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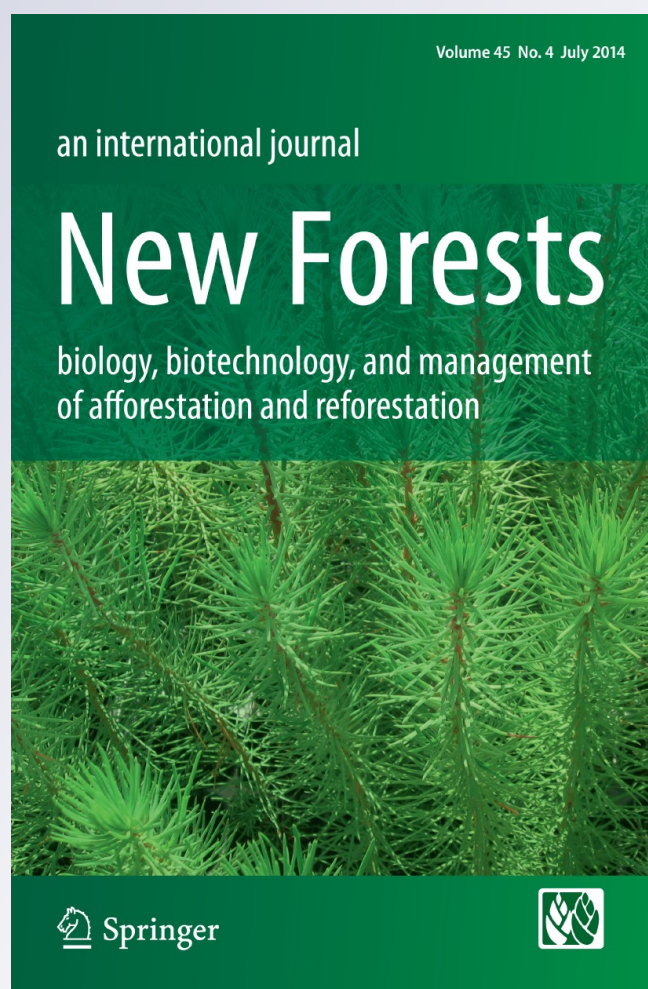
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The influence of alternative plant propagation and stand establishment techniques on survival and growth of eastern cottonwood (*Populus deltoides* Bartr.) clones

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Abstract Four eastern cottonwood clones, including standard operational clone ST66 and three advanced clonal selections were produced and included in a test utilizing five different plant propagation methods. Despite relatively large first-year growth differences among clones, all clones demonstrated similar responses to the treatments and clone × cutting treatment interactions were generally non-significant. The effects of changing cutting lengths are consistent with previous studies which indicated the potential for increased plant survival and growth with increased cutting lengths. Differences in stored carbohydrate reserves alone do not appear to completely control first-year growth and development of cuttings. First-year growth of 51 cm long cuttings planted 30.5 cm deep was greater than the same cuttings planted 48 cm deep. Stem form of plants derived from whip-tip propagation did not differ from plants derived from standard, unrooted cuttings. This propagation method offers the potential of far greater production capacity from a cutting orchard and rapid bulk-up of new or limited clones. Stand uniformity assessments suggest that surviving trees of each individual cutting treatment exhibit similar levels of growth variation. Optimization of plantation establishment techniques has the potential to increase growth of young *Populus* plantations.

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Introduction

In 2005, the Department of Energy and Oak Ridge National Laboratory developed a report titled “Biomass as a feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply” (Perlack et al. 2005). Updated in 2011, this report contains the most up-to-date projections of bioenergy supply in the United States (Perlack et al. 2011). A range of potential bioenergy species for the southeastern United States have been identified and includes *Populus* species or hybrids, loblolly (*Pinus taeda* L.) or slash (*Pinus elliottii* Engelm) pine, and sweetgum (*Liquidambar styraciflua* L.) (Wright 1994; Dale et al. 2011). Tests of *Eucalyptus* species have been established in milder winter climates across the South (Rockwood et al. 2006; Dougherty and Wright 2012). With the exception of pine which has shown dramatic increases from less than 0.9 million hectares in 1952 to approximately 13 million hectares in 1999 (Weir and Gries 2002), most of these other potential options are in early developmental stages and represent small acreages compared to pine.

Populus has been considered a leading bioenergy candidate. Favorable characteristics include a long history of tree improvement work with the genus and the relatively easy vegetative propagation. This allows deployment of clonal *Populus* plantations selected for specific characteristics (Kline and Coleman 2010). Rooting ability is one of the primary factors used in selecting clones for deployment (Robison et al. 2006), but can often vary among general species and interspecific crosses. General clonal variation also occurs (Netzer et al. 2002; Abrahamson et al. 1990; Lo and Abrahamson 1996; Kiernan et al. 2003) with rooting success ranging from 0 to 100 % (Stuhlinger and Tolliver 1985; Farmer et al. 1989; Zalesny et al. 2005). Factors affecting rooting success include date of shoot collection, cutting position along parent shoot, storage conditions, preplant soaking or chemical root stimulation treatments, cutting size, environmental preconditioning, planting date, cultural treatments, soil temperatures, and planting site characteristics (Zalesny and Wiese 2006). Cutting length and cutting diameters have been found to be particularly important since these factors determine total carbohydrate reserves of the initial cutting (Verwijst et al. 2012; Desrochers and Thomas 2003; Zalesny et al. 2003; Robison and Raffa 1996; Krinard 1983; Hansen and Tolsted 1981).

