- Age at which specific leaf area = 0.5(SLA₀+SLA₁): $t_{SLA}$, L, 5
- Age at canopy cover: fullCanAge, M, 5c

Default Values
- Days production lost per frost day: $k_F$, L, 0
- Moisture ratio deficit for $f_q = 0.5$: SWconst, H, 0.7
- Power of moisture ratio deficit: SWpower, L, 9
- Maximum stand age (yrs) used in age modifier: MaxAge, L, 50
- Power of relative age in function for fAge: nAge, L, 4
- Relative age to give fAge = 0.5: rAge, L, 0.95
- Fraction mean tree foliage biomass lost per dead tree: $m_F$, L, 0
- Fraction mean tree root biomass lost per dead tree: $m_R$, L, 0.2
- Fraction mean tree stem biomass lost per dead tree: $m_S$, L, 0.2
- Intercept of net v. solar radiation relationship (W m⁻²): $Q_a$, H, -90°
- Slope of net v. solar radiation relationship: $Q_b$, H, 0.8°
- Molecular weight of dry matter (dry g mol⁻¹): gDM_mol, H, 24d
- Conversion of solar radiation to PAR (mol MJ⁻¹): molPAR_MJ, H, 2.3d

a Estimated from equations and/or values reported in the literature; see Appendix
b Values reported in the literature have been converted to the units and/or ratios required for model input
c Parameter value assigned by iterative manipulation to produce best-fit model
d Conversion factors; values assumed to be constant
Table 2: Plantations from Netzer et al. [35] used for calibration and validation of 3-PG for hybrid poplars. Abbreviated headings are year planted (Y), number of years of data (N), latitude (L), temperature and solar station identification numbers (T/S ID), precipitation station identification number (P ID), mean growing season maximum temperature (T\text{max}) and minimum temperature (T\text{min}), mean annual precipitation (P), mean daily solar radiation (S), depth to water table (D\text{w}), maximum available soil water (ASW\text{max}), and minimum available soil water (ASW\text{min}).

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Site</th>
<th>Location</th>
<th>Y</th>
<th>N</th>
<th>L (°N)</th>
<th>T/S ID\text{a}</th>
<th>P ID\text{b}</th>
<th>T\text{max} (°C)</th>
<th>T\text{min} (°C)</th>
<th>P (mm y\text{-1})</th>
<th>S (MJ m\text{-2} d\text{-1})</th>
<th>Soil Texture\text{e}</th>
<th>D\text{w} (cm)\text{f}</th>
<th>ASW\text{max} (mm)</th>
<th>ASW\text{min} (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calib</td>
<td>ASH87</td>
<td>Ashland WI</td>
<td>1987</td>
<td>6</td>
<td>46.63</td>
<td>14913;727445;475286</td>
<td>17.7</td>
<td>6.1</td>
<td>807</td>
<td>13.0</td>
<td>silty loam</td>
<td>30</td>
<td>131</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ASH88</td>
<td>Ashland WI</td>
<td>1988</td>
<td>5</td>
<td>46.63</td>
<td>14913;727445;475286</td>
<td>17.9</td>
<td>6.4</td>
<td>815</td>
<td>13.0</td>
<td>silty loam</td>
<td>30</td>
<td>131</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRM88</td>
<td>Fairmont MN</td>
<td>1988</td>
<td>6</td>
<td>43.68</td>
<td>14925;726586;212698</td>
<td>20.8</td>
<td>9.7</td>
<td>837</td>
<td>13.8</td>
<td>clay loam</td>
<td>&gt;100</td>
<td>182</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GRF87</td>
<td>Granite Falls MN</td>
<td>1987</td>
<td>7</td>
<td>44.80</td>
<td>14922;726559;215563</td>
<td>20.8</td>
<td>9.8</td>
<td>662</td>
<td>14.0</td>
<td>loam</td>
<td>75</td>
<td>164</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GRF88</td>
<td>Granite Falls MN</td>
<td>1988</td>
<td>6</td>
<td>44.80</td>
<td>14922;726559;215563</td>
<td>20.7</td>
<td>9.8</td>
<td>670</td>
<td>13.9</td>
<td>loam</td>
<td>&gt;100</td>
<td>192</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIL87</td>
<td>Milaca MN</td>
<td>1987</td>
<td>9</td>
<td>45.77</td>
<td>14926;727475;215392</td>
<td>20.4</td>
<td>7.7</td>
<td>660</td>
<td>13.2</td>
<td>silty clay loam</td>
<td>0</td>
<td>196</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MON87</td>
<td>Mondovi WI</td>
<td>1987</td>
<td>9</td>
<td>44.87</td>
<td>14991;726435;475563</td>
<td>21.3</td>
<td>9.1</td>
<td>839</td>
<td>12.9</td>
<td>silty loam</td>
<td>&gt;100</td>
<td>215</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MON88</td>
<td>Mondovi WI</td>
<td>1988</td>
<td>8</td>
<td>44.87</td>
<td>14991;726435;475563</td>
<td>21.4</td>
<td>9.2</td>
<td>843</td>
<td>13.0</td>
<td>silty loam</td>
<td>&gt;100</td>
<td>211</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>CLO88</td>
<td>Cloquet MN</td>
<td>1988</td>
<td>7</td>
<td>46.83</td>
<td>14913;727450;211630</td>
<td>17.5</td>
<td>6.9</td>
<td>826</td>
<td>12.9</td>
<td>loam</td>
<td>&gt;100</td>
<td>163</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FAR87</td>
<td>Fargo ND</td>
<td>1987</td>
<td>6</td>
<td>46.90</td>
<td>14914;727530;322859</td>
<td>21.2</td>
<td>8.6</td>
<td>496</td>
<td>13.3</td>
<td>silty clay</td>
<td>23</td>
<td>158</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SXF87</td>
<td>Sioux Falls SD</td>
<td>1987</td>
<td>6</td>
<td>43.57</td>
<td>14944;726510;397667</td>
<td>22.5</td>
<td>9.8</td>
<td>605</td>
<td>14.0</td>
<td>silty clay loam</td>
<td>&gt;100</td>
<td>190</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SXF88</td>
<td>Sioux Falls SD</td>
<td>1988</td>
<td>6</td>
<td>43.57</td>
<td>14944;726510;397667</td>
<td>22.3</td>
<td>9.8</td>
<td>634</td>
<td>13.9</td>
<td>silty clay loam</td>
<td>&gt;100</td>
<td>181</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\text{a} Temperature and solar radiation data were obtained from the National Renewable Energy Lab (NREL) National Solar Radiation Database [32]. The time period (1987-1998) for the plantations is covered by two different datasets (1961-1990 and 1991-2005); thus, the first station ID refers to the 1961-1990 dataset, and the second station ID refers to the 1991-2005 dataset.

\text{b} Precipitation data obtained from the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center monthly summaries [30].

\text{c} Calculated by averaging monthly temperatures for April through October.

\text{d} Calculated by averaging solar radiation values for all months of the year.

\text{e} Soil data obtained from the Natural Resource Conservation Service (NRCS) Web Soil Survey [34].

\text{f} Estimated using Equation 1 (see text).
Table 3 Classification scheme for assigning soils to the groups found in 3-PG [26]

<table>
<thead>
<tr>
<th>3-PG Soil Groups</th>
<th>Soil Textures</th>
<th>Approximate Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay (C)</td>
<td>Clay, sandy clay, silty clay</td>
<td>&gt;40% clay</td>
</tr>
<tr>
<td>Clay loam (CL)</td>
<td>Clay loam, sandy clay loam, silty clay loam</td>
<td>20-40% clay</td>
</tr>
<tr>
<td>Sandy Loam (SL)</td>
<td>Sandy loam, loam, silt loam, silt</td>
<td>&lt;20% clay, &lt;80% sand</td>
</tr>
<tr>
<td>Sand (S)</td>
<td>Sand, loamy sand</td>
<td>&lt;20% clay, &gt;80% sand</td>
</tr>
</tbody>
</table>

Table 4 Evaluated and best-fit (bold) values for fertility rating (FR) within the estimated upper and lower limits for age at full canopy closure (fullCanAge), with associated fit statistics

<table>
<thead>
<tr>
<th>fullCanAge</th>
<th>FR</th>
<th>Slope</th>
<th>Intercept</th>
<th>R²</th>
<th>RMSE (Mg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.00</td>
<td>0.90</td>
<td>-3.82</td>
<td>0.875</td>
<td>12.86</td>
</tr>
<tr>
<td>3</td>
<td>0.95</td>
<td>0.94</td>
<td>-3.67</td>
<td>0.874</td>
<td>11.04</td>
</tr>
<tr>
<td>3</td>
<td>0.90</td>
<td>0.99</td>
<td>-3.49</td>
<td>0.873</td>
<td>9.69</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>0.92</td>
<td>0.36</td>
<td>0.875</td>
<td>9.77</td>
</tr>
<tr>
<td>4</td>
<td>0.95</td>
<td>0.96</td>
<td>0.46</td>
<td>0.875</td>
<td>8.94</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>0.95</td>
<td>3.60</td>
<td>0.880</td>
<td>8.77</td>
</tr>
</tbody>
</table>
Table 5 Hypothesized values of fertility rating (FR) and age at full canopy (fullCanAge) by site, based on optimization of fit

