ABSTRACT

SHIFFLETT, SHAWN DAYSON. Species Trials of Short Rotation Woody Crops on Two Waste Water Application Sites. (Under the direction of Elizabeth Guthrie Nichols).

Forty-two *Populus spp.* clones, *Eucalyptus benthamii*, and seven tree species native to North Carolina were evaluated for survival and height growth throughout the first-year establishment phase at two municipal wastewater application sites. Groundwater was monitored at each site to determine if establishment of the species trials resulted in exceedances of nutrient mitigation requirements. At the Gibson Wastewater Treatment Facility, 26 *Populus* clones had 100% survival and first year mean height growths between 152 cm to 260 cm. Green ash, planted in 2011 and 2012, had high survivorship (> 95%) with first year mean height growth of 30 cm $\pm$ 28 cm (2012) and second year mean height growth of 101 cm $\pm$ 52 cm (2011). *Eucalyptus benthamii* had moderate survivorship (> 77%) and first year mean height growth of 47 cm $\pm$ 27 cm. At the Jacksonville Wastewater Treatment Facility, green ash and bald cypress had high survivorship (> 96%) and first year mean height growths of 14 cm $\pm$ 25 cm and 27 cm $\pm$ 16 cm, respectively. Survivorship for twelve *Populus* clones was between from 50-94% with mean first year height growths between 58 cm to 121 cm. *Eucalyptus benthamii* had low survivorship (43%) with mean first year height growths of 17 $\pm$ 17 cm. Groundwater concentrations of NO_3+NO_2 and N-NH_4 remained below regulatory requirements at both sites with one exceedance in February 2012 in Jacksonville, NC. The results of this study show that some *Populus* clones are excellent candidates for woody biomass production on municipal wastewater application fields. Native green ash and bald cypress are also

good candidates, but these trees may require longer rotations than *Populus* to achieve similar biomass yields. .

Species Trials of Short Rotation Woody Crops on Two Waste Water Application Sites in North Carolina

by
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BIOGRAPHY

Shawn Dayson Shifflett was born August 11, 1984 in Fairfax, Virginia to Larry Shifflett and Carol Ann Goins. Spending much of his childhood moving around the east coast, Shawn acquired his diploma from Franklinton High School.

Having a strong interest in Public Health, Shawn attending the UNC School of Public Health for his Bachelor of Science and focused on environmental microbiology and toxicology. After gaining his B.S., he volunteered with the United States Peace Corps in El Salvador working in Rural Health and Sanitation until 2008. Shortly after his return, Shawn was employed by Duke Family and Community Medicine as a bilingual health education specialist where he refined his Spanish skills and has since been credentialed as a bilingual interpreter.

In 2010, Shawn decided to return to academia for a higher education degree in Natural Resources at North Carolina State University. His time at NCSU has inspired greater interest in hydrology, environmental impact assessment, and bioenergy. In his spare time, Shawn works on developing vermiculture compost systems, aeroponics for growing an array of vegetables in his apartment, and making coffee.

Shawn plans to continue his research and pursue his PhD in the Spring of 2013.

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CHAPTER 1: Introduction

Short rotation woody crops (SRWCs) are intensively managed tree plantations characterized by closely-spaced tree plantings and shorter harvest rotations (1 to 20 years) than conventional forest practices [1- 3]. Tree density for these systems can range from 5000 – 20,000 stems·ha^{-1} but can be as low as 1000 to 2500 stems·ha^{-1} [4]. In the southeastern U.S.A., common species in SRWC systems are *Populus spp*., *Eucalyptus spp.*, and *Pinus spp.* although research on SRWC systems first began in the 1960s by evaluating "sycamore silage" [4, 5]. In the 1970s and 1980s, SRWCs were part of a national energy research focus to develop renewable energy resources [4, 6].

SRWCs plantations are a key component to meet 2021 renewable energy goals set forth by state and federal agencies in the United States [8-10] although these production systems can also provide fiber and conventional forest products for wood manufacturing [7]. Large scale production of SRWCs may also provide other environmental benefits such as the reduction of greenhouse gases, water and soil conservation, and nutrient sequestration [11, 12]. There are concerns about the potential expansion of SRWC plantations across the United States. One major controversy is where SRWC plantations should be established. Tenenbaum [13] has argued that promoting energy production from bioenergy sources will lead to the conversion of productive food agricultural lands for energy and fiber production, potentially causing food shortages. High water demand by these plantations is a second concern [14, 15].

Some researchers have proposed that degraded or marginal lands can provide significant SRWC biomass production without compromising food production or the conventional management of forested lands [16-19]. Marginal lands represent a small but important source for SRWC production at the global scale [16]. Abandoned and degraded lands could amount to 10% to 52% of current liquid fuel consumption by converting these lands to SRWCs production [18]. Municipal wastewater application fields are a potential marginal land resource for SRWC production, but these lands not always defined within the framework of marginal or degraded lands [19 - 23]. Studies in the 1980s considered these lands to be “disturbed lands and marginal lands,” [24] that represent valuable production acreage to be sourced for biomass and bioenergy generation.

Various studies have evaluated SRWC production, particularly *Populus spp.* and *Eucalyptus spp.*, on municipal wastewater application lands in the United States, Europe, Australia, and the Middle East [25 - 28]. Hopmans et al. [25] observed that species selection played a critical role for biomass production in Australia where biomass production was greatest for *Populus spp*. and *Eucalyptus spp.*, followed by *Casuarina cunninghamiana*, (Miq.), and *Pinus radiate* (D. Don). Borjesson and Berndes [29] showed that SRWC production on wastewater application sites had added economic value due to the removal of nutrients and minimization of eutrophication in Swedish surface waters. Dimitriou and Rosenqvist [30] found that fertilizing *Salix* SRWCs with wastewater increased biomass production and reduced fertilization costs. Shah et al. [31] concluded that *Eucalyptus* seedlings grew more when irrigated with wastewater versus tap water in Pakistan. Zalesny et al. [32] successfully established *Populus spp.*, *Eucalytpus spp.*, *Pinus spp.*, *Khaya ivorensi*, African mahogany *(*A. Chev.), *Tectona* *grandis*, teak (L.), and *Gmelina arborea*, beechwood (Roxb.) plantations using municipal wastewater for irrigation in Egypt. Collectively, these studies note that careful management is required to avoid negative impacts of wastewater irrigation on local water sources. SRWCs on municipal wastewater land application sites must produce biomass, effectively renovate nutrients, and protect surface and groundwater quality.

Potential contamination of groundwater and surface water from municipal wastewater irrigation is a concern in North Carolina (U.S.A.) with its high incidence of nutrient-sensitive waters [33] and a growing bioenergy industry for woody biomass, bioenergy and biofuels [34]. There is some disagreement about the impact of SRWC production on nutrient groundwater quality at wastewater land application sites. Some SRWC studies have reported that groundwater concentrations may initially exceed the regulatory limits during establishment at these sites [35, 36]. Other studies have shown that willow SRWC establishment could maintain nitrogen contamination in groundwater below regulatory requirements [37]. Similar to other studies [29, 38], Minogue et al. [28] observed that nitrogen taken up by *P. deltoides* clones exceeded inputs from irrigation and atmospheric deposition, thus protecting shallow groundwater. One objective of this study was to evaluate the impact of establishment and early growth of SRWCs on nutrient concentrations in groundwaters at two municipal wastewater application facilities in North Carolina.

To date, most studies in the U.S.A. that have documented woody biomass production on municipal wastewater sites were not SRWC systems but studies evaluating conventional forest management on municipal waste application fields using *Platanus* *occidentalis,* American sycamore, (L.); *Taxodium distchum*, bald cypress, (L.); *Robinia pseudoacacia*, black locust, (L.); *Cercis Canadensis*, eastern redbud, (L.); *Liquidambar styraciflua*, sweetgum, (L.); *Fraxinus pennsylvannicas*, green ash, (L.); *Quercus acutissima, sawtooth oak, (Carruth.); Quercus rubra*, northern red oak (L.); *Ulmus parvifolia,* Chinese elm, (Jacq.); *Liriodendron tulipifera*, tulip poplar (L.); *Larix decidua*, European larch, (Mill.); *Larix kaempferi*, Japanese larch, (Carr.); *Juniperus virginiana*, Eastern red cedar, (L.)*;Thuja occidentalis*, white cedar, (L.); *Pseudotsuga menziessi,* Douglas fir, (Mirb.); *Pinus taeda*, loblolly pine, (L.); *Populus spp.* and *Eucalyptus spp*. [24, 28, 35, 39 - 41]. The best SRWC candidate for biomass production and survival is most often eastern cottonwood and its improved hybrid poplar clones [24, 28, 39]. Overman [39] evaluated ten tree species under wastewater irrigation in Florida and found significantly high production for *P. deltoides* (Bartr. ex Marsh.) as well as black locust and American sycamore. These three species grew more than 150 cm in height within the first year, with *Populus deltoides* reaching approximately 250 cm in the first year. Minogue et al. [28] found that improved *P. deltoides* clones produced aboveground biomass yields as high as 112 Mg·hectare^{-1} after 27 months. In this study, *Eucalyptus spp.* grew more than 300 cm in the first 6 months but died during winter. Collectively, these studies show that irrigation with municipal wastewater consistently increased woody biomass production; however, the establishment phase is critical to long-term performance.

In North Carolina (USA), land application of municipal wastewater is a prominent method for reducing contaminant discharges to local surface waters with some economic return by harvesting the land cover. These lands represent 3,540 hectares (~ 0.3% of land) in North Carolina with sites ranging from 0.2 hectares to 944 hectares in size [43]. Only a few of these sites apply municipal wastewater to produce woody biomass. Most municipal facilities in North Carolina produce hay and other herbaceous crops [43]. Based on our review of the literature, only one study has evaluated the productivity of SRWCs on wastewater application sites in North Carolina. Frederick [44] evaluated the growth of *Liquidambar styraciflua* (L.) and *Platanus occidentalis* (L.) at a moderate density (1,788 stems·ha^{-1}) and found trees to have high survival (91 to 93%) and modest biomass production (7.7 – 22.3 oven dry metric tons·hectare^{-1} after 60 months). It is important to note that this plantation was not intentionally established as a SRWC system, but conditions of the study fit within the definition of SRWC provided above.

In North Carolina, native tree species such as *Fraxinus pennsylvanica* (L.), *Taxodium distchum* (L.), *Pinus taeda* (*L*.) and *Liquidambar styraciflua* have potential as SRWC biomass resources and may be better received by land managers [42]. *Populus spp*. clones and *Eucalyptus spp*. are more likely to produce superior biomass, but their growth and survival is not well documented in North Carolina. Thus, a second objective of this study was to evaluate the establishment, survival, and growth of North Carolina native trees to a variety of *Populus spp.* clones and *Eucalyptus benthamii* (Maiden & Cambage) on two wastewater land application sites.

CHAPTER 2: Materials and Methods

2.1 Site description, groundwater monitoring, and site establishment: SRWC plantations were established on two municipal wastewater application facilities in eastern North Carolina (Figure 1). Each location was selected based on the availability of an established irrigation system, active permits for land application of municipal wastewater, and cooperation of facility administration. Both facilities receive similar rainfall precipitation and wastewater irrigation but differ by land application size due to the volume of municipal wastewater treated. The study site at the Gibson Wastewater Treatment Facility (Gibson, NC) has well-drained soils and receives little shading from surrounding land cover. This site has loamy sands (Table 1) and moderate soil slopes ranging from 0 – 6% to 12 – 15% [45]. The study site at the Jacksonville Wastewater Treatment Facility (Jacksonville, NC) has poorly-drained, loamy fine sand soils and substantial shading due to an adjacent 35-year old stand of loblolly pine. The site is moderately flat with 2 to 6% slopes [45] and is prone to pooling after either rain or wastewater applications.