On alluvial sites, eastern cottonwood¹ (*Populus deltoides* Bartr.) productivity levels can be high (Nelson et al. 1987) and this productivity has been exploited for commercial production (Robison et al. 2006). Standard operational recommendations for alluvial sites along the Mississippi River are available (McKnight 1970), but have not been developed for upland sites in the southeastern United States. Based on previous results from Mead-Westvaco’s (MWV) research, optimal cottonwood cutting parameters on alluvial sites were cutting lengths of 51 cm with diameters from 16 to 26 mm (Rousseau, unpublished data). The research described in this paper was part of a comprehensive effort by MWV to fully develop the knowledge-base and genetic resources to support rapid development of an upland *Populus* plantation program. Research focused on developing short-rotation

¹ Hereafter referred to as cottonwood.

cottonwood plantations grown with optimal water and nutrient additions. Goals and objectives were similar to hybrid poplar plantations developed in the Northwestern United States (Stanton et al. 2002). Soils within the potential deployment area are significantly different from the alluvial soils in the Mississippi River Delta. We designed the current, study which utilized three advanced cottonwood clones and compared their responses to a standard, commercially available check clone (ST66). This clone is well-characterized, commercially available, and commonly planted on Mississippi River alluvial sites (Mohn et al. 1970). It also has favorable combinations of volume production, wood specific gravity, and alpha cellulose concentrations (Olson et al. 1985). The three advanced clones were developed with the goals of high inherent rooting capacity, increased growth rates, and enhanced leaf disease resistance, which primarily focused on *Melampsora*, *Mars-sonina*, and *Septoria*. These advanced clones were projected to comprise a significant proportion of future plantations established.

In the current study, we examined the influence of several key cutting parameters on cottonwood growth and survival. These factors included:

- The influence of changes in cutting carbohydrate reserves by altering cutting lengths.
- The influence of changes in rooting depths or variation in potential root-shoot ratios by altering the relative proportion of cuttings remaining aboveground.
- Development of actively-growing, containerized cottonwood plants versus plants derived from dormant cuttings.

The specific objectives were:

- To investigate alternative plantation establishment methods for cottonwood plantations grown under irrigated conditions in the Southeastern United States.
- To understand if a range of cottonwood clones exhibit consistent growth, survival, and stem form responses under alternative establishment techniques.
- To determine if actively-growing, containerized cottonwood propagated by rooted whip-tip cuttings exhibit comparable growth responses to standard, unrooted cuttings.
- To develop regression equations to estimate key developmental parameters.
- To investigate plant uniformity arising from each propagation method and clonal combination.

Materials and methods

Study location

The experiment was conducted on an upland site located in the Upper Atlantic Coastal Plain in Bamberg County, South Carolina (33°10'N, 81°09'W). The climate characterized by long, hot summers and short, mild winters. The annual growing season averages 226 days (March 25 to November 7). Daily high and low summer temperatures (May through September) average 31.2 and 18.6 °C. Winter (December through February) daily high and low temperatures average 15.3 and 2.4 °C. Mean annual precipitation is 121.1 cm with rainfall fairly well-distributed throughout the year. Rainfall between March and October averages 84.9 cm (Norfleet 2007). The study site is flat (slopes $\leq 2\text{--}4\%$) with well-drained, deep, sandy surface horizons. The soil is a Wagram sand classified as a loamy, kaolinitic, thermic Arenic Kandiudult (Norfleet 2007). The upper soil surface horizons to depths of 60–90 cm are sands to loamy-sands. Deeper horizons have sandy-

loam transitioning to sandy-clay-loam textures. Previous vegetation at the site consisted of other short rotation woody crop (SRWC) research plantings that were removed. Historically, this site had been used for row crop agriculture dating to the late 1800s.

Prior to test establishment, soil samples were collected at depths of 0–15 and 15–45 cm. We measured soil pH, carbon and nitrogen concentrations and extractable phosphorus, calcium, magnesium, and potassium concentrations (kg ha^{-1}). Carbon and N concentrations were determined with a C and N analyzer. (LECO FP-528, St. Joseph, MI). Soil extractable P was determined by extracting the soils with Mehlich I extractant and analyzing the extracts via Inductively Coupled Plasma—Atomic Emission Spectroscopy (ICP analysis, Perkin Elmer Optima 3000, Wellesley, MA). Exchangeable K, Ca, Mg were determined by ammonium acetate extraction followed by ICP analysis of the extract. Soil bulk density values were determined by collecting soil cores in brass rings from each respective depth and oven-drying these samples at 105 °C to a constant weight (Blake and Hartge 1986). All cation nutrient concentrations were converted to per hectare values using bulk density values. Soil textural analysis (sand, silt, and clay percentages) (Gee and Bauder 1986) and base saturation and cation exchange capacity (CEC) were determined. Results are presented in Table 1.