<table>
<thead>
<tr>
<th>Site</th>
<th>FR</th>
<th>fullCanAge</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASH87</td>
<td>0.85</td>
<td>6</td>
</tr>
<tr>
<td>ASH88</td>
<td>0.95</td>
<td>4</td>
</tr>
<tr>
<td>FRM88</td>
<td>0.85</td>
<td>6</td>
</tr>
<tr>
<td>GRF87</td>
<td>0.90</td>
<td>5</td>
</tr>
<tr>
<td>GRF88</td>
<td>0.95</td>
<td>4</td>
</tr>
<tr>
<td>MIL87</td>
<td>0.95</td>
<td>4</td>
</tr>
<tr>
<td>MON87</td>
<td>0.95</td>
<td>4</td>
</tr>
<tr>
<td>MON88</td>
<td>1.00</td>
<td>3</td>
</tr>
<tr>
<td>CLO88</td>
<td>0.95</td>
<td>4</td>
</tr>
<tr>
<td>FAR87</td>
<td>0.90</td>
<td>5</td>
</tr>
<tr>
<td>SXF87</td>
<td>0.85</td>
<td>6</td>
</tr>
<tr>
<td>SXF88</td>
<td>0.90</td>
<td>5</td>
</tr>
</tbody>
</table>
Fig. 1 Fit of the calibrated model to the data used for validation for total aboveground biomass (Mg ha⁻¹). The dashed line represents 1:1 ratio of actual versus predicted dry biomass
Fig. 2 Results of linear regression for predicted biomass versus actual biomass by site. Asterisks represent significant differences at Pr < 0.05 (*), Pr < 0.01 (**), and Pr < 0.001 (***) from contrasts of the surrogate site for the overall model (MON87) versus all other sites. Calibration sites are ASH87, ASH88, FRM88, GRF87, GRF88, MIL87, MON87, and MON88; validation sites are CLO88, FAR87, SXF87, and SXF88.
Fig. 3 Actual (a) and predicted (b) biomass productivity for hybrid poplar plantations established in 1987. Calibration sites include ASH87 (◊), GRF87 (□), MIL87 (○), and MON87 (Δ); validation sites include FAR87 (+) and SXF87 (×)
**Fig. 4** Actual (a) and predicted (b) biomass productivity for hybrid poplar plantations established in 1988. Calibration sites include ASH88 (◊), FRM88 (Δ), GRF88 (□), and MON88 (○); validation sites include CLO88 (+) and SXF88 (×).
Fig. 5 Sensitivity (mean bias and RMSE; Mg ha\(^{-1}\) yr\(^{-1}\)) of the model for calibration, validation, and all (calibration + validation) sites for various levels of (a) the full canopy age (fullCanAge) parameter (at FR = 1), and (b) the fertility rating (FR) growth modifier (at fullCanAge = 5)
Fig. 6 Model fit (RMSE; Mg ha\(^{-1}\) yr\(^{-1}\)) by site for various levels of (a) the full canopy age (fullCanAge) parameter, and (b) the fertility rating (FR) growth modifier. Calibration sites are ASH87, ASH88, FRM88, GRF87, GRF88, MIL87, MON87, and MON88; validation sites are CLO88, FAR87, SXF87, and SXF88.
Fig. 7 Map of predicted mean annual aboveground biomass productivity (dry Mg ha$^{-1}$ yr$^{-1}$) for hybrid poplars on a 10-year rotation in Minnesota and Wisconsin. Calibration sites are ASH87, ASH88, FRM88, GRF87, GRF88, MIL87, MON87, and MON88; validation sites are CLO88, FAR87, SXF87, and SXF88
CHAPTER 5. BIOMASS FLY ASH AS FOLIAR FERTILIZER FOR HYBRID ASPEN TREES: NUTRIENT UPTAKE, GROWTH RESPONSE, AND COMPATIBILITY WITH NITROGEN FERTILIZER

A paper submitted to *Journal of Plant Nutrition*

William L. Headlee and Richard B. Hall

**ABSTRACT**

Biomass ash is an important and potentially useful by-product of the bioenergy industry. As a “proof of concept” for using biomass fly ash as a foliar fertilizer, we tested (i) whether the nutrients in the ash were absorbed by hybrid aspen trees, (ii) whether the ash affected tree growth, and (iii) whether the ash was compatible with nitrogen foliar fertilizer. Four foliar treatments (water [control], ash suspended in water, nitrogen fertilizer solution, and ash suspended in nitrogen fertilizer solution) were evaluated. Several nutrients in the fly ash were absorbed by hybrid aspen both in the greenhouse and in the field; however, this absorption did not significantly affect tree growth in either setting. Nitrogen fertilization was associated with significantly higher tree growth in the greenhouse; inclusion of the fly ash with the nitrogen fertilizer solution did not significantly alter this growth response.
INTRODUCTION

When biomass is burned to produce bioenergy, a significant portion of the dry mass may be converted to ash: for example, 0.3-5.2% ash has been reported for various wood fuels, and 3.9-20.3% for various herbaceous residues (Miles et al., 1996). While the value of biomass ash as a soil-applied fertilizer and liming agent is known (Moilanen et al., 2005; Moilanen et al., 2002; Hytonen, 1998), so too are the difficulties of utilizing dry ash. Achieving uniform coverage is challenging, and windy conditions easily transport the ash from the intended application site. Theoretically, these difficulties may be overcome by mixing the ash with water to form a foliar fertilizer suspension. Though not all nutrients are readily absorbed through foliage, several nutrients found in biomass ash (such as potassium, calcium, and iron) can be absorbed this way, perhaps more efficiently than if applied to the soil (Fageria et al., 2009).

In general, foliar fertilization is less well-studied than dry fertilization, and to our knowledge has never been reported for a suspension of biomass ash. Thus, the overarching goal of this study was to test the concept of using biomass ash as a foliar fertilizer for the hybrid aspen ‘Crandon’ (*Populus alba* L. × *P. grandidenta* Michx.), which has demonstrated high yield potential as a bioenergy crop in Iowa (Goerndt and Mize, 2008) as well as the southern portions of Minnesota and Wisconsin (Zalesny et al., 2009). To achieve this goal, we first evaluated the basic characteristics of the ash which are important for foliar applications, and then tested the fly ash as a foliar fertilizer in both greenhouse and field settings.

As a “proof of concept” for using biomass fly ash as a foliar fertilizer, we specifically tested the following: (i) whether the nutrients in the ash were absorbed by hybrid aspen trees,
(ii) whether the ash affected tree growth, and (iii) whether the ash was compatible with nitrogen foliar fertilizer (which is an important consideration due to the low nitrogen content of ash). The first of these questions was answered via nutrient analyses of trees treated with ash-based foliar fertilizer (ash suspended in water) compared to trees treated with a control (water). Similarly, the second question was answered by comparing the effects of the foliar treatments on tree growth. The third question was answered by comparing the effects of a nitrogen fertilizer solution alone to that of a nitrogen fertilizer solution with ash added (ash suspended in nitrogen fertilizer solution).

**MATERIALS AND METHODS**

**Source and Characteristics of the Ash**

The ash for this study was provided by POET from an ethanol plant which burns biomass at approximately 980°C in a 150,000 PPH watertube boiler. The ash was produced from a mix of dry and wet wood (60-65% and 35-40%, respectively, on a mass basis), as well as trace amounts of soluble corn biomass. Two types of ash are produced at the plant: wet front ash, which is shaken off the front of the boiler grates during combustion and is doused with water during removal; and dry fly ash, which is recovered after exiting the boiler via the flue. The dry fly ash was selected for this study, due to its lower moisture content and lack of contamination from soil and other foreign objects which may be found in the front ash.

Using a 60-gram sample, a preliminary evaluation of the particle size distribution of the fly ash was conducted, and each particle size fraction was tested to determine sprayer compatibility (based on clogging of spray equipment nozzles); from this, it was determined that the ash particles ≤ 150 µm were sprayer-compatible. On a mass basis, the sprayer-
compatible ash comprised 68.8% of the total ash in this initial sample. A larger quantity (13.5 kg) of fly ash was then separated into sprayer-compatible and non-compatible fractions using a 150 µm nylon sieve measuring 20 cm across; approximately 1 kg of ash was sieved (hand-shaken for 3 to 4 minutes) at a time. With this larger sample, it was found that 75.4% of the fly ash was of a sprayer-compatible size.

Both the sprayer-compatible and non-compatible ash fractions were tested for plant nutrient content (Table 1). The smaller, sprayer-compatible fraction was enriched in most nutrients, which is consistent with previous research (Dahl et al., 2009; Obernberger et al., 1997). Conductivity of the foliar treatments containing ash was also evaluated, to gauge the risk of damage to the trees due to salinity. The conductivity of the ash treatments used in this study were found to be 3.4 and 2.8 mmhos cm⁻¹, for ash suspended in water and ash suspended in fertilizer, respectively. Conductivity of 2-4 mmhos cm⁻¹ is considered problematic for sensitive plant species, and 4-8 mmhos cm⁻¹ is considered problematic for most crops (Ayers & Westcot, 1976; Bernstein, 1975); however, it is important to distinguish that these guidelines are based upon irrigation water applied on a frequent (e.g. daily) basis. Less appears to be known about the risks of infrequent (e.g. weekly or monthly) applications of foliar fertilizers having similar conductivity values.