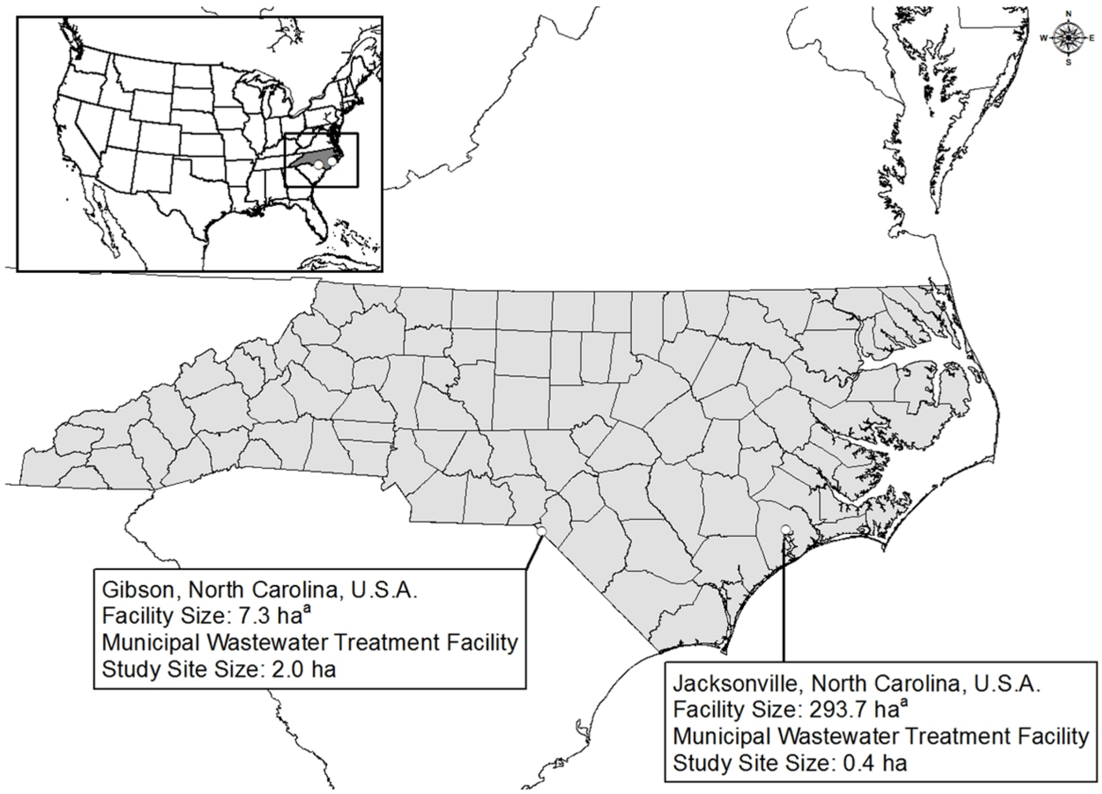

Figure 1: Location and description for Gibson Wastewater Treatment Facility and Jacksonville Wastewater Treatment Facility. [a][43]

Shallow groundwater monitoring wells were installed at Gibson (n = 9) and Jacksonville (n = 12) across the planting sites as shown in the Appendix. These wells were used to collect groundwater samples to evaluate the impact of SRWCs on nitrate and ammonia concentrations in groundwater before, during, and after site establishment. Two-inch (50-mm) boreholes were hand-augured to one meter below groundwater depth (two to seven meters below ground surface) in accordance with the North Carolina Department of Environment and Natural Resources, Division of Water Quality Well Construction Standards [47]. PVC-screens (50-mm diameter by 1.5-m height) were installed in the boreholes, supplemented with two-inch PVC piped as needed to reach 1.5 meters above the land surface. Capped and concreted galvanized shrouds (1.6 m) were installed over the PVC wells. A 1-L Teflon dedicated bailer (Forestry Supplies Inc., Jackson, MS, USA) was provided for each well and used for required purging and sample collection. Wells were conditioned by repeated purging for one month prior to the first sample collection.

Table 1: Climate, site, and experiment characteristics for Gibson and Jacksonville, North Carolina, U.S.A.

	Gibson	**Jacksonville**
Location	34°45'N, 79°36'W	34°45'N, 77°25'W
Average Annual Precipitation [a]	1300 mm	1400 mm
Average Annual Irrigation	1102 $mm \cdot ha^{-1}$ [b]	1109 $mm \cdot ha^{-1}$ [c]
Daily Wastewater Outflow	275 m^3 [b]	19700 m^3 [d]
Soil Series [e]	Ailey loamy sand & Pelion loamy sand	Norfolk loamy fine sand
Soil pH	5.8 – 6.4	5.3 – 7.0
Mean Depth to Groundwater	1.9 ± 0.24 m	0.83 ± 0.26 m
Mean Depth of Monitoring Wells	3.2 ± 1.3 m	2.8 ± 0.26 m
Previous Use	Managed American sycamore plantation	Fallow ground
Planting Date(s)	03/2011, 03/2012, 05/2012[f]	03/2012, 05/2012[f]
Experimental Design	Randomized block design	Randomized block design
Tree Spacing	1.8 m x 1.8 m	1.8 m x 1.8 m

[a] Climate data provided by the State Climate Office of North Carolina; [b] personal communication – Greg Leonard; [c] personal communication – Jill Puff ; [d] Data provided by City of Jacksonville website; [e] Data provided by USDA Web Soil Survey [45]; [f] *E. benthamii* planting date.

At Gibson, site preparation began in October 2010 with the removal of an existing stand of American sycamore that had chronic bacterial leaf scorch [46]. In February 2011, the terrain was disced to 18 cm and a 41% glyphosate treatment was applied for weed control. All native tree species were provided by Claridge State Nursery (Goldsboro, NC) and planted as 1-year old bare rooted seedlings. The planting design consisted of a randomized complete block design where sixteen variously sized blocks were planted with seven native tree species: Atlantic white cedar (*Chamaecyparis thyoides*, (L.)); green ash (*Fraxinus pennsylvanica*, (Marshall)); loblolly pine (*Pinus taeda*, (L.)); cheerybark oak (*Quercus pagoda*, (Raf.)); water oak (*Quercus nigra*,(L.)); willow oak (*Quercus phellos*, (L.)); and bald cypress (*Taxodium distichum*, (Rich.)). Water oak and willow oak were planted within the same blocks; thus, each block of water oak and willow oak serves as a single cohort. The *Populus* clones were all *Populus deltoides* provided by ArborGen, LLC (Ridgeville, SC) and were planted as dormant 20 - 60 cm cuttings. Three blocks of six, *Populus* clones were planted in a separate, randomized block design. Table 2 shows the total number of plantings for each tree species in 2011; a complete planting layout is provided in Appendix A.

Table 2: Species and number planted in Gibson and Jacksonville, North Carolina, U.S.A

Species	Gibson 2011	Gibson 2012	Jacksonville 2012
Chamaecyparis thyoides	94	-	-
Eucalyptus benthamii	-	247	84
Fraxinus pennsylvanica	78	399	82

Table 2 (cont.)

Species	Gibson 2011	Gibson 2012	Jacksonville 2012
Liquidambar styraciflua	-	270	82
Pinus taeda	102	355	50
Quercus pagoda	93	-	-
Q. phellos, Q. nigra	93	-	-
Taxodium distichum	93	370	82
Populus spp. –Clone ID (Parentage)			
140 (*P. deltoides*)	-	5	-
176 (*P. deltoides*)	-	5	-
185 (*P. deltoides*)	97	5	-
198 (*P. deltoides*)	-	5	-
200 (*P. deltoides*)	-	5	-
221 (*P. deltoides*)	-	25	-
224 (*P. deltoides*)	-	5	-
245 (*P. deltoides*)	-	5	-
379 (*P. deltoides*)	-	5	-
380 (*P. deltoides*)	-	30	16
381 (*P. deltoides*)	-	5	-
406 (*P. deltoides*)	-	5	-
409 (*P. deltoides*)	-	5	-
410 (*P. deltoides*)	-	5	-
411 (*P. deltoides*)	-	5	-
412 (*P. deltoides*)	-	5	-
413 (*P. deltoides*)	-	5	-
414 (*P. deltoides*)	-	5	-
423 (*P. deltoides*)	-	5	-
427 (*P. deltoides*)	-	5	-
429 (*P. deltoides*)	-	5	-
432 (*P. deltoides*)	-	5	-
434 (*P. deltoides*)	-	5	-
439 (*P. deltoides*)	97	-	-
443 (*P. deltoides*)	96	-	-
444 (*P. deltoides*)	93	25	-
445 (*P. deltoides*)	82	-	-
448 (*P. deltoides*)	-	5	16
449 (*P. deltoides*)	-	5	16
450 (*P. deltoides*)	-	5	16
451 (*P. deltoides*)	-	5	16
187 (*P. trichocarpa* x *P. deltoides*)	-	30	16
188 (*P. trichocarpa* x *P. deltoides*)	-	5	16

Table 2 (cont.)

Species	Gibson 2011	Gibson 2012	Jacksonville 2012
Populus spp. –Clone ID (Parentage)			
229 (*P. trichocarpa* x *P. deltoides*)	-	5	16
302 (*P. trichocarpa* x *P. deltoides*)	-	30	16
303 (*P. trichocarpa* x *P. deltoides*)	-	30	16
304 (*P. trichocarpa* x *P. deltoides*)	-	30	16
337 (*P. trichocarpa* x *P. deltoides*)	-	5	-
339 (*P. trichocarpa* x *P. deltoides*)	-	30	16
341 (*P. trichocarpa* x *P. deltoides*)	-	5	-
342 (*P. trichocarpa* x *P. deltoides*)	-	5	-
6 (Unknown)	297	-	-
138 (Unknown)	-	5	-
147 (Unknown)	-	5	-
147 (Unknown)	-	5	-
230 (*P. deltoides* x *P. maximowiczii*)	-	5	-

An early summer drought in 2011 resulted in minimal irrigation by the wastewater treatment facility, and 62% of the trees died. In preparation for replanting in 2012, all *Populus* clones were removed from the study area, except for one block of *Populus* clone 6 that grew exceptionally well. In October and November 2011, the planting area was expanded by removing another diseased section of American sycamore, treating the entire planting area with herbicide, Karmex (Dupont, Wilmington, Delware, U.S.A.), and discing again to 18 cm. In December 2011, wheat was planted to meet facility permit requirements for a cover crop until trees were planted in March 2012. Tree locations were spot-sprayed with herbicide to maintain a surrounding wheat cover crop.

In March 2012, dead native trees from 2011 were replanted with green ash, loblolly pine, or bald cypress as bare-rooted seedlings and provided by Claridge State

Nursery (Table 2). The former 2011 *Populus* section and the new expanded section were planted with *Populus*, native trees, and *Eucalyptus benthamii* (Maiden & Cambage) using a randomized block design. In March 2012, forty *Populus* clones were planted at Gibson using a randomized design with five blocks containing **37** *Populus* clones of three parentages (*P. deltoides x P. trichocarpa, P. deltoides,* and *P. deltoides x P. maximowiczii*) and three clones with unknown parentage (ArborGen, LLC, Ridgeville, SC). Eight additional blocks were planted homogenously with eight clones, including clones 187, 221, 302, 303, 304, 339, 380, and 444. All of the *Populus* clones were represented in the randomized block design except for clones 221 and 444. Native trees (sweetgum, bald cypress, green ash, loblolly pine) and *E. benthamii* were planted in randomized block design in March 2012 and April 2012, respectively. *E. benthamii* seedlings were provided and planted by the North Carolina State Tree Nutrition program. Clone ID and parentage can be found in Table 2, and planting layouts for 2011 and 2012 are provided in Appendix B & C.

In October 2011, the study site at Jacksonville was disced to 18 cm and treated with 41% glyphosate to reduce weeds. *Populus* and native trees were planted in March 2012, and *E. benthamii* was planted in April 2012 using a complete randomized block design. Four native tree species (sweetgum, bald cypress, green ash, and loblolly pine) and *E. benthamii* were planted separately in six blocks while twelve *Populus* clones were planted in four blocks. The site layout is provided in Appendix D**.**

2.2 Sampling and Analyses: All tree locations and monitoring wells were recorded using a handheld unit GPS (Tremble GeoXT Handheld, Sunnyvale, CA, USA). Recorded locations were plotted using ArcGIS 10 (ESRI ArcGIS, Redlands, CA, USA) for site maps. All height (cm) measurements were collected using a Crain CMR series Measuring Rod (Crain Enterprises, Inc., Memphis, TN, USA). Because *Eucalyptus benthamii* and native tree species were planted as one-year old seedlings with a pre-existing height, two measurements were required to determine height growth. Height was determined at the time of planting and then again several months afterward. Because *E. benthamii* was planted in April 2012 to avoid late frost, initial height measurements were not taken until July 2012. Initial heights for all other trees were determined in July 2012 at Gibson and in April 2012 at Jacksonville. Initial height growth measurements were not required for *Populus.* Mortality measurements were scored as binary traits (0 = Dead, 1= Alive). Computing the total number of dead trees and dividing by the total number of plantings determined percent mortality. Tree height and mortality were quantified in September 2011, July 2012 and October 2012 for Gibson and in March 2012, July 2012 and October 2012 for Jacksonville.