**Greenhouse Experiment**

To address the questions of interest (absorbance, growth response, and nitrogen fertilizer compatibility), four different foliar treatments were evaluated: (i) a control in which only water was applied to the leaves, (ii) a suspension of fly ash in water, (iii) a nitrogen fertilizer solution, and (iv) a suspension of fly ash in nitrogen fertilizer solution. A 20-0-0
urea-ammonium nitrate (UAN) fertilizer was used as the nitrogen source. For treatments containing ash and/or nitrogen fertilizer, mixtures were prepared in 1 L plastic spray bottles at rates of 7.25 g of ash and/or 2.55 g of nitrogen fertilizer per liter of water. Based on the nutrient analysis previously described for the sprayer-compatible ash, the resulting nutrient concentrations are estimated to be 510 mg kg\(^{-1}\) for total N, 350 mg kg\(^{-1}\) for P\(_2\)O\(_5\), and 435 mg kg\(^{-1}\) for K\(_2\)O.

Dormant hardwood cuttings (each measuring 10 cm long) of the hybrid aspen ‘Crandon’ (\(P. alba \times P. grandidentata\)) were planted in peat moss in mid-March, 2011. Initial growth and survival were poor due to widespread infection with black stem rot. Based on visual appearance, the healthiest trees were selected for inclusion in the experiment and were transplanted into 236 cm\(^3\) Accelerator\(^{®}\) (Nursery Supplies Inc., Chambersburg, PA) containers in mid-April. After transplanting, the trees were watered via sub-irrigation for 45 minutes two times per day. Initial tree heights were recorded prior to the first foliar treatment on April 21; heights were also recorded prior to each weekly foliar treatment thereafter. Treatments were applied with a spray bottle by saturating the upper and lower surfaces of the leaves.

The initial design consisted of a randomized complete block design, with each of the four foliar treatments represented by eight randomly selected trees per block (32 trees per block), with three blocks (96 trees total). However, over the course of the experiment trees in two of the blocks developed black stem rot, which resulted in highly variable tree growth as well as mortality. As a result, measurements of these two blocks were discontinued after six foliar applications. The experiment was continued with the remaining block of healthy trees
(which received a total of nine foliar applications), and was analyzed as a completely randomized design, with eight trees per foliar treatment (32 trees total).

On July 1 the trees were destructively sampled, washed thoroughly under running water, and oven-dried at 60° C to determine the effects of the treatments on the final dry biomass of the shoots (stems + leaves), roots, and cuttings. The plant tissues were ground through a 0.4 mm screen and sent to the U.S. Forest Service Institute for Applied Ecosystem Studies in Rhinelander, WI, for analysis of nutrients (N, P, K, Ca, Mg, and Na). N content was determined using a Flash EA1112 N-C analyzer with a model MAS 200 autosampler (Thermo Electron, via CE Elantech, Inc., Lakewood, NJ); P content was determined using a Varian 720 ICP-OES (EPA 200.7 method); and the remaining nutrients (K, Ca, Mg, and Na) were determined via atomic emission (AE) spectroscopy using a Varian Agilent model 240 FS AA unit (Agilent Technologies, Englewood, CO). Due to additional costs for the P analysis, only shoots were analyzed for this nutrient.

The growth and nutrient data were evaluated for treatment effects with analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute Inc., Cary, NC). The response variables analyzed (covariables in parentheses) included: final tree height (initial tree height), shoot biomass (number of shoots), root biomass (number of shoots), final cutting biomass (initial cutting diameter), shoot:root biomass ratio (final tree height), shoot nutrients (shoot biomass), root nutrients (root biomass), and cutting nutrients (cutting biomass). Where treatment effects were statistically significant (P < 0.05), multiple comparisons analyses (with Tukey adjustment) were conducted to determine differences among the adjusted means of the treatments. In addition, statistical contrasts which pooled observations within nitrogen treatments (“N” and “N+Ash”) versus non-nitrogen treatments (“Control” and “Ash”), as
well as ash treatments (“Ash” and “N+Ash”) versus non-ash treatments (“Control” and “N”),
were conducted to further evaluate plant responses to applications of nitrogen and ash.

Field Experiment

For the field study, the second treatment (ash suspended in water) was excluded to
allow greater replication of the remaining treatments (control, nitrogen fertilizer solution, and
ash suspended in nitrogen fertilizer solution). This decision was based on the results of the
greenhouse study (presented in the following section), which demonstrated that (i) a growth
response is unlikely to occur without nitrogen fertilizer, and (ii) the ash is likely to be
compatible with the nitrogen fertilizer.

Each of the three foliar treatments was randomly assigned to a plot within each of
four blocks (12 total plots) at a field site located near the Iowa State University campus in
Ames, IA. Each plot measured 4 m × 5 m in area; the inner 2 m × 3 m area was used for
biomass measurements, and the 1-m-wide outer perimeter was used for tissue sampling. The
plots were marked and cleared of existing vegetation (3-year-old hybrid aspen root sprouts)
in mid-May. For the treatments containing ash and/or nitrogen fertilizer, mixtures were
prepared in a 15 L Solo® (Solo Inc., Newport News, VA) backpack sprayer at rates of 8.0 g
of ash and/or 2.4 g of nitrogen fertilizer per liter of water. Based on the nutrient analysis for
the sprayer-compatible ash, the resulting nutrient concentrations are estimated to be 480 mg
kg⁻¹ for total N, 385 mg kg⁻¹ for P₂O₅, and 480 mg kg⁻¹ for K₂O, which is similar in
magnitude to the greenhouse experiment but more evenly balanced among the primary
nutrients. The foliar treatments were applied to the plots at a rate of 1,875 L ha⁻¹. This
equated to 3.6, 2.9, and 3.6 kg ha⁻¹ yr⁻¹ of N, P₂O₅, and K₂O, respectively; although these are
modest rates by row-crop standards, research suggests that similarly low fertilizer rates - with directed placement - can significantly increase the growth of young hybrid poplar trees in the field (van den Driessche, 1999). Monthly foliar applications to the regenerating trees were conducted on June 24, July 17, August 13, and September 7.

Tissue samples were collected on September 16 by clipping stems (four per plot) just below the tenth leaf measuring at least 2 cm long, per the Leaf Plastochron Index (LPI) method (Larson and Isebrands, 1971). The samples were washed under running water, the leaves were removed from the stems, and the material was oven-dried at 60° C. The tissue samples were then ground through a 0.4 mm screen and sent to the U.S. Forest Service Institute for Applied Ecosystem Studies in Rhinelander, WI, for analysis of nutrients (N, P, K, Ca, Mg, and Na), using the same equipment and methods previously described for the greenhouse experiment. Due to additional costs for the P analysis, only stems were analyzed for this nutrient. Upon cessation of growth, the trees in the inner plots were harvested and oven-dried at 100° C to determine the final biomass productivity for each plot.

The growth and nutrient data were evaluated for treatment effects with analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute Inc., Cary, NC). The response variables analyzed (covariables in parentheses) included: percent biomass regrowth (initial biomass [log-transformed]), stem nutrients (sample biomass), and leaf nutrients (sample biomass). Where treatment effects were statistically significant (P < 0.05), multiple comparisons analyses (with Tukey adjustment) were conducted to determine differences among the adjusted means of the treatments. In addition, statistical contrasts were conducted (“N” versus “Control”; and, “N+Ash” versus “N”) to further evaluate plant responses to the presence of nitrogen and ash in the treatments.
RESULTS

Greenhouse Experiment

The treatments were associated with significant differences (P < 0.001) in final tree height following nine foliar applications. Multiple comparisons analysis showed that the treatments containing nitrogen fertilizer significantly increased tree height, both with and without ash (Fig. 1). Significant treatment differences (P < 0.01) were also observed for shoot biomass, while root and cutting biomass were not significantly affected by the treatments (Fig. 2). Multiple comparisons analysis for shoot biomass showed that the treatments containing nitrogen fertilizer significantly increased shoot biomass, both with and without ash. Treatment differences were found to be non-significant (P = 0.82) for the ratio of shoot:root biomass (not shown). The statistical contrasts confirmed the aforementioned trends for tree height and shoot biomass; in addition, root biomass and cutting biomass were found to be significantly higher for the treatments containing nitrogen fertilizer, whereas the ash treatments did not significantly affect root or cutting biomass (Table 2).

The foliar treatments were associated with significant differences in the plant tissue content of N (shoots), P (shoots), K (roots and cuttings), and Na (shoots, roots, and cuttings), as illustrated in Figure 3. Multiple comparisons analysis for shoot N showed that the nitrogen fertilizer treatments significantly increased shoot N, both with and without ash (Fig. 3a). The ash alone significantly increased shoot P and root K compared to the control, and when mixed with nitrogen fertilizer the ash significantly increased shoot P and root K compared to the nitrogen fertilizer alone (Figs. 3b and 3c). For the cuttings, the nitrogen fertilizer treatments were associated with a significant decrease in K content relative to the control, whereas the ash treatments had no significant effect (Fig. 3c). The ash treatments
significantly increased the Na content of the shoots, roots, and cuttings, both with and without nitrogen fertilizer, with the exception of the roots in the ash-only treatment (Fig. 3f).

Most of these trends were confirmed by the statistical contrasts: significant increases for N (shoots) were found for the nitrogen treatments, and significant increases for P (shoots), K (roots), and Na (shoots, roots, and cuttings) were found for the ash treatments (Table 3). However, shoot N was found to be significantly lower in the ash treatments (whereas multiple comparisons had detected no difference). Additional trends were also identified by the contrasts: N (roots) and Na (roots) were significantly higher in the nitrogen treatments, while P (shoots) and K (cuttings and roots) were significantly lower; and, K (shoots) was significantly higher in the ash treatments, while Ca (shoots) and Mg (shoots and roots) were significantly lower.

**Field Experiment**

Treatment effects were not significant (P = 0.68) for biomass regrowth (not shown). The foliar treatments were, however, associated with significant differences in the plant tissue content of Ca (leaves) and Na (leaves and stems), as illustrated in Figure 4. Multiple comparisons analysis for leaf Ca showed that the ash with nitrogen fertilizer significantly increased Ca content compared to the nitrogen fertilizer solution alone, but not relative to the control (Fig. 4d). For stem and leaf Na, multiple comparisons analysis showed that the ash with nitrogen fertilizer significantly increased Na content, relative to both the control and the nitrogen fertilizer solution alone (Fig. 4f).

These trends were confirmed by the statistical contrasts: significant increases for Ca in the leaves and for Na in the stems and leaves were found with the ash with nitrogen
fertilizer compared to the nitrogen fertilizer alone (Table 4). Additional trends were also identified by the contrasts: K (leaves) was significantly higher for the nitrogen fertilizer treatment compared to control, while Ca (leaves) was significantly lower; and, Ca (stem) was significantly higher for the ash with nitrogen fertilizer versus the nitrogen fertilizer alone.

DISCUSSION

Greenhouse Experiment

The results of the greenhouse study show that hybrid aspen trees respond positively to foliar nitrogen fertilization, and that the increased growth does not alter the trees’ ratio of shoot:root biomass. This is consistent with previous research showing that fertilization accelerates poplar growth without altering biomass allocation, after accounting for differences in tree size (Coyle and Coleman, 2005). Because the presence of the ash did not reduce the growth effects of the nitrogen fertilizer solution, relative to the nitrogen fertilizer solution alone, the ash appears to be compatible with nitrogen fertilizers derived from UAN. The results also show no effects (positive or negative) of the ash on tree growth. Possible reasons for this include (i) the trees were nitrogen-limited, even with foliar nitrogen applications, which prevented the plants from benefiting from the nutrients in the ash, and/or (ii) hybrid aspen are not highly susceptible to deficiencies of the nutrients found in the ash.

The increases in tissue N associated with the nitrogen fertilizer treatments (see Table 3) indicate that the growth response described above is due to the uptake of foliar-applied N by the trees. The increases in P, K, and Na associated with the ash treatments indicate that some of the nutrients contained in the ash can be absorbed by the target plants; however, decreases in N, Ca, and Mg in certain plant tissues were also associated with the ash
treatments. Similarly, decreases in shoot P were also associated with the nitrogen fertilizer treatments.

Some of these changes in nutrient concentrations may be explained by specific nutritional needs of the plants, coupled with the need to maintain electrochemical balance in the plant tissue. Fernández and Eichert (2009) describe foliar uptake of plant nutrients through pores in the cuticle via the exchange of cations; some cations are absorbed, but in order to maintain electrochemical balance other cations are leached. Here, it would appear the cations K and Na were absorbed, while the cations Ca and Mg were leached. Based on the optimum nutrient levels described for hybrid poplars by Lteif et al. (2008), the hybrid aspen trees in our study were below sufficiency for K, but were within the sufficiency ranges for Ca and Mg; thus, it would appear the trees selectively exchanged cations based on nutritional needs. Sufficiency levels for Na in hybrid poplars were not available in the literature, but the absorbance of Na here may be explained by the ability of Na to substitute for K in certain physiological functions (Subbarao et al., 2003; Wakeel et al., 2011); as previously noted, shoot K was below sufficiency in this experiment. The cation exchange hypothesis is supported by a comparison of individual and total cations in the shoots (Table 5); the total cation content of the shoots was similar among treatments (3.0-3.3%), and was less variable than that of the individual cations, as demonstrated by the lower coefficient of variation (standard deviation of the treatment means as a percentage of the overall mean) for the total cations (5.2%) compared to the individual cations (6.5-95.1%).

One explanation for the decreases in shoot P associated with the nitrogen treatments and the decreases in shoot N associated with the ash treatments (see Table 3) may be that these two nutrients were affecting each other’s nutrient-use-efficiencies within the shoots.
Specifically, the increases in shoot P resulting from the ash treatments may have increased N-use-efficiency in the shoots, thereby reducing the need to transfer stored N from the cuttings to the shoots. Comparison of the distribution of nitrogen among plant tissues (Table 6) supports this hypothesis; a shift of 1-2% of total plant nitrogen from the cuttings to the shoots was observed for the “Control” treatment compared to “Ash”, and for the “N” treatment compared to “N+Ash”, while total N (mg tree\(^{-1}\)) was similar within each of these pairings. Similarly, the increases in shoot N resulting from the foliar nitrogen treatments may have increased P-use-efficiency in the shoot, which in turn may result in a shift of P from the shoot to other tissues (i.e. cutting or roots). However, because the cost of the P analysis precluded the other tissues from being analyzed for P, this particular hypothesis cannot be evaluated here.

**Field Experiment**

The results of the field study showed that neither the nitrogen fertilizer nor the ash significantly affected tree growth, nor did the ash significantly alter the effects of the nitrogen fertilizer. Plant tissue N for the control trees fell within the range of sufficiency for hybrid poplars (Lteif et al., 2008); thus, higher N availability in the field soil (compared to the potting media used in the greenhouse study) appears to explain this lack of response to nitrogen fertilizer. Because the trees did not respond to the ash treatment, even with sufficient nitrogen, it appears that hybrid aspen are not highly susceptible to deficiencies of the nutrients found in the ash.

The increases in Ca and Na associated with the ash treatments (see Table 4, Fig. 4d, Fig. 4f) suggests that some of the nutrients contained in the ash can be absorbed by the target
plants. Although the control trees were generally within the ranges of sufficiency for hybrid poplars (Lteif et al., 2008) for most nutrients, Ca and K were near their lower limits for sufficiency. Thus, similar to the greenhouse experiment, it appears nutrient uptake in the field may have been selective and based on nutritional needs, assuming that Na substitutes for K as previously discussed.

In summary, the results of the field experiment suggest that the ash is compatible with nitrogen fertilizer solution and contains nutrients that can be absorbed by the target plants, which is consistent with the results of the greenhouse experiment. However, the absorption of nutrients from the ash did not result in any statistically significant benefits to biomass productivity (even with sufficient levels of N in the plant tissues). This is consistent with findings from willow bioenergy plantations (Park et al., 2004), where increases in leaf litter nutrients were associated with ash applied to agricultural soils but biomass productivity was unaffected. Targeting crop species that are known to benefit from foliar applications of the nutrients prevalent in the ash (and particularly those nutrients shown here to be available for foliar absorption) should be considered for future testing.

**Crop Selection**

The effectiveness of foliar fertilizers varies by crop and by nutrient (Fernandez and Eichert, 2009). In general, though, crops are not highly responsive to foliar applications of primary nutrients (Fageria et al., 2009). This stems from the fact that crops often have large requirements for primary nutrients, and application rates for foliar fertilizers are limited to relatively small amounts by (i) the risk of foliar damage associated with high nutrient concentrations, and (ii) the desire of growers to minimize the number of passes through the
field with spraying equipment. Because fertilization rates for secondary nutrients and micronutrients are generally smaller than those for primary nutrients, a greater proportion of the crop’s requirement can potentially be met with foliar fertilization. Thus, responses to foliar fertilization may be more easily attained, particularly for nutrients having low availability in the soil (as may occur with iron) and/or poor mobility within the plant (as may occur with calcium).

For example, calcium deficiency in fruit trees is treated with approximately 10 kg ha\(^{-1}\) yr\(^{-1}\) of foliar calcium (for example Neilsen et al., 2005; Raese and Drake, 2006; Rosenberger et al., 2004). Further evaluation of the fly ash as a source of foliar calcium appears to be warranted based on (i) the prevalence of calcium in the fly ash (see Table 1), (ii) the ability of the fly ash to raise calcium levels of plant tissues as demonstrated in the field study (see Table 4), and (iii) the pre-existence of a market for foliar calcium in fruit production. The other nutrients found in the ash are not as likely to be feasible for foliar application due to the high application rates typically required for primary nutrients such as P and K, the apparent lack of markets for Na, and the relatively low levels of the remaining secondary nutrients and micronutrients in this particular ash. However, the nutrient content of biomass ash is known to vary by feedstock (Miles et al., 1996), and by other factors including boiler temperature (Misra et al., 1993); as such, additional work should be done to evaluate the possible foliar fertilizer uses of a wider selection of biomass ashes.

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The authors would like to thank POET and the U.S. Forest Service Institute for Applied Ecosystem Studies (IAES) for supporting this study. They would also like to thank
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REFERENCES


Table 1. Nutrient content of the non-compatible (NC) and sprayer-compatible (SC) biomass fly ash.