Groundwater samples were collected monthly from November 2011 until September 2012 and were analyzed for nitrate and nitrite ($NO_3 + NO_2$) and ammonium ($N\text{-}NH_4$). Before collecting samples, wells were purged using dedicated Teflon® bailers for three well volumes or until wells were empty to assure removal of any stagnant water [48]. Samples were stored in pre-cleaned high density 125-mL polyethylene Nalgene® bottles (Rochester, N.Y., U.S.A.), preserved with sulfuric acid to a pH less than 2.0, kept cool at 4°C or below, and analyzed at the Center for Applied Aquatic Ecology (CAAE, Raleigh, N.C., U.S.A.) within 28 days of collection. Analysis for $NO_3 + NO_2$ was performed on a Bran and Luebbe QuAAtro Segmented Flow Analyzer (Bran+Luebbe Inc., Delavan, WI, USA) following Standard Method 4500NO_3F and United States Environmental Protection Agency (USEPA) Method 353.2 by means of Automated Cadmium Reduction with a reportable detection limit of 5.6 $\mu g \cdot L^{-1}$. Analysis of N-NH_4^+ followed the Standard Method 4500 NH_3H, USEPA Method 350.1, by means of automated phenate, with a reportable detection limit of 7.0 $\mu g \cdot L^{-1}$.

2.3 Statistical Analyses: Mortality data was not subjected to statistical analyses because binary data with a mean incidence outside certain boundaries (e.g., 30% to 70%) are not advised for analysis using common statistical practices [49]. Data on height growth was subjected to either one-way or two-way analyses of variance (ANOVA) according to the randomized complete block design with an $\alpha = 0.05$ (SAS, PROC GLM or PROC MIXED, Cary, NC, USA). Block and interaction effects were evaluated for significance, except for native trees and *E. benthamii* from Gibson 2012. Trees that died during the experiment were not analyzed for height growth and were thus not included in statistical models.

2.4 Quality Assurance and Quality Control: Field duplicates were collected for eight percent of the total number of trees measured at Gibson (n = 179) and ten percent of the total number of trees measured at Jacksonville (n = 56) to evaluate field data precision for height (cm). Relative percent difference, or RPD, was calculated for duplicate height measurements of the same tree then averaged to assess a mean RPD ± one standard deviation for height precision. Mean RPD for tree height was 4% ± 6% for Gibson and 4% ± 6% for Jacksonville. For each groundwater sampling event, quality control included a field duplicate, a field blank, and two field matrix spikes to evaluate precision and accuracy of groundwater collection and analysis for nitrate/nitrite ($NO_3 + NO_2$) and ammonia ($NO_3 + NO_2$). The precision of field duplicate measurements and field matrix spikes, reported as mean RPD ± one standard deviation, was 1.7 ± 1.0% for $NO_3 + NO_2$ (n=22) and 14% ± 9.1% for $N\text{-}NH_4^+$ (n=22) at Gibson and 10% ± 10% for $NO_3 + NO_2$ (n=16) and 10% ± 4.1% for $N\text{-}NH_4^+$ (n=16) at Jacksonville. Field blanks were used to identify potential contamination in sample collection and analysis. The method detection limits (MDL) are 5.6 $\mu g \cdot L^{-1}$ for nitrate/nitrite and 7.0 $\mu g \cdot L^{-1}$ for ammonia. At Gibson, two of ten field blanks had $NO_3 + NO_2$ concentrations above the nitrate MDL at concentration ranges between 5.8 $\mu g \cdot L^{-1}$ and 49 $\mu g \cdot L^{-1}$. Nine of ten field blanks reported ammonia concentrations above the MDL between 8.4 $\mu g \cdot L^{-1}$ and 29 $\mu g \cdot L^{-1}$. At Jacksonville, four of eight field blanks were above the nitrate/nitrite MDL with concentrations between 6.7 $\mu g \cdot L^{-1}$ and 73 $\mu g \cdot L^{-1}$. Eight of nine ammonia field blanks were above the ammonia MDL between 11 $\mu g \cdot L^{-1}$ and 33 $\mu g \cdot L^{-1}$. Analysis of laboratory distilled, deionized water (Pure Water, Mebane, NC) showed that field blanks above MDLs derived from nitrate and ammonia in the distilled, deionized water used for field blanks. Concentrations above the MDL were less than 0.5% (nitrate) and 1.7% (ammonia) of the regulatory levels in groundwater at 10,000 $\mu g \cdot L^{-1}$ and 1,500 $\mu g \cdot L^{-1}$, respectively. Field matrix spikes of nitrate and ammonia in groundwater collected on site were used to assess the accuracy of field sample collection. Accuracy, reported as mean percent error, was 7.6% ± 4.2% (n = 7) for $NO_3 + NO_2$ and 8.5% ± 6.6% (n = 9) for N-

NH_4^+ at Gibson and 6.7 ± 9.1% (n = 9) for NO_3 + NO_2 and 5.1% ± 4.8% (n = 10) for N-NH_4^+ at Jacksonville.

CHAPTER 3: Results

3.1 Study Challenges: This study compared differences between first year establishment and initial growth (height) for trees native to North Carolina, *Populus spp.* clones, and *E. benthamii* at two study sites irrigated with municipal wastewater. Identical protocols were followed for site preparation and planting spacing at each site, but the two sites differed in the number of trees planted and some specific site attributes. The Jacksonville site had a 35-year old loblolly pine stand that shaded part of the study site. In addition, soils at Jacksonville tended to be saturated, leading to pooling of water on the surface after irrigation. Lastly, weed competition at Jacksonville was highly problematic. *Cyperus esculentus*, yellow nutsedge, and *Sorghum halepense*, Johnsongrass, dominated the field site and required multiple herbicide treatments paired with mowing to control through the growing season. Weed competition did not require as much chemical treatment or mowing at Gibson, and surface pooling was not observed after wastewater irrigation. Shading was not an issue at the Gibson experimental site. The Gibson site was planted with a greater variety of species and number of plantings for 2011 and 2012 (see Table 2). For presentation clarity, percent mortality is presented in Figure 2 instead of survivorship; thus, for consistency, the results and discussion sections discuss percent mortality rather than percent survivorship.

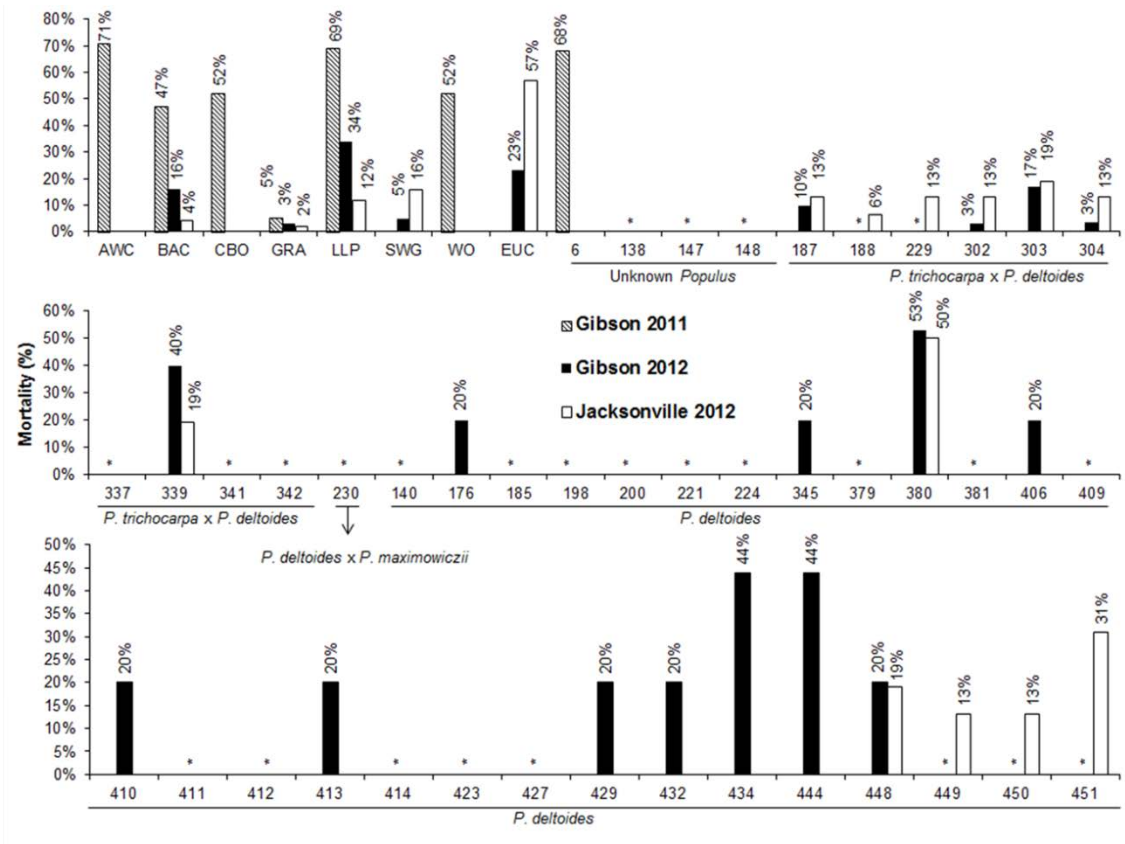

Figure 2: Mortality among tree species and clones planted in 2011 and 2012. * indicates 0% mortality for the corresponding tree planting in 2012 at Gibson. Clones sharing similar parentage are underlined. AWC = Atlantic white cedar, BAC = bald cypress, CBO = cherry bark oak, GRA = green ash, LLP = loblolly pine, SWG = sweetgum, WO = white oak, EUC = *Eucalyptus benthamii*.

3.2 Tree Species Establishment and Mortality – Gibson 2011: Figure 2 shows percent mortality data for trees planted in 2011 and 2012; actual inventory data are provided in the Appendix. In 2011, the percent mortality for all tree plantings was 62% due to a late spring and early summer drought. Drought conditions limited irrigation with municipal wastewater at Gibson due to permit restrictions. In 2011, native tree species survived better and had lower percent mortalities than *Populus* (67%). Green ash had the lowest percent mortality (3%) and best survival of all species planted at Gibson, followed by bald cypress (42%), cherrybark oak (44%), water/white oak (57%), Atlantic white cedar (65%) and loblolly pine (66%). *Populus* clones (n=6) had higher percent mortalities between 50% and 90%. Thus, in 2011, most native tree species, particularly green ash, survived short-term drought conditions better than *Populus* clones in the early establishment period. *E. benthamii* was not planted at Gibson in 2011. Most of the *Populus* clones planted in 2011 were removed except for *Populus* clone 6 which had high mortality (50%) but good growth.

3.2.1 Tree Species Establishment and Mortality – Gibson 2012: In 2012, rainfall was normal throughout the growing season, and all trees, including surviving trees from 2011, were inventoried for mortality in October 2012 (see the Appendix). Overall, tree survivorship improved with declines in mortality from 62% in 2011 to 26% in 2012. For trees planted in 2012, native trees had a lower percent mortality (14%) than *Populus* and *E. benthamii* (18%). For native trees planted in 2011, green ash had the lowest mortality (5%) and best survival followed by bald cypress (47%), cherry bark oak (52%), water/white oak (52%), loblolly pine (69%), and Atlantic white cedar (71%) (Figure 2). A similar mortality trend was observed for native trees planted in 2012, with higher mortalities observed for loblolly pine (34%) and lower mortalities observed for bald cypress (16%), sweetgum (5%), and green ash (3%) (Figure 2). *Populus* clone 6, that was planted in 2011 and not removed in 2012, had a percent mortality of 20% in 2012.

Populus clones planted in 2012 had lower mortalities (20%) to clones planted in 2011 (60%). Twenty six of the forty two clones planted in 2012 had no mortality (Figure

2). The remaining sixteen clones had mortalities ranging from 3% to 53%. Evaluating mortality for clones by parentage revealed that clones with unknown parentage (n=3, 0% mortality) and *P. deltoides* x *P. maximowiczii*) parentage (n=1, 0% mortality survived superior to clones with *P. deltoides* (n=28, 13%) or *P. trichocarpa* x *P. deltoides* (n=10, 18%) parentage. Fifty percent of *P. trichocarpa* x *P. deltoides* clones and 45% of *P. deltoides* clones had no mortality.