<table>
<thead>
<tr>
<th>Type</th>
<th>Plant Nutrient</th>
<th>NC (%)</th>
<th>SC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Nutrients</td>
<td>Total N</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>P&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>2.43</td>
<td>4.86</td>
</tr>
<tr>
<td></td>
<td>K&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>3.66</td>
<td>6.01</td>
</tr>
<tr>
<td>Secondary Nutrients</td>
<td>Ca</td>
<td>6.44</td>
<td>9.09</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>1.71</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.97</td>
<td>2.44</td>
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<tr>
<td>Micronutrients</td>
<td>Cu</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>1.88</td>
<td>1.78</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>0.13</td>
<td>0.13</td>
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<tr>
<td></td>
<td>Na</td>
<td>2.43</td>
<td>6.22</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>0.05</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 2. Results of statistical contrasts for tree growth parameters in the greenhouse experiment. Levels of statistical significance are P < 0.10 (*), P < 0.05 (**), and P < 0.01 (***)

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>Height</th>
<th>Shoot Biomass</th>
<th>Cutting Biomass</th>
<th>Root Biomass</th>
<th>Shoot:Root Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Effect</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>o</td>
</tr>
<tr>
<td>Sig.</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>**</td>
<td>--</td>
</tr>
<tr>
<td>Ash Effect</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>Sig.</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 3. Results of statistical contrasts for plant tissue nutrients in the greenhouse experiment. Levels of statistical significance are $P < 0.10$ (*), $P < 0.05$ (**), and $P < 0.01$ (***)$. Where significant for a particular nutrient, the effects are identified as being associated with an increase (+) or decrease (−) in the concentration of that nutrient in the plant. Cuttings and roots were not analyzed for phosphorus.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>---------------</th>
<th>---------------</th>
<th>---------------</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoots</td>
<td>Cuttings</td>
<td>Roots</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>N Effect</td>
<td>+</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Sig.</td>
<td>***</td>
<td>***</td>
<td>--</td>
</tr>
<tr>
<td>Ash Effect</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sig.</td>
<td>**</td>
<td>***</td>
<td>*</td>
</tr>
</tbody>
</table>

Table 4. Results of statistical contrasts for the field experiment. Levels of statistical significance are $P < 0.10$ (*), $P < 0.05$ (**), and $P < 0.01$ (***)$. Where significant, the effects are identified as being associated with an increase (+) or decrease (−) in the parameter. Leaves were not analyzed for phosphorus.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Biomass</th>
<th>---------------</th>
<th>---------------</th>
<th>---------------</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Regrowth</td>
<td>Stems</td>
<td>Leaves</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
<td>Ca</td>
</tr>
<tr>
<td>N Effect</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>Sig.</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Ash Effect</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>Sig.</td>
<td>--</td>
<td>--</td>
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</tr>
</tbody>
</table>
Table 5. Adjusted mean shoot content (% tree\(^{-1}\)) of individual cations (K, Ca, Mg, Na) and total cations in the greenhouse experiment. Standard deviations and coefficients of variation (CV, %) represent the variability among treatment means.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot K (% tree(^{-1}))</th>
<th>Shoot Ca (% tree(^{-1}))</th>
<th>Shoot Mg (% tree(^{-1}))</th>
<th>Shoot Na (% tree(^{-1}))</th>
<th>Total Cations (% tree(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.89</td>
<td>1.59</td>
<td>0.49</td>
<td>0.01</td>
<td>2.98</td>
</tr>
<tr>
<td>Ash</td>
<td>1.02</td>
<td>1.40</td>
<td>0.41</td>
<td>0.16</td>
<td>2.99</td>
</tr>
<tr>
<td>N</td>
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<td>0.53</td>
<td>0.02</td>
<td>3.31</td>
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<tr>
<td>N+Ash</td>
<td>0.97</td>
<td>1.51</td>
<td>0.39</td>
<td>0.14</td>
<td>3.01</td>
</tr>
<tr>
<td>St. Dev.</td>
<td>0.06</td>
<td>0.20</td>
<td>0.07</td>
<td>0.08</td>
<td>0.16</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.5</td>
<td>12.3</td>
<td>14.5</td>
<td>95.1</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Table 6. Adjusted mean plant tissue nitrogen (% of total tree nitrogen) and total tree nitrogen (mg tree\(^{-1}\)) for hybrid aspen in the greenhouse experiment.

<table>
<thead>
<tr>
<th>Treatment Pairings</th>
<th>Shoot N (% total N)</th>
<th>Cutting N (% total N)</th>
<th>Root N (% total N)</th>
<th>Total N (mg tree(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.1</td>
<td>37.7</td>
<td>11.2</td>
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<tr>
<td>Ash</td>
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<td>38.6</td>
<td>11.2</td>
<td>22.5</td>
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<tr>
<td>N</td>
<td>56.3</td>
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<td>N+Ash</td>
<td>54.4</td>
<td>34.7</td>
<td>10.9</td>
<td>24.8</td>
</tr>
</tbody>
</table>
Figure 1. Effects of treatments on final tree height (adjusted for initial tree height) after nine foliar applications. Error bars represent +/- 1 standard error of the mean. Statistically significant differences (P < 0.05) are represented by different letters above the treatments.
Figure 2. Effects of foliar treatments on adjusted mean biomass of cuttings, roots, and shoots after nine foliar applications in the greenhouse. Error bars represent +/- 1 standard error of the mean. Statistically significant differences (P < 0.05) are represented by different letters above the treatments.
Figure 3. Effects of treatments on plant tissue concentrations (adjusted for tissue biomass) of nitrogen (a), phosphorus (b), potassium (c), calcium (d), magnesium (e), and sodium (f), after nine foliar applications in the greenhouse. Error bars represent +/- 1 standard error of the mean. Statistically significant differences (P < 0.05) are represented by different letters above the treatments. Cuttings and roots were not analyzed for phosphorus.
Figure 4. Effects of treatments on plant tissue concentrations (adjusted for tissue biomass) of nitrogen (a), phosphorus (b), potassium (c), calcium (d), magnesium (e), and sodium (f), after four applications in the field. Error bars represent +/- 1 standard error of the mean. Statistically significant differences (P < 0.05) are represented by different letters above the treatments. Leaves were not analyzed for phosphorus.
CHAPTER 6. BIOCHAR AS A SUBSTITUTE FOR VERMICULITE IN POTTING MIX FOR HYBRID POPLAR ‘NM6’

A paper to be submitted to *Plant and Soil*

William L. Headlee, Catherine E. Brewer, and Richard B. Hall

**ABSTRACT**

The purpose of this study was to evaluate biochar as a substitute for vermiculite in potting mixes for unrooted vegetative cuttings of the hybrid poplar ‘NM6’ (*Populus nigra* L. × *P. suaveolens* Fischer subsp. *maximowiczii* A. Henry). We compared three treatments (peat moss [control], peat moss mixed with vermiculite, and peat moss mixed with biochar) at three times (pre-experiment, pre-fertilization, and post-fertilization). The biochar mix had significantly higher pH and cation exchange capacity, similar concentration and content of shoot N and K, and similar shoot and total tree biomass relative to the vermiculite mix; all of these were significantly higher than the control, except for shoot N concentration (pre- and post-fertilization) and shoot and total biomass (pre-fertilization). The biochar mix was also associated with lower root biomass than the vermiculite mix. Vector analyses indicate that all treatments were deficient in N at pre-fertilization, and that the control was also deficient in K. While the improved availability of K (and concomitant increase in shoot and total biomass) for the biochar mix may be due in part to greater K adsorption associated with higher CEC, luxury consumption of K at pre-fertilization for both the biochar and vermiculite mixes suggests further study is needed to separate CEC effects from the effects of “pre-
loaded” nutrients. We conclude that when substituted for vermiculite, biochar provides similar benefits to ‘NM6’ in terms of nutrient availability and growth. Additional research is recommended to determine whether these results hold for a wider selection of crops and biochars, as well as over longer time periods that include evaluation of survival and growth following out-planting to the field.

INTRODUCTION

Biochar is a high-carbon, porous co-product of biomass fast pyrolysis for the production of bio-oil (Brown 2003, Sohi et al. 2010). Biochar’s porosity results in high surface areas for biochar particles, which can serve a number of functions such as adsorbing nutrients and increasing cation exchange capacity (CEC) in soils (Atkinson et al. 2010, Laird et al. 2010a, Peng et al. 2011). Other observed benefits of adding biochar to the soil include increased water holding capacity (Jeffery et al. 2011, Karhu et al. 2011), improved pH (Yuan and Xu 2011), increased levels of certain plant nutrients (Major et al. 2010, Unger and Killorn 2011), and reduced nitrogen leaching and/or volatilization (Laird et al. 2010b, Taghizadeh-Toosi et al. 2012, Zhang et al. 2012).

While much research to date has focused on applying biochar to agricultural soils, biochar’s properties may also make it useful for greenhouse applications. For example, Graber et al. (2010) found that potting mix amended with biochar enhanced tomato and pepper plant growth, and Dumroese et al. (2011) found that peat moss amended with biochar pellets showed improved hydraulic water conductivity and water availability. However, we are unaware of any peer-reviewed studies in the published literature which have tested biochar as a substitute for vermiculite, which is commonly used in greenhouses to improve
the CEC of potting mixes. Such an application would capitalize on biochar’s ability to adsorb nutrients, while avoiding issues of reduced efficacy of herbicides that may occur with field applications (Grabert et al. 2012, Nag et al. 2011, Sun et al. 2011). Daily greenhouse watering may help to further capitalize on this biochar property, as an environment of alternating saturated and unsaturated conditions appears to speed the development of CEC in biochar (Nguyen and Lehmann 2009, Singh et al. 2010).