3.2.2 Jacksonville: In 2012, site conditions at Jacksonville were often very wet with frequent, if not constant, soil saturation and pooling from rainfall and wastewater application. The overall site percent mortality for all trees planted in 2012 was 18%. For native trees, green ash had the lowest percent mortality (2%) and best survival, followed by bald cypress (4%), loblolly pine (12%), and sweetgum (16%). *Populus* clones had a cumulative percent mortality of 19% and *E. benthamii* had a percent mortality of 57% (Figure 2). Only one *Populus* clone, HP 188, had a percent mortality less than 10% (HP 188, 6%) (Figure 2). The remaining *Populus* clones had mortalities between 13% and 50% (see the Appendix). Aggregating *Populus* mortality data by clone parentage showed similar trends to Gibson in that *P. trichocarpa* x *P. deltoides* parentage (n = 7 clones) had a lower percent mortality (14%) than *P. deltoides* parentage (28%) (n = 5 clones).

3.3 Tree height growth: Height growth for all trees was determined by measuring the change in height from the initial height measurement for each tree at the start of the growing season and at the end of the growing season. In cases where height measurements were not collected at the time of planting, the earliest available measurement was substituted as the initial height growth. Figure 3 shows box plots of

Populus and *E. benthamii* tree height growth for 2012 plantings at Gibson and Jacksonville. Figure 4 shows height growth for native trees planted at Gibson in 2011, and height growth for native trees planted at Jacksonville and Gibson in 2012. Results of analysis of variance for height growth between clones, parentage types, species, and blocks can be found in Table 3.

Table 3: Results of analysis of variance for height growth between *Populus* clones, *E.benthamii*, and native tree species at Gibson and Jacksonville, North Carolina, U.S.A. Results are reported based on the use of PROC GLM unless otherwise noted.

	SOURCE [£]	DF	Mean Square	Z/F	p
2011 Gibson Poplar Clones	Clone	5	5786	0.85*	0.55
	Block	2	1221	0.18*	0.84
	Clone x Block	9	6845	5.11	< 0.0001
	Residual	166	1339		
2012 Gibson Poplar Clones – Clone ID[§]	Clone	39	280	1.50†	0.067
	Block	4		7.94†	<0.0001
	Residual	149	428	8.60†	<0.0001
2012 Gibson Poplar Clones – Parentage	Parent	2	3668.5	0.72*	0.52
	Block	4	32842	6.44*	0.013
	Parent x Block	8	5101.4	1.25	0.27
	Residual	176	4068.9		
2012 Gibson Native Tree Species and *E. benthamii*	Species	4	33069	72.95	<0.0001
	Residual	1068	453.32		
2012 Jacksonville Poplar Clones – Clone ID	Clone	11	4432	1.99*	0.063
	Block	3	3361	1.61*	0.23
	Clone x Block	32	2222	1.07	0.39
	Residual	108	2077		
2012 Jacksonville Poplar Clones – Parentage	Parent	1	37590	48.84*	0.0060
	Block	3	2862.4	1.37*	0.25
	Parent x Block	3	769.70	0.37	0.78
	Residual	147	2087.7		
2012 Jacksonville Native Tree Species and *E. benthamii*	Species	3	1046	2.81*	0.078
	Block	5	813.5	2.18*	0.11
	Species x Block	14	372.8	2.68	0.0012
	Residual	215	139.3		

[£]Computed in SAS v. 9.3 (Cary, NC, USA), †- Z-values, * - Interaction term used to calculate F-value,
[§]- Results reported from PROC MIXED procedure

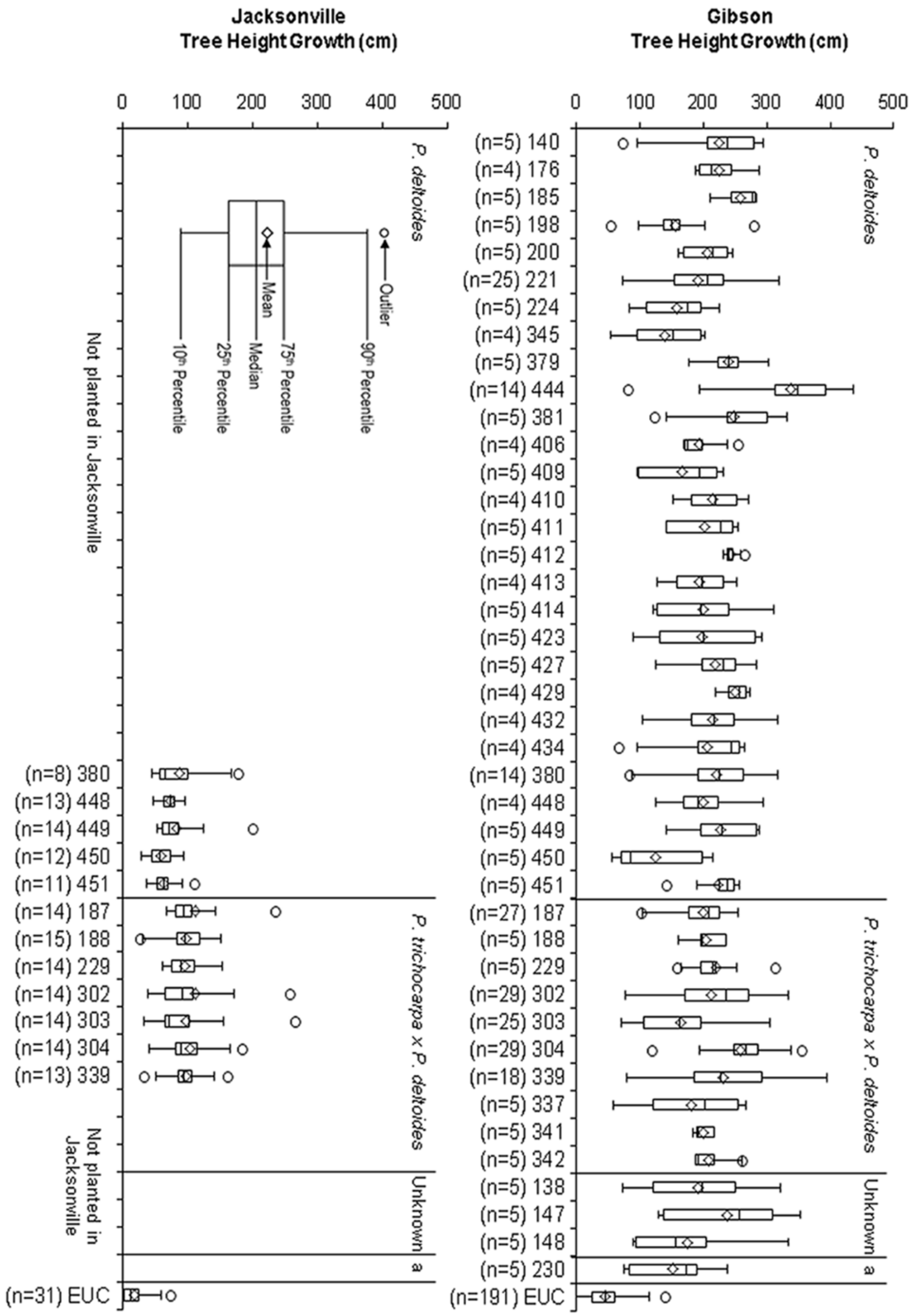

Figure 3: Tree height growth (cm) for *Populus* clones and *Eucalyptus benthamii*. *Populus* clones are grouped by parentage; "a" - *P. deltoides* x *P. maximowiczii*.

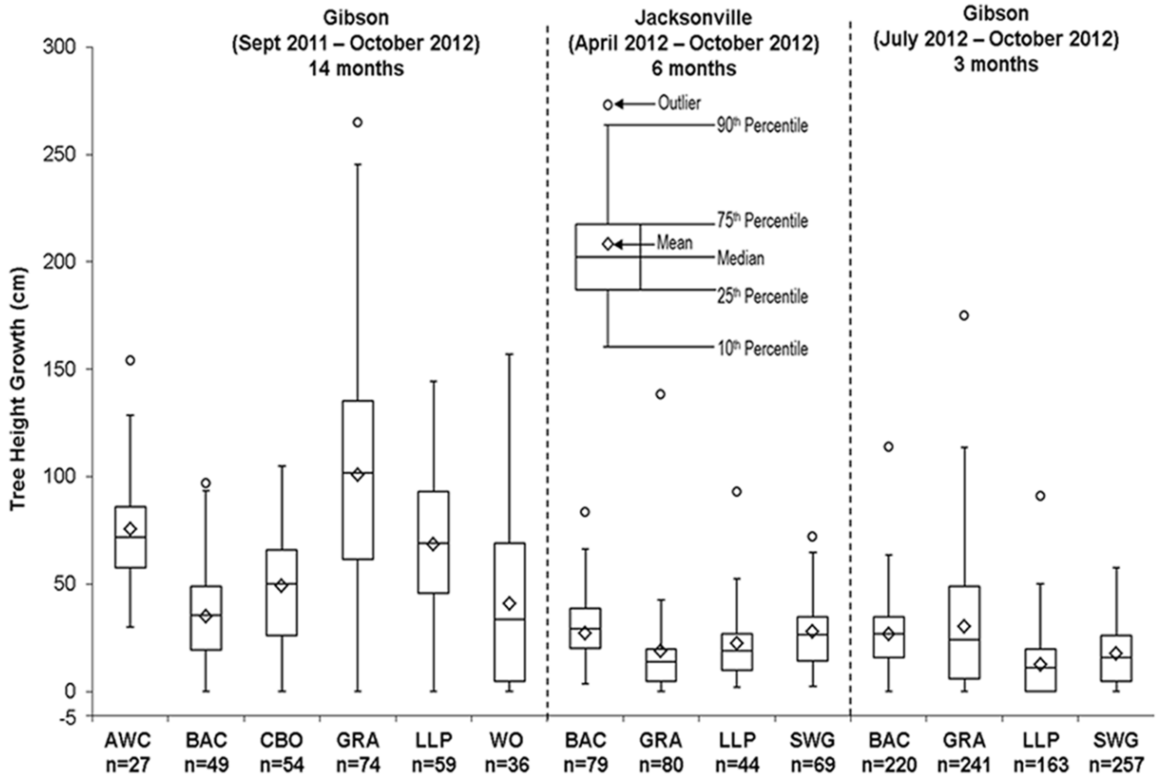

Figure 4: Tree height growth in centimeters for native trees in Gibson and Jacksonville, North Carolina, U.S.A. Location and months of growth are noted at the heading of each section. Species noted along the horizontal axis are: AWC Atlantic White Cedar, BAC Bald Cypress, CBO Cherrybark Oak, GRA Green Ash, LLP Loblolly Pine, WO White oak/Water oak, SWG Sweetgum.

3.3.1 Gibson 2011: Tree height growth for all 2011 plantings at Gibson can be found in the Appendix. *Populus* clones were planted in March 2011 and final measurements were collected in September 2011. Four out of six clones grew to a mean height of 100 cm or more. However, statistical analysis of *Populus* clones found clone ID not to be significant ($p = 0.55$).

Height growth for native tree species planted in 2011 at Gibson is shown in Figure 4, representing 19 months of height growth after planting. As block integrity was compromised after replanting in March 2012, native tree species from 2011 were not subjected to statistical analysis. Five of the six species had plantings with little to no growth from September 2011 to October 2012. Green ash grew the most with a mean height growth ± one standard deviation of 99 cm ± 52 cm, followed by Atlantic white cedar (75 cm ± 28 cm), loblolly pine (69 cm ± 34 cm), cherrybark oak (48 cm ± 30 cm), white/willow oak (42 cm ± 33 cm), and bald cypress (35 cm ± 22 cm). These results suggest that green ash was the only native species at Gibson that achieved height growth comparable to the most successful *Populus* clones. Otherwise, *Populus* appear to outperform natives in height growth. This finding is reinforced by the longer growth period permitted for native tree species (19 months) versus *Populus* (6 months).

3.3.2 Gibson 2012: Figure 3 shows box plots of *Populus* and *E. benthamii* tree height growth for 2012 plantings at Gibson. Figure 4 shows height growth of native tree species. Damage to *Populus* and native species was evident in October 2012. Evidence of herbivory was present on 75% of green ash and 25% of *Populus* clones. Three percent of *Populus* clones endured physical damage, most likely due to rutting from *Odocoileus virginianus*, white tail deer. Ten percent of bald cypress appeared to endure some damage to branches and primary shoots as many appeared to have been broken. In cases where damage to trees resulted in negative height growth (n = 156), growth was modified to be zero growth; this number accounted for 11% of all trees planted.

Despite damage to trees, *Populus* clones grew much better in 2012 than in 2011. Final height measurements were collected in October of 2012 and evaluated for differences in mean height growth. Mean height growth was 207 ± 68 cm for all *Populus* compared to 121 cm ± 48 cm in 2011. Height growth for individual clones can be found in the Appendix. The difference in height growth from 2011 to 2012 is largely attributed to the regular application of wastewater and precipitation. The 2012 *Populus* clones did not differ significantly in height growth ($p = 0.067$, Table 3). Clones were evaluated by parentage type and segregated into three groups: *P. deltoides*, *P. deltoides* x *P. trichocarpa*, and unknown. The unknown group included *Populus* 138, 147, 148, and 230. Clone 230 was included as an unknown because no other clones with *P. deltoides* x *P. maximowiczii* parentage were planted. Grouping clones by parentage did not show significant differences between parentage types ($p = 0.52$, Table 3). These results suggest that tree height growth does not appear to be a sensitive parameter to differentiate between *Populus* clones during the establishment period for *Populus* in 2012.

Native tree species and *E. benthamii* were separately evaluated for height growth. Evaluation of these tree species was based on growth between July 2012 and October 2012. Tree heights are reported in the Appendix. Growth differed significantly ($p < 0.0001$) between tree species with *E. benthamii* achieving the most height growth at 47 cm ± 27 cm. Damage to native trees as discussed above resulted in a wide distribution of height growth within each tree species. Green ash and bald cypress grew to 30 cm ± 28 cm and 26 cm ± 17 cm during the three month period, respectively. Despite having low mortality, sweetgum grew very little with 18 cm ± 15 cm of growth. Loblolly pine grew the least with 13 cm ± 14 cm of growth from July to October. These results suggest that both *Populus* and *E. benthamii* can be highly productive on wastewater application sites, despite higher mortality. Native tree species like green ash are productive, but further evaluation of growth over longer periods is needed to determine whether *Populus*, *E. benthamii*, or native tree species are best for meeting wastewater application requirements and biomass production at wastewater land application sites.

3.3.3 Jacksonville 2012: Figure 3 shows height growth for *E. benthamii* and *Populus* clones with native trees height growth provided in Figure 4. Severe damage from herbivory, weeds, and other unknown sources appeared throughout the growing season to both native trees and trees. Nearly all (99%) green ash and all *Populus* clones were damaged with most foliage and some of the terminal buds pruned by the time of final height measurement. In addition to damage from herbivory, weed competition appeared to damage and limit growth of *Populus* plantings. *Cardiospermum grandiflorum* was found overgrowing plantings on many occasions, and required manual removal. Rapidly growing *Cyperus esculentus* and *Sorghum halepense* were also observed across the site and were managed by mowing. Bald cypress had several breakages to both primary and secondary shoots. The only species that did not have significant external damage were loblolly pine and *E. benthamii*. In cases where damage to trees resulted in negative height growth (n = 156), growth was corrected to be zero growth, accounting for 34% of all trees planted.

Populus clones grew 93 cm ± 48 cm in height over seven months at Jacksonville compared to 207 cm ± 68 cm in Gibson. Height growth did not differ significantly between clones (p = 0.063, Table 3). Clones were segregated into two groups to evaluate if height growth showed significant differences between parentage types (*P. deltoides* and *P. trichocarpa* x *P. deltoides*). Clones with parentage type *P. trichocarpa* x *P. deltoides* grew significantly more than clones with *P. deltoides* parentage (p = 0.0060, Table 3). *P. trichocarpa* x *P. deltoides* clones grew a mean height of 106 cm ± 52 cm and *P. deltoides* clones grew a mean height of 73 cm ± 32 cm. Four out of six *P. trichocarpa* x *P. deltoides* clones grew more than 100 cm on average during the experimental period whereas no *P. deltoides* clones grew more than 100 cm on average in the same period. This difference may be partly due to herbivory. These results suggest selecting clones with *P. trichocarpa* x *P. deltoides* parentage may be preferred when sites are shaded with high weed competition, herbivory, and surface pooling.

Native tree species and *E. benthamii* were evaluated for height growth separately from *Populus* at Jacksonville. Comparisons between trees were made based on height growth between July 2012 and October 2012. Mean height growth for all trees was found to be 9.0 cm ± 13 cm. A two way ANOVA showed no significant differences between tree species (p = 0.078, Table 3). The small height growth at Jacksonville is likely the result of poor site conditions. Despite damage and subdued growth, bald cypress and green ash still showed some promise due to their low percent mortalities and final heights. In addition, the short period of evaluation may not have been appropriate for evaluating height growth for tree species at a site with poor conditions. Continued monitoring will better evaluate if these tree species are good biomass candidates for wastewater application sites.

3.4 Groundwater Nutrient Concentrations: Both facilities are required to meet permit requirements to protect surface water and groundwater quality on and off site; hence, concentrations of nitrate (NO_3+NO_2) and ammonia (N-NH_4^+) were monitored in groundwater across both sites. The USEPA has established the maximum contaminant level (MCL) for NO_3+NO_2 concentration at 10 mg·L^{-1}. North Carolina's wastewater treatment facilities are required to meet criteria set by the USEPA, but states can have stricter guidelines than EPA federal standards. The USEPA does not have a MCL for N-NH_4^+, but the North Carolina Department of Environment and Natural Resources [NCDENR] has a provisional N-NH_4^+ MCL of 1.5 mg·L^{-1} in groundwater (NCDENR DWQ Groundwater Standards, 2010). Monthly concentrations of ammonia and nitrate at both sites are provided in Figure 5. NO_3+NO_2 ranged from 0.2 mg·L^{-1} to 9.5 mg·L^{-1} for Gibson and < 0.0056 mg·L^{-1} to 10.95 mg·L^{-1} for Jacksonville. N-NH_4^+ ranged from 0.01 mg·L^{-1} to 0.90 mg·L^{-1} for Gibson and 0.02 mg·L^{-1} to 1.42 mg·L^{-1} for Jacksonville. One sample exceeded the MCL for NO_3+NO_2 at Jacksonville in February 2012; otherwise, concentrations of nitrate and ammonia in groundwater were below MCLs for both sites.

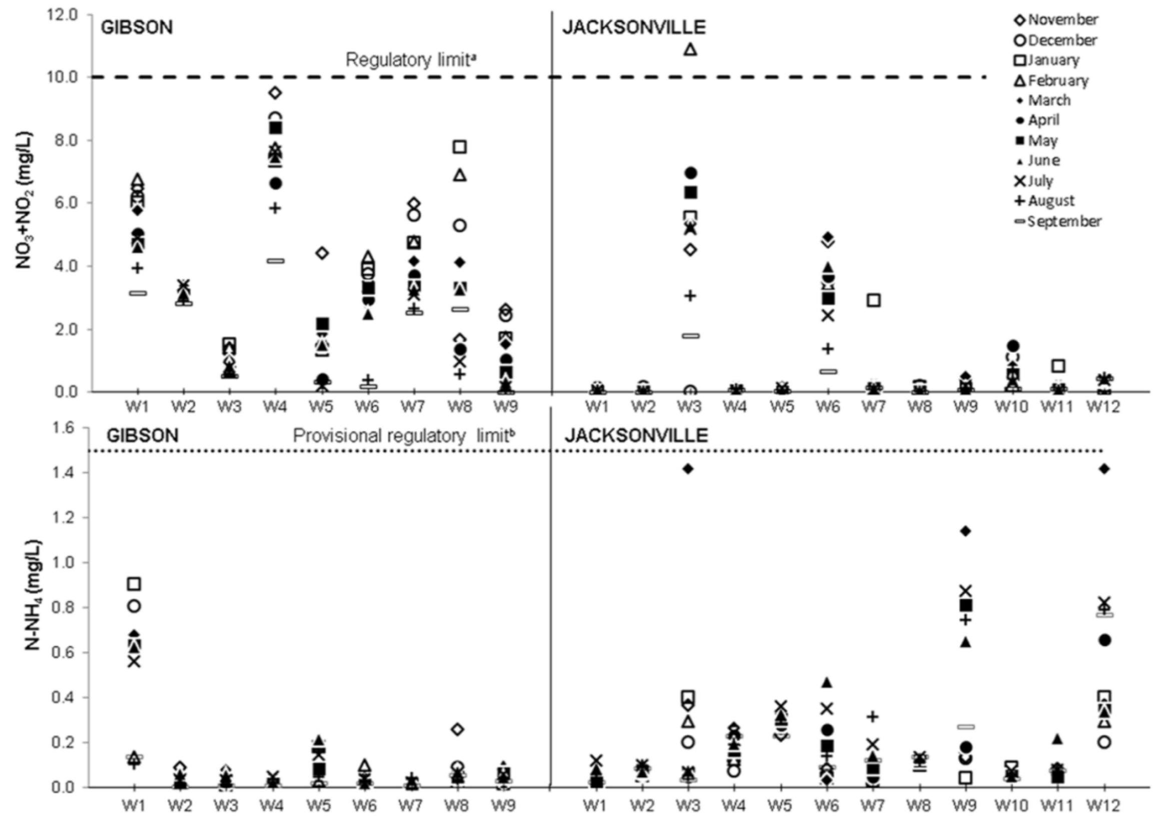

Figure 5: Nitrate/nitrite (NO_3+NO_2) and ammonia (N-NH4) concentrations in groundwater monitoring wells from Gibson and Jacksonville, North Carolina, U.S.A. [a] NCDENR State Standards, 15A NCAC 02L.0202 Groundwater Quality Standards; [b] NCDENR State Standards, IMAC 15A NCAC 02OL.202 Groundwater Quality Standards [64].

CHAPTER 4: Discussion

Forty-two *Populus spp.* clones, one *Eucalyptus* species, and seven native North Carolina tree species were evaluated for survival, height growth, and nutrient mitigation at two municipal wastewater application sites throughout the first year establishment phase. Superior performance for some species was indicated by relatively high tree growth and low percent mortalities, but selection criteria differed between sites. At Gibson, *Populus* clones 185, 140, 412, 379, 381, 147, 449, 451, 229, 427, 342, 200, 188, 411, 414, and 341 had low percent mortalities (<10%) and greater than 200 cm of height growth. Growth was generally lower at Jacksonville compared to Gibson. As a result, selection of superior candidates required a lower threshold for height growth. Bald cypress, green ash, *P. deltoides* x *P. trichocarpa* clones 187, 188, 229, 302, 304, and *P. deltoides* clone 449 had greater than 75 cm of height growth and low percent mortality (<13%) at Jacksonville. The difference in selection criteria between these two sites shows that site differences can be sufficient to vary species and clone selection, despite similar waste treatment, application rates, and general climate. These results emphasize the need for site specific screening of tree species and clonal varieties as noted in prior studies [27, 39, 51].

Populus and *Eucalyptus* are good candidates for wastewater land application because of rapid tree growth and high water use efficiency [12, 53, 65]. In this study, *Eucalyptus* had poor survival in saturated soil conditions, particularly at Jacksonville; however, a few surviving trees did demonstrate rapid height growth superior to *Populus* clones. *Eucalyptus* at Gibson survived better, and some surviving trees grew as well as

Populus and green ash. *Populus* clones, in general, had good survival and growth; however, select clones (380, 444, 439, and 345) did not perform well. Minogue *et al*. [28] compared *Eucalyptus* and *Populus* survival and growth at a municipal wastewater application facility in Florida, USA and concluded that *P. deltoides* clones outperformed both *E. amplifolia* and *E. grandis c*lones after two years. Both *Eucalyptus spp.* had high mortality due to low temperatures. *Populus* was also found to be the superior species for biomass production in Florida by Overman [39]. These findings and results from our study suggest that *Populus* is a good candidate for biomass production in the southeastern USA, particularly for wastewater land application sites.

A unique aspect of this study is the comparison of *Populus* and *Eucalyptus* to trees indigenous to North Carolina. Native trees may offer pest resistance, drought tolerance, and climate adaption traits that non-native species do not possess. Other studies have reported initial height growth of 200 cm or more in a single year for *Populus spp.*, *Eucalyptus spp.* and *Salix spp*. [25, 36, 52]. Native trees did not achieve this height growth at either site throughout the establishment period. These findings support *Populus* clones as a potential source of woody biomass at these municipal wastewater land application sites [28]. The low performance of all tree species at Jacksonville is largely ascribed to the shading by surrounding trees, saturated soils and weed competition, which are significant factors that can impede biomass production [4, 52]. However, the low percent mortality among bald cypress and green ash suggest that native trees might be better candidates than improved *Populus* clones at this particular site.