The purpose of this study was to evaluate biochar as a substitute for vermiculite in potting mixes for hybrid poplars grown from vegetative cuttings. Hybrid poplars were selected because they are a short-rotation woody crop with potential as a feedstock for bio-energy production (Goerndt and Mize 2008, Zalesny et al. 2009), and they are readily propagated from vegetative cuttings. We compared three treatments: peat moss (control), peat moss mixed with vermiculite, and peat moss mixed with biochar. Chemical properties (pH, CEC, and effective CEC [ECEC]) and nutrient content (total N and exchangeable K, Ca, Mg, and Na) of the potting mixes were measured at three times (pre-experiment, pre-fertilization, and post-fertilization) to gauge their inherent nutrient content and their ability to adsorb nutrients. Trees were destructively sampled at pre-fertilization and at post-fertilization to determine the effects of the treatments on selected variables: tree biomass (shoot, root, cutting, and total); nutrient concentrations (N, K, Ca, Mg, and Na); and total nutrient content (N, K, Ca, Mg, and Na). These variables were first analyzed individually with analysis of variance, and then analyzed simultaneously using the vector analysis method. In vector analysis, the variables are graphed for each treatment relative to a reference condition (i.e. control), whereby the direction and magnitude of the differences from the reference condition indicate the nature and strength of nutrient responses. This method allows for the diagnosis
of plant nutrient status, and it has been applied to hybrid poplars in previously-published studies (Timmer 1985, Lteif et al. 2008).

MATERIALS AND METHODS

Biochar Material

The biochar used in this study was produced at the Iowa State University BioCentury Research Farm (Boone, IA) on a pilot scale (8 kg hr⁻¹) bubbling fluidized bed fast pyrolyzer. The red oak feedstock was ground to a particle size of <600 μm prior to fast pyrolysis at 500°C. The sand bed was fluidized with N₂. Biochar was collected by cyclone from the product stream with an approximate yield of 12-15%.

Greenhouse Experiment

Three potting media treatments were evaluated: peat (100% peat moss; control), vermiculite mix (75% peat moss and 25% vermiculite by volume), and biochar mix (75% peat moss and 25% biochar by volume). A randomized complete block design was used; treatments were randomly assigned to 236 cm³ Accelerator® containers (Nursery Supplies Inc., Chambersburg, PA) in each of three trays (blocks). Each tray held 32 containers: 12 peat, 10 vermiculite mix, and 10 biochar mix, for a total of 96 containers (each of which was filled with 225 cm³ of the assigned mix). Unrooted vegetative cuttings (10 cm long) of the hybrid poplar ‘NM6’ (*Populus nigra* L. × *P. suaveolens* Fischer subsp. *maximowiczii* A. Henry) were soaked in water for 24 hours, planted in the containers (one tree per container), and initial cutting diameters were recorded. The trays were placed in a bench-scale humidity tent (consisting of opaque plastic sheeting supported by PVC pipe) for the first three weeks,
and then on open benches for the remainder of the experiment. They were sub-irrigated continuously over the first four weeks, and twice daily for 30 minutes at a time over the remainder of the experiment.

Half of the trees per treatment were destructively sampled prior to fertilization (six weeks after planting), and the other half were destructively sampled following fertilization (eight weeks after planting). Fertilizer solution was prepared by dissolving dry 15-30-15 fertilizer in water (3.6 g L\(^{-1}\)); the solution was applied at a rate of 35 mL tree\(^{-1}\) at the start of week seven and 70 mL tree\(^{-1}\) at the start of the week eight. Destructive sampling consisted of separating the tree tissues (shoots [stems + leaves], roots, and cuttings) and oven-drying the tissues at 50°C to obtain the dry weights prior to tissue nutrient content analysis. Due to the small amount of root material available for most trees in the pre-fertilization harvest, root samples from up to five trees were bulked by treatment, resulting in a total of 18 root samples (rather than 48) for the pre-fertilization harvest.

For the potting media, samples of the unused mixes were collected to determine their pre-experiment chemical properties and nutrient contents. To determine pre-fertilization and post-fertilization effects, the media from each container was collected during destructive sampling of the trees and bulked by tray for each treatment. The media was then oven-dried at 50°C prior to analysis of chemical properties and nutrient contents. Because the potting mixes were bulked by tray, the data was evaluated as a completely randomized design, with the three trays serving as replicates (3 treatments × 3 sample times × 3 replicates = 27 total samples).
Laboratory Analyses

The plant tissues and potting mixes were sent to the U.S. Forest Service Institute for Applied Ecosystem Studies in Rhinelander, WI, where they were ground through a 0.5 mm screen prior to analysis. For both the plant tissues and the potting mixes, total N content was determined with a Flash EA1112 N-C analyzer with a model MAS 200 autosampler (Thermo Electron, via CE Elantech, Inc., Lakewood, NJ). For the remaining plant tissue nutrients, atomic emission spectroscopy (AES) was conducted using a Varian Agilent model 240FS atomic absorption spectrophotometer (Agilent Technologies, Englewood, CO) following nitric acid digestion. For the potting mixes, exchangeable base cations (K, Ca, Mg, and Na) were extracted with hexa-amine cobalt (Co) chloride and analyzed via AES. CEC was determined by summing the base cations, and ECEC was determined from the difference of the Co level measured compared to the initial Co level as described by Ciesielski and Sterckeman (1997). Potting mix pH was measured by adding potting mix (1 g) to 5 mL of dilute CaCl (0.01 mol L⁻¹), shaking for 1 hour, then measuring with an AccuCap combination pH electrode and Accumet Model No. XL50 pH meter (Fisher Scientific, Waltham, MA, USA).

Data Analyses

For the statistical analyses, all data were evaluated as a two-way factorial (treatment × time) with analysis of variance (ANOVA) using PROC GLM in SAS (SAS Institute Inc., Cary, NC). Each tissue was evaluated (with initial cutting diameter as a covariate) for biomass and nutrient concentration. In addition, the nutrient content of each tissue (determined by multiplying the measured nutrient concentration by the dry weight of the
tissue) and total biomass (determined by summing the dry weights of the tissues) were similarly evaluated, again with cutting diameter as a covariate. Potting mix nutrients, CEC, and ECEC data were converted to units of mg container$^{-1}$ and meq container$^{-1}$ by multiplying the measured values (mg kg$^{-1}$ or meq kg$^{-1}$) by the potting media bulk density (kg container$^{-1}$) prior to statistical analysis. Whenever treatment, time, or treatment × time interactions were found to be significant (P < 0.05), multiple comparisons analyses with Tukey adjustments were conducted to identify statistically significant differences between the adjusted least-squares means.

Vector analysis was conducted using the adjusted least-squares means of the shoot parameters (specifically shoot biomass, nutrient concentrations, and nutrient contents). The peat treatment at pre-fertilizer was used as the reference condition (relative value = 100 for all shoot parameters), and the relative values for all other treatment × time combinations were calculated by dividing the measured value by that of the reference condition and then multiplying by 100. These relative values were graphed for each nutrient to compare treatment effects on nutrient status, based on the typical interpretation of vector analysis diagrams (Fig. 1; adapted from Lteif et al. 2008). These interpretations reflect the status of the treatment plants relative to the control plants, and can be summarized as: (A) growth dilution (increased biomass and nutrient content with decreased nutrient concentration); (B) sufficiency (increased biomass and nutrient content with no change in nutrient concentration); (C) deficiency (increased biomass, nutrient content, and nutrient concentration); (D) luxury consumption (no change in biomass with increased nutrient content and concentration); (E) toxicity (decreased biomass with increased nutrient concentration and increased or decreased nutrient content); (F) antagonism (decreased
biomass, nutrient concentration, and nutrient content), and (G) retranslocation (little or no increased biomass with decreased nutrient concentration and nutrient content). For a more thorough description of vector analysis and its applications, see Haase and Rose (1995).

RESULTS

Potting Mix Properties and Nutrients

Analyses of potting mixes’ chemical properties and nutrient contents indicated significant treatment and time effects for most of the parameters, with significant interactions for pH (Table 1). The biochar mix had significantly higher CEC and ECEC than both the peat and the vermiculite mix (Table 2). The vermiculite mix had significantly lower total N and higher exchangeable K than both the biochar mix and the peat, with the biochar mix also being significantly higher in K than the peat. The biochar mix had significantly higher exchangeable Ca and Mg than the peat and the vermiculite mix, with the peat also being significantly higher than the vermiculite in both cases. The biochar mix had significantly higher Na than the peat, with the vermiculite mix being intermediate.

Time effects are also shown in Table 2. CEC increased significantly from pre-experiment to pre-fertilization, along with exchangeable Ca, Mg, and Na (which were likely introduced via the tap water used for irrigation). ECEC increased significantly from pre-experiment to post-fertilization, with pre-fertilization being intermediate. Exchangeable K significantly decreased from pre-experiment to pre-fertilization, and significantly increased from pre-fertilization to post-fertilization. Time differences were not significant for total N.

As noted above, significant treatment × time interactions were found for pH. The peat showed no significant change in pH over time, whereas the other two treatments both showed
significant increases from pre-experiment to pre-fertilization and significant decreases from pre-fertilization to post-fertilization (Fig. 2).