Native species, like green ash and bald cypress, have been used at other wastewater application sites in North Carolina. Frederick [50] documented high survival and growth for green ash, bald cypress and sweetgum on portions of a municipal wastewater irrigation facility that had been degraded due to poor species selection, high hydraulic loading from wastewater application, and soil compaction due to hay production. Frederick [44] also documented survival and biomass production of American sycamore and sweetgum at 2.4 m x 2.4 m spacing on a 145 hectare wastewater application facility located in Edenton, NC. By year five, biomass from American sycamore had seven percent mortality with approximately 4.92 dry $Mg \cdot hectare^{-1} \cdot yr^{-1}$ accumulated biomass (total biomass = 24.6 dry $Mg \cdot hectare^{-1}$). Sweetgum, grown on the same site, had no mortality and accumulated approximately 1.7 $Mg \cdot hectare^{-1} \cdot yr^{-1}$ for a total of 8.5 dry $Mg \cdot hectare^{-1}$ at year 5[44].These findings suggest that native species like sweet gum and bald cypress may grow too slowly to meet woody biomass production objectives. On the other hand, the moderate performance of green ash suggests that further evaluation of 10 to 20 year biomass production from these species is needed.

Poor survival and low growth of many native tree species in this study may result from ecosystem preferences of the species. Native trees such as Atlantic white cedar, cherrybark oak, white oak, and water oak displayed high mortality and low growth throughout the first growing season. Initially, this performance was attributed to drought conditions in Gibson. However, slow growth persisted for many native trees throughout 2012 when rainfall and irrigation were adequate. Cherrybark oak, loblolly pine, white oak are facultative or facultative-upland species [see 54] and prefer upland sites with well drained soils. These trees are not naturally found in saturated, poorly drained soils. *Populus spp.*, bald cypress, and green ash are known to be a facultative wetland species that indigenously grow on mesic sites and may be readily adapted for wastewater application lands where soil saturation is frequent [28, 55]. One exception to this finding is Atlantic white cedar, which exhibited high mortality during 2011. This finding should be considered in future studies when evaluating new and less traditional species for wastewater application.

An unexpected finding in this study was the lack of significance in statistical tests for height growth between *Populus* clones at Gibson. Many studies have found significant differences in biomass production between *Populus* clones with and without wastewater irrigation [27, 56, 57, 60]. One potential reason for the non-significant finding is the early evaluation of all plantings. Many studies evaluate growth two to three years after planting and as much as seven to twelve years after [27, 28, 41, 57, 65] to capture responses for pest and disease or cold tolerance. One year growth is less common, but has been used to find differences in clone responses to fertilizer [66] and carbon dioxide concentration [67]. Though this study did not find significant differences between clones in year 1, differences between *Populus* clones are likely to emerge in subsequent years. Coyle et al. [27] documented this effect over three growth seasons with 31 *Populus* clones in South Carolina, USA. At year 10, many of the clones that were documented as preferred clones at year 3 continued to exhibit superior growth [65]. Further monitoring of *Populus* clones at Gibson and Jacksonville will discern if similar findings are true for municipal waste water application sites.

Although biomass production potential is frequently considered one of the most important traits when growing SRWC species, this study has shown that percent mortality is equally as important when selecting species for plantation establishment [27, 59]. A wide range of percent mortality was documented in this study (see Figure 2). At Gibson, two of the species with greatest height growth demonstrated the highest percent mortalities. *Populus* clones 444 and 380 both grew more than two meters throughout the experimental period but had percent mortalities of 44% and 53%, respectively. In contrast, many of the other improved clones and native tree species demonstrated lower height growth as well as lower percent mortalities or higher survival (e.g. green ash, clone 230, 450). Some unlikely candidate species showed moderate percent mortality and relatively little growth (sweetgum and loblolly pine). These species are clearly undesirable for biomass production at these two wastewater application sites. The differences in percent mortality stress the importance of field trials when selecting for biomass production potential at the stand level.

In this study, nitrate+nitrite (NO_3+NO_2) and ammonia (N-NH_4) in groundwaters at both sites were below regulatory limits throughout the establishment period. These results were different than many previous studies on nitrate and ammonia leaching from SRWC systems on wastewater application sites [35, 57, 62]. Generally, nutrient concentrations are expected to increase during the establishment phase due to disturbed organic matter and undeveloped root systems [3, 58]. The ammonification of organic nitrogen and nitrification of ammonia increases water soluble nitrogen. Thus, high concentrations of NO_3+NO_2 and N-NH_4 would be expected during the establishment phase. Other wastewater application sites may find these two nutrients to temporarily exceed regulatory limits during the establishment phase, but reductions would be expected shortly thereafter either through denitrification or through root uptake. The EPA reports that denitrification is expected to account for 10 to 25% of nitrogen loss as nitrous oxide emissions from forested land treatment systems [64]. However, lysimeter studies have shown that nitrogen uptake can vary through time and by soil type for *Salix* spp. but, overall, soluble nitrogen loss decreases with time [61, 62]. Combined, these findings suggest that nitrogen will either be absorbed by tree uptake or lost to atmospheric release as nitrous oxide. Nutrient budgets are needed for other SRWC growth on wastewater application sites to determine how much nitrogen gas and other greenhouse gases are released to the atmosphere. If some species show signs of decreased gas emissions due to high uptake, the presence of greenhouse gases would be expected to decrease. More research is needed in this arena to understand what long-term impacts and environmental benefits can be expected from generating biomass on other marginal and low-productivity lands in the United States.

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APPENDICES

Appendix A: Locations of Monitoring Wells at Gibson Wastewater Application Facility.

Appendix B: Locations of Monitoring Wells at Gibson Wastewater Application Facility.

Appendix C: 2011 Planting Layout for the Gibson Wastewater Application Facility

Appendix D: Number of dead trees, total number of trees planted, percent mortality, and final height as of September 2011 for trees planted at Gibson Waste Water Treatment Facility in March 2011. Trees are ranked by low to high mortality then high to low seasonal growth if mortalities are the same.

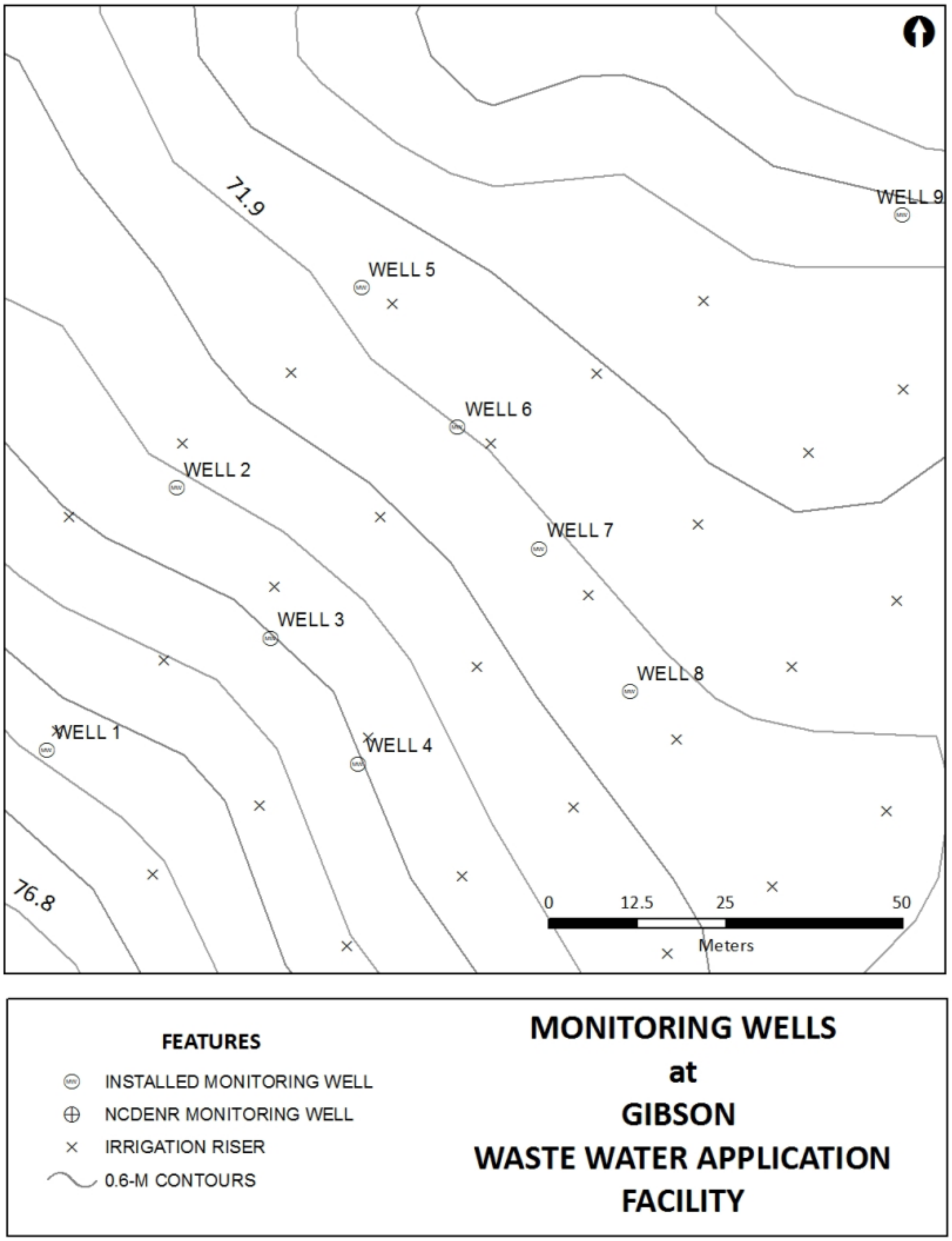
Appendix E: 2012 Planting Layout for the Gibson Wastewater Application Facility

Appemdix F: Number of dead trees, total number of trees planted, final percent mortality, initial height, mid-season height, and final height as of October 2012 for trees planted at Gibson Waste Water Treatment Facility in 2011 and 2012. Trees are ranked by low to high mortality then high to low seasonal growth if mortalities are the same.

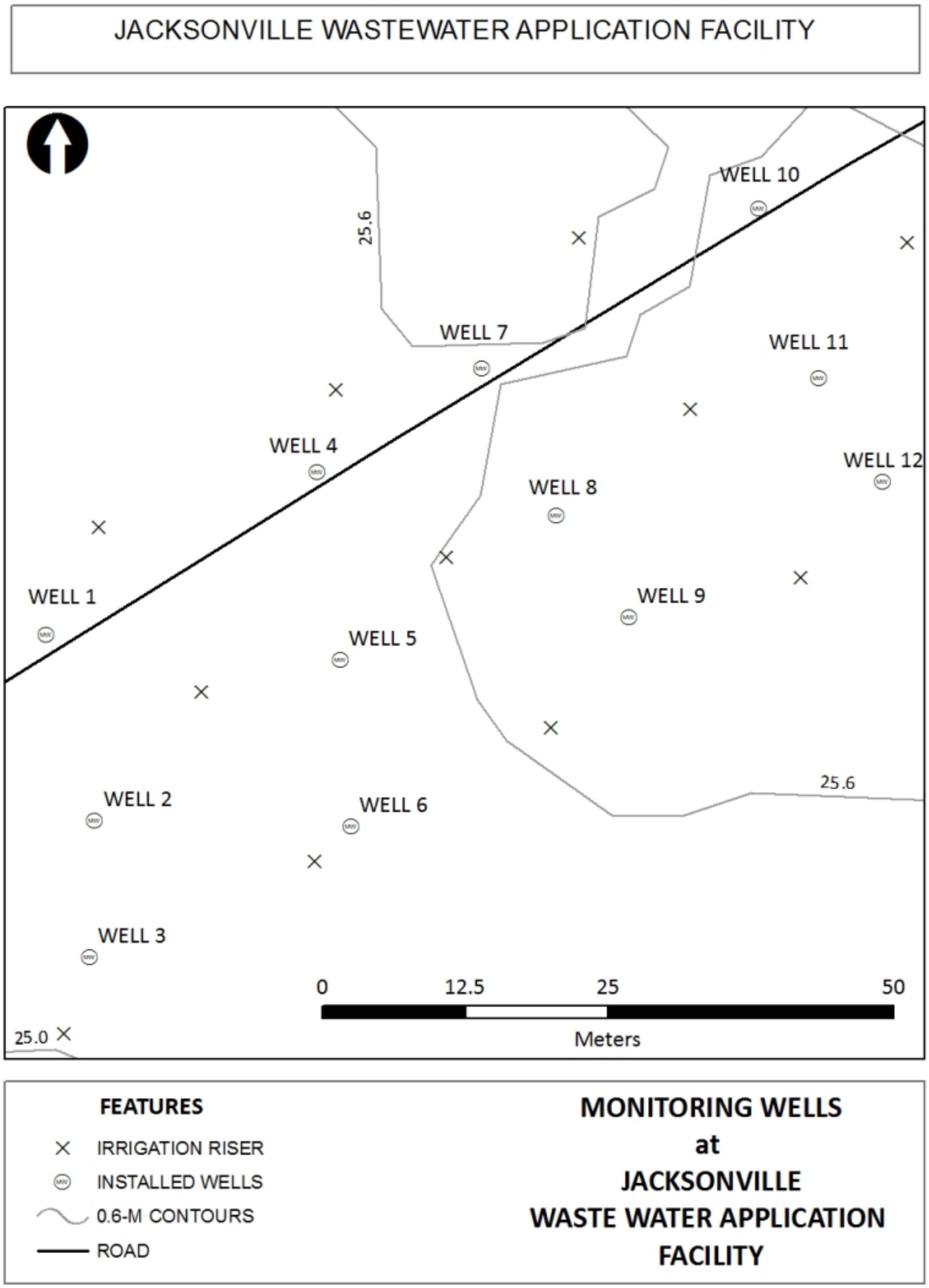
Appendix G: 2012 Planting Layout for the Jacksonville Wastewater Application Facility.

Appendix H: Number of dead trees, total number of trees planted, percent mortality, initial height, mid-season height, and final height as of October 2012 for trees planted at Jacksonville Waste Water Treatment Facility in 2012. Trees are ranked by low to high mortality then high to low seasonal growth if mortalities are the same.

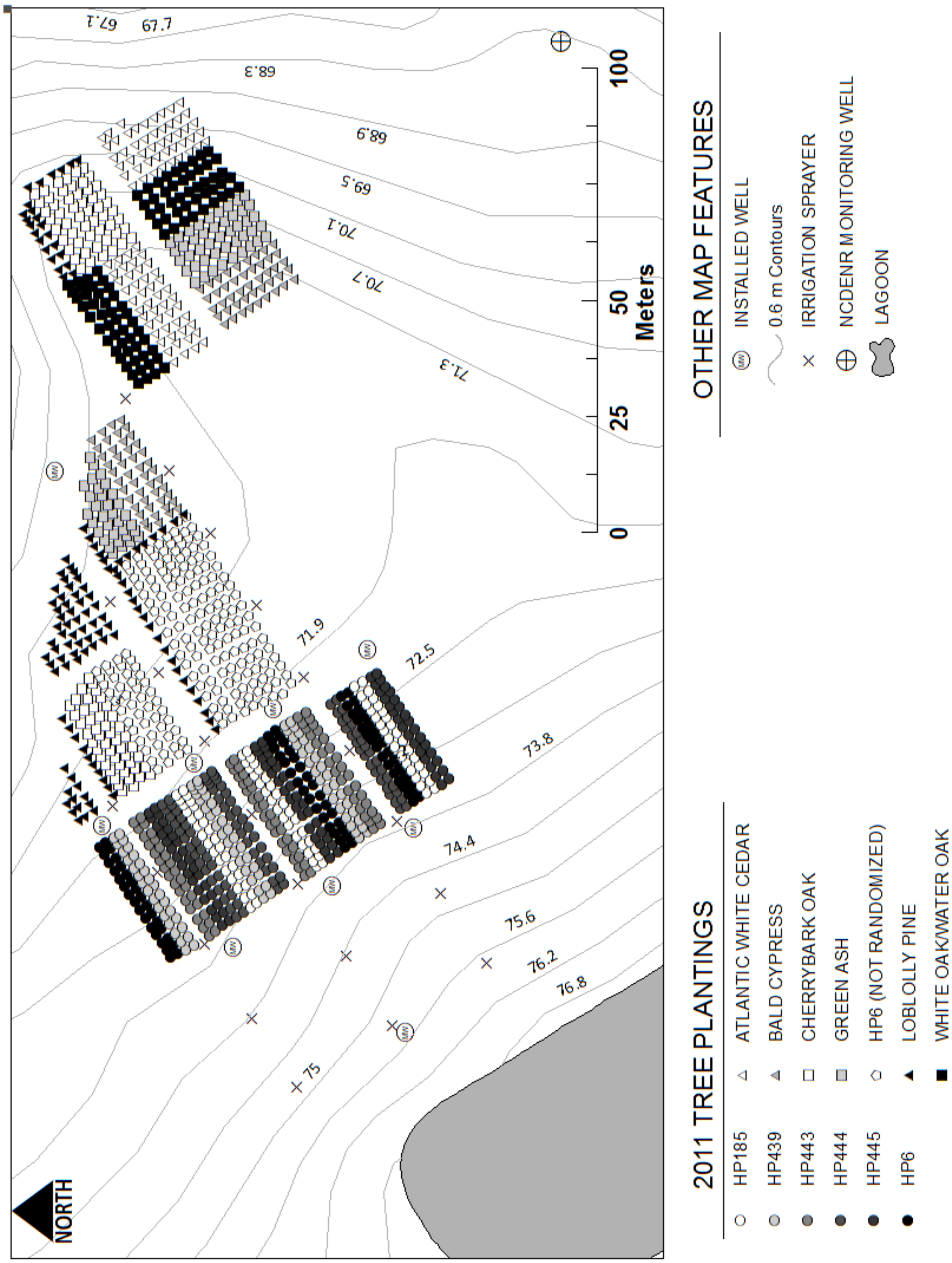
Appendix A: Locations of Monitoring Wells at Gibson Wastewater Application Facility.



Appendix B: Locations of Monitoring Wells at Gibson Wastewater Application Facility.



Appendix C: 2011 Planting Layout for the Gibson Wastewater Application Facility

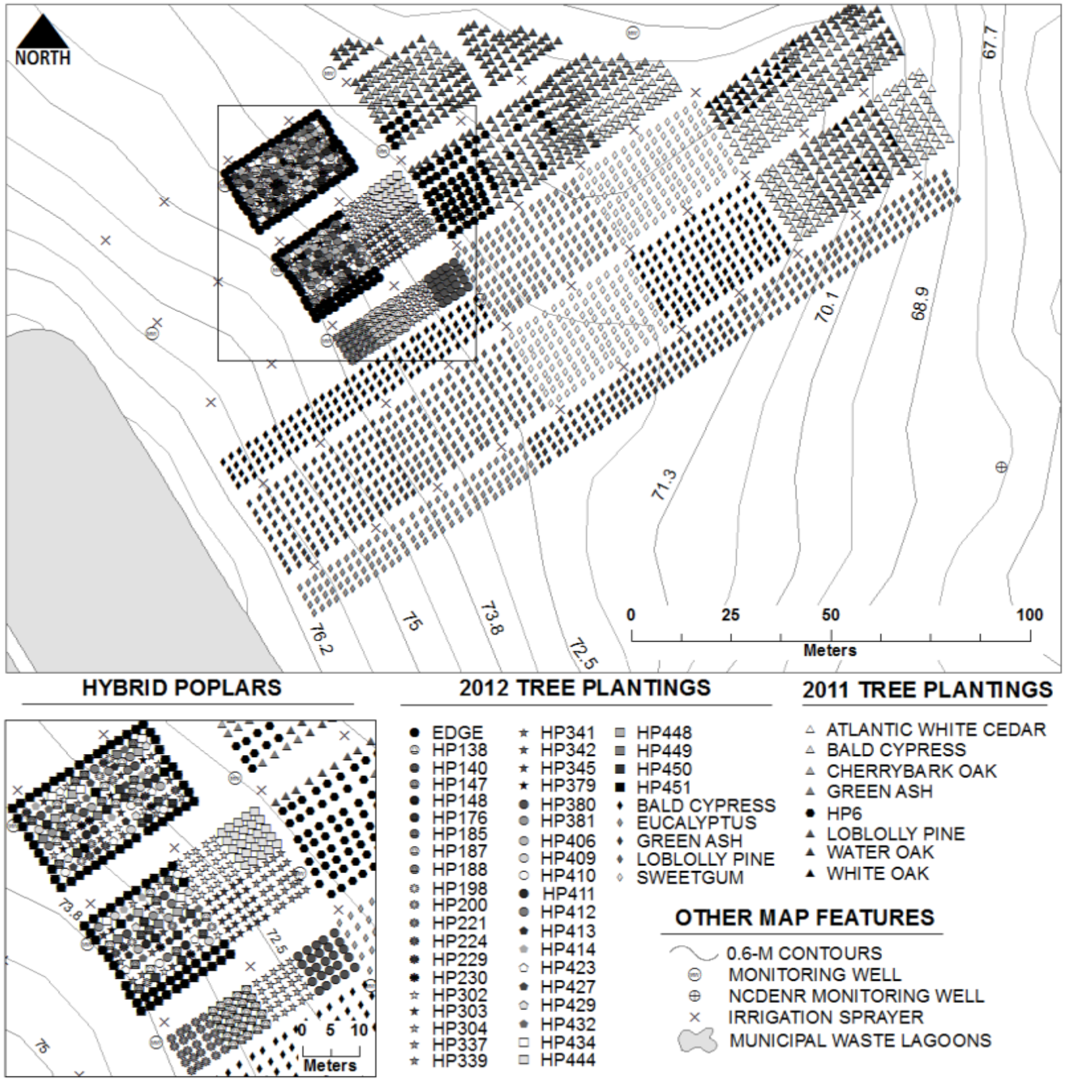


Appendix D: Number of dead trees, total number of trees planted, percent mortality, and final height as of September 2011 for trees planted at Gibson Waste Water Treatment Facility in March 2011. Trees are ranked by low to high mortality then high to low seasonal growth if mortalities are the same.

Species year planted	**Dead**	**Total**	**Percent Mortality (%)**	**Height March 2011 (cm)**	**Height September 2011 (cm)**	**Seasonal Height Growth (cm)**
Green Ash	2	77	3%	NM	125 ± 34	NM
Bald Cypress	39	93	42%	NM	91 ± 20	NM
Cherrybark Oak	41	93	44%	NM	67 ± 16	NM
White Oak	53	93	57%	NM	64 ± 20	NM
Atlantic White Cedar	60	93	65%	NM	35 ± 10	NM
Loblolly Pine	130	196	66%	NM	55 ± 17	NM
Populus spp.	487	732	67%	0	121 ± 48	121 ± 48
Populus by clone						
6	140	282	50%	0	134 ± 50	134 ± 50
185	47	90	52%	0	109 ± 27	109 ± 27
443	65	90	72%	0	107 ± 46	107 ± 46
444	75	90	83%	0	95 ± 45	95 ± 45
439	80	90	89%	0	100 ± 39	100 ± 39
445	81	90	90%	0	86 ± 45	86 ± 45

Atlantic white cedar - *Chamaecyparis thyoides*, Bald cypress – *Taxodium distchum*, Cherrybark oak – *Quercus pagoda*, Green ash – *Fraxinus pennsylvanica*, Loblolly pine – *Pinus taeada*, Sweetgum – *Liquidambar styraciflua*, White oak – *Quercus alba*, Water oak – *Quercus nigra*. NM = not measured

Appendix E: 2012 Planting Layout for the Gibson Wastewater Application Facility



Appendix F: Number of dead trees, total number of trees planted, final percent mortality, initial height, mid-season height, and final height as of October 2012 for trees planted at Gibson Waste Water Treatment Facility in 2011 and 2012. Trees are ranked by low to high mortality then high to low seasonal growth if mortalities are the same.