**Tree Biomass Productivity**

The biomass productivity data showed significant treatment × time interactions for total biomass and for most tissues (Table 3), with the exception of cutting biomass which showed only a significant time effect (whereby cutting biomass increased significantly from 2.71 g plant$^{-1}$ at pre-fertilization to 2.94 g plant$^{-1}$ at post-fertilization). Treatment × time interactions for the remaining biomass parameters are illustrated in Figure 3. While the treatments did not differ significantly in shoot or total biomass at pre-fertilizer, and all treatments increased from pre-fertilizer to post-fertilizer, the biochar and vermiculite treatments had significantly higher shoot and total biomass than the peat at post-fertilizer (Fig. 3a, 3d). Root biomass also did not differ significantly between treatments at pre-fertilizer, and increased for all treatments from pre-fertilizer to post-fertilizer, but at post-fertilizer the vermiculite treatment had significantly higher root biomass than biochar while peat was intermediate (Fig. 3b). No significant interactions were detected for cutting biomass (Fig. 3c); however, it was included in order to illustrate its contribution to total biomass.

**Plant Nutrient Concentrations and Contents**

Shoot nutrient concentrations showed mainly treatment and/or time effects, with only shoot K concentration showing a significant treatment × time interaction (Table 4). Conversely, shoot nutrient contents showed significant interactions for all nutrients evaluated.
Shoot N concentration was significantly higher with the peat than with the other two treatments (Table 5). Shoot Ca concentration was significantly lower with the vermiculite treatment than with the others, and shoot Mg concentration was significantly lower with the biochar treatment than with the others. No significant treatment differences were observed for shoot Na concentration. The treatment × time interaction for shoot K concentration (Fig. 4) shows that the biochar and vermiculite treatments were significantly higher than the peat at both pre-fertilizer and post-fertilizer, but did not change from pre-fertilizer to post-fertilizer whereas the peat treatment increased in shoot K concentration.

Treatment × time interactions for shoot nutrient contents are illustrated in Figure 5. For shoot N, Mg, and Na content, no significant differences between treatments were observed at pre-fertilizer and all treatments increased from pre-fertilizer to post-fertilizer, but the biochar and vermiculite were significantly higher than peat at post-fertilizer. For shoot Ca content, no significant differences between treatments were observed at pre-fertilizer and all treatments increased from pre-fertilizer to post-fertilizer, but the biochar was significantly higher than the other two treatments at post-fertilizer. For shoot K content, the biochar and vermiculite treatments were significantly higher than peat both at pre-fertilizer and at post-fertilizer, with the difference being larger at post-fertilizer.

Vector Analysis

The vector analysis diagrams (Fig. 6) illustrate the relative shoot nutrient concentrations, shoot nutrient contents, and shoot biomass for each treatment at pre-fertilization (small symbols), and at post-fertilization (large symbols), relative to the control (peat) at pre-fertilization. Vectors show the differences between treatments at pre-fertilization
(dotted lines), and the changes for each treatment from pre-fertilization to post-fertilization (dashed lines).

For nitrogen (Fig. 6a), the biochar and vermiculite vectors at pre-fertilization showed a shift toward lower N concentration and slightly lower N content along with slightly higher shoot mass, which is indicative of possible retranslocation to other tissues (although statistical analyses of cuttings and roots showed no significant treatment effects for N concentration or content; results not shown). At post-fertilization, all treatments shifted toward higher N concentration, N content, and shoot mass; this indicates all treatments were deficient in N prior to fertilization.

The biochar and vermiculite vectors at pre-fertilization showed a shift toward higher K concentration and higher K content along with only slightly higher shoot mass, which is indicative of luxury consumption (Fig. 6b). At post-fertilization, the biochar and vermiculite treatments shifted toward higher total K content and shoot mass with little change in K concentration, while with peat all three of these increased; this indicates the biochar and vermiculite were sufficient in K prior to fertilization, whereas the peat was deficient.

With calcium (Fig. 6c), the vermiculite vector at pre-fertilization showed a shift toward lower Ca concentration and slightly lower Ca content along with slightly higher shoot mass; this indicates possible retranslocation of Ca to other tissues (although statistical analyses of cuttings and roots showed no significant treatment effects for Ca concentration or content; results not shown). At post-fertilization, all three treatments increased slightly in Ca concentration, with relatively larger increases in total Ca and shoot mass; this is indicative of a slight deficiency for all treatments.
As shown in Fig. 6d, the biochar vector at pre-fertilization showed a shift toward lower Mg concentration but slightly higher Mg content and shoot mass, whereas the vermiculite vector showed a shift toward higher Mg concentration along with slightly higher Mg content and shoot mass; this is indicative of growth dilution for biochar, and luxury consumption for vermiculite. At post-fertilization, all three treatments shifted toward higher Mg content and shoot mass with little change in Mg concentration; this indicates the treatments were sufficient in Mg.

The biochar and vermiculite vectors at pre-fertilization showed a shift toward slightly lower Na concentration along with slightly higher Na content and shoot mass, which indicates slight growth dilution for biochar and vermiculite (Fig. 6e). At post-fertilization, all three treatments increased slightly in Na, with larger increases in total Na and shoot mass; this is indicative of a slight deficiency for all treatments.

**DISCUSSION**

The results of this study demonstrate that biochar is a suitable replacement for vermiculite in potting mixes for the hybrid poplar ‘NM6’ when substituted on a volume basis. The biochar mix had higher pH, CEC, and ECEC than both peat and the vermiculite mix (see Fig.2 and Table 2), resulted in similar shoot and total biomass productivity as the vermiculite mix (both mixes being higher than the peat; see Fig. 3), and resulted in similar concentrations and contents of shoot N and K as the vermiculite mix (see Figs. 4 and 5, and Table 6). The vector analysis diagrams suggest that the trees growing in the biochar and vermiculite mixes were limited primarily by N, whereas the trees growing in peat were
limited by both N and K (see Figs. 6a and 6b); thus, the improved biomass productivity of the trees growing in biochar and vermiculite is likely due to higher K availability.

The luxury consumption of K associated with the biochar and vermiculite treatments at pre-fertilizer (see Fig. 6b) indicate that the increased availability of K was due at least in part to the nutrient being “pre-loaded” in the mixes, rather than simply a superior ability to adsorb K from the soil solution. However, the higher CEC and ECEC values for biochar suggest that superior availability of K (as well as other cations) may be sustained over longer periods. Additional research to test this hypothesis is therefore recommended.

Other differences between the biochar and vermiculite mixes were observed, but did not appear to be significant factors in shoot or total biomass productivity. For example, total N was higher in the potting mix for biochar compared to vermiculite (Table 2), and exchangeable K was higher in the potting mix for vermiculite compared to biochar (Fig. 3); however, these differences did not translate to differences in shoot or total productivity, nor even to differences in concentrations or contents of the nutrients within the plants. In the case of N, it is possible that the higher total N for biochar represents a difference in fixed N rather than available N; whereas for K, it is likely that the higher exchangeable K for vermiculite represents a surplus supply.

Similarly, significant differences between the biochar and vermiculite treatments were observed for Ca and Mg in the potting mixes. Specifically, the biochar mix had higher exchangeable Ca and Mg than the vermiculite mix (see Table 2). This corresponded with higher shoot nutrient concentration and content of Ca for the biochar mix, whereas the vermiculite mix resulted in higher shoot nutrient concentrations and content of Mg (see Table 5 and Fig. 5). Although these differences did not significantly impact shoot or total biomass
(see Fig. 3), they may be important for other species which frequently suffer from deficiencies in these nutrients (such as Ca deficiency in tomatoes), and therefore additional research with a wider variety of crops is recommended.

Previous research by Graber et al. (2010) showed that pepper and tomato plant growth was significantly enhanced by addition of biochar to their potting mix. They concluded this was not due to improved nutrient availability (based on a lack of significant differences in leaf nutrient concentrations), and hypothesized that instead the biochar may have stimulated beneficial soil microbes and/or contained non-nutrient chemicals that directly stimulated plant growth. However, it is important to note that their fertilizer regime (fertigation applied 2-3 times daily throughout the experiment) may have supplied sufficient plant nutrients via the soil solution, making the ability of the growing media to adsorb nutrients from the soil solution a moot point. Our study, on the other hand, purposefully induced sub-optimal nutrient conditions to test for differences in nutrient adsorption by the soil and, in turn, uptake by the plants. As such, our study demonstrates that biochar may enhance plant growth via improved nutrient availability under sub-optimal nutrient conditions.

It is important to note that the biochar treatment was associated with lower root biomass than the vermiculite treatment at post-fertilization (see Fig. 3). This may constitute a plant response to the improved availability of certain nutrients in the biochar; alternatively, it may be related to the bulk density of the biochar mix being approximately 50% greater than that of the vermiculite mix, which may result in lower oxygen availability and thereby reduce root growth. The latter hypothesis appears to be supported by Dumroese et al. (2011), who found that pelleted biochar mixed with peat at the same ratio used in our study (25% biochar
and 75% peat by volume) reduced air-filled porosity from 47% to 38% and lowered relative oxygen diffusivity by approximately half compared to peat alone. While it did not translate to significant differences in shoot or total biomass productivity in our study, this reduction in root biomass could affect survival and growth of the trees when planted in the field, where nutrients and water (especially) may be less available than in the greenhouse. Thus, additional research is recommended to evaluate out-planting success.