Year Planted Species	**Dead**	**Total**	**Percent Mortality (%)**	**Height March 2012 (cm)**	**Height July 2012 (cm)**	**Height October 2012 (cm)**	**Seasonal Height Growth (cm)**
2011							
Green Ash	4	77	5%	NM	192 ± 52	227 ± 60	101 ± 52
*Populus**	16	82	20%	NM	133 ± 78	205 ± 75	105 ± 58
Bald Cypress	44	93	47%	NM	109 ± 31	129 ± 28	35 ± 22
White Oak	48	93	52%	NM	63 ± 19	97 ± 38	42 ± 33
Cherry Bark Oak	48	93	52%	NM	41 ± 30	105 ± 43	48 ± 30
Loblolly Pine	136	196	69%	NM	93 ± 37	124 ± 42	69 ± 34
Atlantic White Cedar	67	94	71%	NM	62 ± 17	98 ± 25	75 ± 28
Populus by clone							
6	16	82	20%	NM	133 ± 78	205 ± 75	105 ± 58
2012							
Green Ash	7	248	3%	NM	96 ± 33	125 ± 37	30 ± 28
Sweetgum	13	270	5%	NM	51 ± 17	68 ± 25	18 ± 15
Populus	58	400	15%	0	113 ± 18	214 ± 73	214 ± 73
Bald Cypress	43	263	16%	NM	54 ± 18	78 ± 26	27 ± 17
Eucalyptus benthamii	56	247	23%	NM	58 ± 18	109 ± 41	47 ± 27
Loblolly Pine	85	248	34%	NM	34 ± 10	47 ± 19	13 ± 14

Atlantic white cedar - *Chamaecyparis thyoides*, Bald cypress – *Taxodium distchum*, Cherrybark oak – *Quercus pagoda*, Green ash – *Fraxinus pennsylvanica*, Loblolly pine – *Pinus taeada*, Sweetgum – *Liquidambar styraciflua*, White oak – *Quercus alba*, Water oak – *Quercus nigra*. NM = not measured

Appendix F: (Continued)

Year Planted Species	**Dead**	**Total**	**Percent Mortality (%)**	**Height March 2012 (cm)**	**Height July 2012 (cm)**	**Height October 2012 (cm)**	**Seasonal Height Growth (cm)**
2012							
Populus clone by parentage							
unknown parentage	0	15	0%	0	90 ± 41	202 ± 97	202 ± 97
Clone 148	0	5	0%	0	106 ± 44	237 ± 41	237 ± 41
Clone 138	0	5	0%	0	93 ± 47	193 ± 99	193 ± 99
Clone 147	0	5	0%	0	75 ± 39	176 ± 101	176 ± 101
P.delotides x *P. maximowiczii*	0	5	0%	0	122 ± 32	152 ± 71	152 ± 71
Clone 230	0	5	0%	0	122 ± 32	152 ± 71	152 ± 71
P. trichocarpa x *P. deltoides*	22	175	13%	0	120 ± 40	213 ± 67	213 ± 67
Clone 229	0	5	0%	0	130 ± 63	221 ± 58	221 ± 58
Clone 342	0	5	0%	0	111 ± 46	210 ± 32	210 ± 32
Clone 188	0	5	0%	0	120 ± 28	205± 32	205± 32
Clone 341	0	5	0%	0	128 ± 22	200 ± 16	200 ± 16
Clone 337	0	5	0%	0	146 ± 26	181 ± 89	181 ± 89
Clone 304	1	30	3%	0	149 ± 41	260 ± 50	260 ± 50
Clone 302	1	30	3%	0	139 ± 40	203 ± 84	203 ± 84
Clone 187	3	30	10%	0	123 ± 28	200 ± 37	200 ± 37
Clone 303	5	30	17%	0	109 ± 37	171 ± 66	171 ± 66
Clone 339	12	30	40%	0	122 ± 51	231 ± 89	231 ± 89
P. deltoides	36	205	18%	0	100 ± 43	217 ± 76	217 ± 76
Clone 185	0	5	0%	0	164 ± 20	260 ± 31	260 ± 31
Clone 140	0	5	0%	0	124 ± 19	258 ± 41	258 ± 41

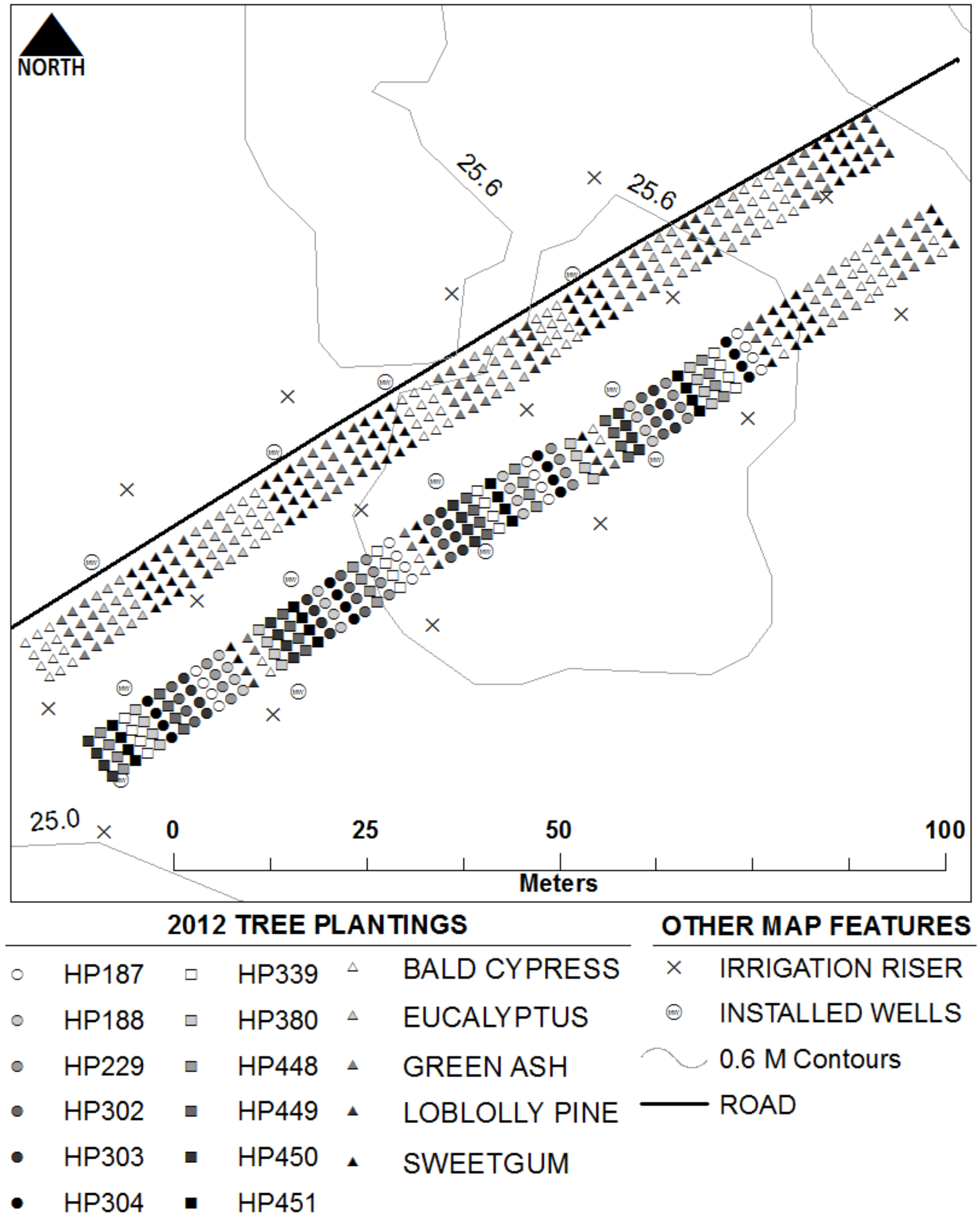
Appendix F: (Continued)

Year Planted Species	**Dead**	**Total**	**Percent Mortality (%)**	**Height March 2012 (cm)**	**Height July 2012 (cm)**	**Height October 2012 (cm)**	**Seasonal Height Growth (cm)**
Populus clone by parentage							
P. deltoides (continued)							
Clone 412	0	5	0%	0	106 ± 22	245 ± 14	245 ± 14
Clone 379	0	5	0%	0	109 ± 22	239 ± 46	239 ± 46
Clone 381	0	5	0%	0	115 ± 35	249 ± 79	239 ± 46
Clone 449	0	5	0%	0	115 ± 33	228 ± 62	228 ± 62
Clone 451	0	5	0%	0	99 ± 18	223 ± 45	223 ± 45
Clone 427	0	5	0%	0	104 ± 32	218 ± 60	218 ± 60
Clone 200	0	5	0%	0	62 ± 18	206 ± 39	206 ± 39
Clone 411	0	5	0%	0	85 ± 27	203 ± 56	203 ± 56
Clone 414	0	5	0%	0	98 ± 21	200 ± 80	200 ± 80
Clone 423	0	5	0%	0	76 ± 51	199 ± 90	199 ± 90
Clone 221	0	25	0%	0	111 ± 44	192 ± 60	192 ± 60
Clone 409	0	5	0%	0	69 ± 19	168 ± 67	168 ± 67
Clone 224	0	5	0%	0	70 ± 58	158 ± 60	158 ± 60
Clone 198	0	5	0%	0	95 ± 38	158 ± 81	158 ± 81
Clone 450	0	5	0%	0	89 ± 52	125 ± 75	125 ± 75
Clone 429	1	5	20%	0	78 ± 22	251 ± 24	251 ± 24
Clone 176	1	5	20%	0	60 ± 25	226 ± 46	226 ± 46
Clone 410	1	5	20%	0	82 ± 15	216 ± 54	216 ± 54
Clone 432	1	5	20%	0	92 ± 15	214 ± 87	214 ± 87
Clone 434	1	5	20%	0	80 ± 44	206 ± 92	206 ± 92

Appendix F: (Continued)

Year Planted Species	**Dead**	**Total**	**Percent Mortality (%)**	**Height March 2012 (cm)**	**Height July 2012 (cm)**	**Height October 2012 (cm)**	**Seasonal Height Growth (cm)**
Populus clone by parentage							
P. deltoides (continued)							
Clone 448	1	5	20%	0	112 ± 41	201 ± 70	201 ± 70
Clone 406	1	5	20%	0	73 ± 31	195 ± 42	195 ± 42
Clone 413	1	5	20%	0	77 ± 32	194 ± 57	194 ± 57
Clone 345	1	5	20%	0	87 ± 18	140 ± 71	140 ± 71
Clone 444	11	25	44%	0	157 ± 47	338 ± 87	338 ± 87
Clone 380	16	30	53%	0	71 ± 31	221 ± 67	221 ± 67

Appendix G: 2012 Planting Layout for the Jacksonville Wastewater Application Facility.



Appendix H: Number of dead trees, total number of trees planted, percent mortality, initial height, mid-season height, and final height as of October 2012 for trees planted at Jacksonville Waste Water Treatment Facility in 2012. Trees are ranked by low to high mortality then high to low seasonal growth if mortalities are the same.

Species (planted 2012)	**Dead**	**Total**	**Percent Mortality (%)**	**Height March 2012 (cm)**	**Height July 2012 (cm)**	**Height October 2012 (cm)**	**Seasonal Height Growth (cm)**
Green Ash	2	82	2%	74 ± 27	85 ± 30	86 ± 37	14 ± 25
Bald Cypress	3	82	4%	48 ± 18	69 ± 20	75 ± 27	27 ± 16
Loblolly Pine	6	50	12%	25 ± 6	40 ± 10	48 ± 19	22 ± 18
Sweetgum	13	82	16%	31 ± 9	58 ± 17	68 ± 26	33 ± 21
*Populus**	37	192	19%	0	68 ± 35	93 ± 48	93 ± 48
Eucalyptus benthamii	41	72	57%	NM	42 ± 29	72 ± 31	17 ± 17
Populus clone by parentage							
P. trichocarpa x *P. deltoides*	15	112	14%	0	84 ± 31	106 ± 52	106 ± 52
HP188	1	16	6%	0	82 ± 19	99 ± 31	99 ± 31
HP187	2	16	13%	0	90 ± 34	112 ± 52	112 ± 52
HP302	2	16	13%	0	84 ± 36	112 ± 70	112 ± 70
HP304	2	16	13%	0	94 ± 24	104 ± 42	104 ± 42
HP229	2	16	13%	0	80 ± 21	95 ± 30	95 ± 26
HP303	3	16	19%	0	74 ± 52	121 ± 90	121 ± 90
Populus deltoides	22	80	28%	0	56 ± 22	73 ± 32	73 ± 32
HP449	2	16	13%	0	60 ± 25	85 ± 40	85 ± 40
HP450	2	16	13%	0	52 ± 21	58 ± 21	58 ± 21
HP339	3	16	19%	0	85 ± 21	99 ± 36	99 ± 36
HP448	3	16	19%	0	66 ± 23	73 ± 13	73 ± 13
HP451	5	16	31%	0	53 ± 16	64 ± 21	64 ± 21
HP380	8	16	50%	0	46 ± 23	87 ± 51	87 ± 51