Finally, it has been established that biochars derived from different feedstocks and under different pyrolysis conditions have different physical and chemical properties (Brewer et al. 2009). As such, additional testing with a variety of biochars is needed to compare how the selection of feedstocks and processes affect the ability of different biochars to serve as substitutes for vermiculite. The costs associated with different feedstocks and processes will also be important in determining the most economical substitute for vermiculite, which in the greenhouse industry commands a price of US $135 to $155 m⁻³ (approximately $1,500 Mg⁻¹) based on supplier catalog pricing (BFG Supply Co., Burton, OH).

ACKNOWLEDGEMENTS

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Table 1. ANOVA results for potting mix chemical properties (pH, CEC, ECEC) and nutrient content (total N and exchangeable K, Ca, Mg, Na). Statistically significant effects (P < 0.05) are depicted in bold.

<table>
<thead>
<tr>
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<tbody>
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<td>0.2642</td>
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</table>

Table 2. Adjusted least-squares means for potting mix chemical properties and nutrients, by treatment and time. Significant differences between means (P < 0.05) are indicated with different letters within the column. Units of measure for the parameters are: CEC and ECEC (meq container⁻¹); total N and exchangeable K, Ca, Mg, and Na (mg container⁻¹). Results for pH are not shown here due to significant treatment × time interactions.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>ECEC</th>
<th>N</th>
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<td>34.6 b</td>
<td>306 a</td>
<td>61.3 a</td>
<td>19.2 a</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Experiment</td>
<td>16.4 b</td>
<td>19.9 b</td>
<td>318</td>
<td>38.2 a</td>
<td>230 b</td>
<td>46.1 b</td>
<td>2.6 b</td>
</tr>
<tr>
<td>Pre-Fertilizer</td>
<td>20.8 a</td>
<td>23.2ab</td>
<td>336</td>
<td>24.0 b</td>
<td>288 a</td>
<td>57.7 a</td>
<td>23.6 a</td>
</tr>
<tr>
<td>Post-Fertilizer</td>
<td>21.7 a</td>
<td>24.5 a</td>
<td>356</td>
<td>42.1 a</td>
<td>294 a</td>
<td>58.9 a</td>
<td>25.2 a</td>
</tr>
</tbody>
</table>

Table 3. ANOVA results for tree biomass (shoot, BS; root, BR; cutting, BC; and total, BT).

Statistically significant effects (P < 0.05) are depicted in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>BS</th>
<th>BR</th>
<th>BC</th>
<th>BT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
<td>0.4976</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt x Time</td>
<td>&lt;0.0001</td>
<td>0.0271</td>
<td>0.2483</td>
<td>0.0057</td>
</tr>
</tbody>
</table>
Table 4. ANOVA results for shoot nutrient (N, K, Ca, Mg, and Na) concentrations and content. Statistically significant effects (P < 0.05) are depicted in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>N</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>N</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0.0011</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.1998</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time</td>
<td>&lt;0.0001</td>
<td>0.2190</td>
<td>&lt;0.0001</td>
<td>0.7090</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt x Time</td>
<td>0.0551</td>
<td>&lt;0.0001</td>
<td>0.4567</td>
<td>0.1912</td>
<td>0.4196</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>0.0021</td>
</tr>
</tbody>
</table>

Table 5. Adjusted least-squares means for shoot nutrient concentrations (N, Ca, Mg, and Na; %), by treatment and time.

Significant differences between means (P < 0.05) are indicated with different letters within the column for a given effect. Results for shoot K concentration and shoot nutrient contents (N, K, Ca, Mg, and Na) are not shown here due to significant treatment × time interactions.

<table>
<thead>
<tr>
<th>Effect</th>
<th>N</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peat</td>
<td>2.19 a</td>
<td>0.77 a</td>
<td>0.23 b</td>
<td>0.028</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>2.03 b</td>
<td>0.64 b</td>
<td>0.26 a</td>
<td>0.026</td>
</tr>
<tr>
<td>Biochar</td>
<td>1.97 b</td>
<td>0.77 a</td>
<td>0.21 c</td>
<td>0.028</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Fertilizer</td>
<td>1.60 b</td>
<td>0.68 b</td>
<td>0.23</td>
<td>0.025 b</td>
</tr>
<tr>
<td>Post-Fertilizer</td>
<td>2.52 a</td>
<td>0.78 a</td>
<td>0.23</td>
<td>0.030 a</td>
</tr>
</tbody>
</table>
Figure 1. Example of vector analysis diagram and the interpretations associated with shifts in shoot biomass (m), shoot nutrient concentration (c), and shoot nutrient amount (a), for each vector (A-G) relative to the reference condition (R); adapted from Lteif et al. (2008).
Figure 2. Adjusted least squares means (+/- 1 standard error) for potting mix pH. Statistically significant differences (P < 0.05) are indicated with different letters above the bars.
Figure 3. Adjusted least squares means (+/- 1 standard error) for shoot (a), root (b), cutting (c), and total (shoot + root + cutting) biomass (d). Statistically significant differences (P < 0.05) are indicated with different letters above the bars.
Figure 4. Adjusted least squares means (+/- 1 standard error) for shoot K concentration. Statistically significant differences (P < 0.05) are indicated with different letters above the bars.
Figure 5. Adjusted least squares means (+/- 1 standard error) for total shoot content of N (a), K (b), Ca (c), Mg (d), and Na (e). Statistically significant differences (P < 0.05) are indicated with different letters above the bars.
Figure 6. Vector diagrams showing relative shifts associated with biochar (◇), vermiculite (Δ), and peat (□) treatments at pre-fertilizer (small symbols) and post-fertilizer (large symbols) for shoot N (a), K (b), Ca (c), Mg (d), and Na (e). In all cases the initial reference condition (shoot mass, nutrient concentration, and nutrient content = 100) is peat at pre-fertilizer.
CHAPTER 7. GENERAL CONCLUSIONS

KEY FINDINGS AND RECOMMENDATIONS

The results of the alleycropping study demonstrated that total aboveground biomass productivity was not significantly affected by topographic position, with the exception of the floodplain in the first year, where weed pressure was high. Thus, the hybrid aspen ‘Crandon’ appears to be relatively versatile in its placement on the landscape. Fertilizer placed in the planting hole had positive and statistically significant effects; the trees receiving the highest fertilizer rate had nearly twice as much biomass as those receiving none. Fertilizer rate and age were found to be relatively strong predictors for total aboveground biomass, but not for branch fraction which was better predicted by tree size. The significant effects observed for blocks were likely due to higher rates of deer damage at the north end of the site, where deer were frequently observed. In general, productivity was lower than that observed in previous studies of ‘Crandon’ in Iowa; further research is recommended to determine whether these trees may be negatively affected by triticale (e.g. competition and/or allelopathy).

The study of root sprout inventory methods and row thinning indicated that variable-radius plot sampling is a feasible approach for inventorying dense stands of hybrid aspen root sprouts. Similar estimates of root sprout density and harvestable biomass were attained with BAFs of 1.56 and 2.78 m² ha⁻¹. The latter did so with roughly half as many measurement trees, but also had a larger confidence interval; thus, a trade-off exists between reducing sampling time and obtaining more precise estimates. Using a BAF of 6.22 m² ha⁻¹ resulted in even wider confidence intervals, and lower estimates of root sprout density. The row-thinning equation developed in the study was effective for predicting the size of the largest
gap in the row based on the number of sprouts in the row, which (in conjunction with inventory data for sprout density) dictates the appropriate width of the unharvested row for a desired maximum gap size. Additional testing of the equation under a wider variety of environmental, age, and stocking conditions is recommended.

The results of the regional modeling study suggest that, as parameterized and calibrated here, 3-PG appears well-suited for modeling hybrid poplar aboveground biomass productivity. Linear regression of actual versus predicted total aboveground biomass for the validation dataset demonstrated a strong fit ($R^2 = 0.89$, RMSE = 8.1 Mg ha$^{-1}$). When used to map mean annual biomass productivity (total aboveground dry biomass divided by age), predicted values ranged from 4.4 to 13.0 Mg ha$^{-1}$ yr$^{-1}$ across Minnesota and Wisconsin, with the highest productivity mainly concentrated in the area stretching from south-central Minnesota across southern Wisconsin. Additional work can and should be done to further improve model fit by determining clone-specific values for the physiological parameters, estimating full canopy age and fertility rating without prior knowledge of yields, and adapting the model to other clones and regions.

The study of biomass fly ash as foliar fertilizer demonstrated that several nutrients in the fly ash were absorbed by hybrid aspen both in the greenhouse and in the field; however, this absorption did not significantly affect tree growth in either setting. The ash appeared to be compatible with foliar nitrogen fertilizer, as inclusion of the fly ash did not significantly alter the effects of the nitrogen fertilizer on tree growth. Additional research should be done with crops known to benefit from foliar application of the nutrients found in the ash, particularly the nutrients shown in this study to be available for uptake by plants.
The results of the biochar study suggest that, when substituted on a volume basis, biochar can be an effective substitute for vermiculite. The biochar and vermiculite mixes similarly produced higher shoot and total biomass than the peat moss control; vector analysis indicates that this was primarily due to superior availability of K. The increased availability of K may be related to the elevated initial levels of K detected in the biochar and vermiculite mixes, rather than a superior ability of the mixes to adsorb cations from the soil solution. As such, future studies should attempt to separate such “pre-loading” effects from CEC effects, as well as test a wider selection of crops and biochars. In addition, the biochar mix was associated with lower root biomass than the vermiculite mix. Additional research is needed to determine whether this affects long-term growth and survival of the trees, particularly after out-planting to the field.

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