Plant material

Clone ST66 and three advanced cottonwood clones (clones 4, 5, and 6) were included in this test. All cuttings were obtained from MWV's cottonwood cutting orchards. Cuttings were harvested in January, cut to 51 cm lengths, and stored and shipped frozen to South Carolina. Cuttings were thawed for 2 days in water to assure full hydration and optimum rooting (Desrochers and Thomas 2003). Cuttings were sorted to assure that they had viable, undamaged buds within 5 cm of the terminal end (Radwan et al. 1987; Wiese et al. 2006). Cuttings had diameters ranging from 20 to 28 mm in diameter.

Production of rooted planting stock

Whip-tip material was collected from the same cottonwood cutting orchard in January 1998. Whip-tip material consisted of lateral branches as well as terminal shoots with diameters less than 16 mm. This material is not utilized for field propagation. Plants were propagated under greenhouse and nursery conditions from the first week in February through early May 1998 using Ray Leach SC-10 Super Cells. Cottonwood whip-tips were soaked in water for 24 h before being cut to 7.5–15 cm lengths. The distal end of each cutting was dipped in Rootone F rooting hormone before planting. The photo period was extended to 16 hours using high-pressure sodium lights. Initial plant density was 527 plants per square meter at the start of the propagation cycle, but decreased to 183 per square meter approximately 6 weeks into the propagation cycle. In late April, all plants were moved outdoors to acclimate prior to planting. Quantifying propagation success was not an objective, but all these clones would be classified as having good-to-excellent rooting potential. Propagation success exceeded 75 % for all clones. Plants were sorted for uniformity for test establishment. Plants were 25–35 cm in height when planted.

Cutting treatments

- Treatment 1: Containerized, in-leaf, rooted cuttings.

Table 1 Initial soil characteristics in the 0–15 and 15–45 cm depths

Soil parameter	0–15 cm depth	15–45 cm depth
Soil pH	5.89	5.55
Carbon concentration (g kg^{-1})	7.40	4.20
Nitrogen concentration (g kg^{-1})	0.40	0.30
Phosphorus content (kg Ha^{-1})	82	45
Calcium content (kg Ha^{-1})	458	315
Magnesium content (kg Ha^{-1})	71	53
Potassium content (kg Ha^{-1})	136	94
CEC ($\text{cmol}_c \text{ kg}^{-1}$)	4.50	3.49
Base saturation %	20.7	18.5
Sand %	88.2	84.7
Silt %	5.4	7.6
Clay %	6.4	7.7
Soil texture	Sand	Loamy-sand

- Treatment 2: Dormant unrooted, 51 cm cuttings planted 48.5 cm deep (Standard treatment).
- Treatment 3: Dormant unrooted, 40.6 cm cuttings planted 38 cm deep.
- Treatment 4: Dormant unrooted, 30.5 cm cuttings planted 28 cm deep.
- Treatment 5: Dormant unrooted, 51 cm cuttings planted 30.5 cm deep.

Study design and establishment

The statistical design utilized was a Randomized Complete Block Design with 25 replications. Blocking was used to account for differences in depth to sandy-clay-loam subsoil textures. Within each replication, each clone and propagule type were represented by a single-tree plot. The test site was planted on May 7 and 8, 1998. Tree spacing was $2.438 \text{ m} \times 3.048 \text{ m}$ ($1,346 \text{ trees ha}^{-1}$). Each row of trees had a single drip-line with emitters spaced 76 cm apart running the length of the planting row. Trees were irrigated up to 3.56 cm per week based on ambient rainfall patterns and soil moisture holding capacities. Plots were fertilized 3 days per week from planting through October 31. Fertilization rates were 50 kg ha^{-1} of nitrogen (Total N applied for the entire year or approximately 2.4 kg ha^{-1} of N per week). All nutrients were applied in the form of 7-0-7 (nitrogen, phosphorus, and potassium,) liquid fertilizer applied through the drip irrigation system. The fertilizer applications also included calcium, magnesium, and micronutrients. We applied oxyflourfen (Goal 2XL) and pendimethalin (Pendulum) immediately before planting for competition control. Before planting, the surface layer of the herbicide treated soil was scraped away to prevent it from being deposited into the planting hole. Cuttings, with the exception of treatment 5, were planted with 2.5 cm of the cutting remaining above the soil surface. Directed backpack sprays of glyphosate were used in midsummer and early fall to control competing vegetation at the test site. Weed competition was minimal throughout the first growing season. Cottonwood leaf beetle (*Chrysomela scripta*) was controlled with directed spray applications of Sevin and damage throughout the first growing season was minimal. All clones included in the test had moderate to high levels of resistance to cottonwood leaf rust (*Melampsora medusae*) and premature defoliation was minimal.

Survival and growth measurements

In December 1998 survival, and growth measurements were made. Tree heights (m) and stem diameters (cm) at 1.37 m height (Referred to as diameter at breast height or DBH) were measured. Aboveground year-one stem Volume Index was calculated as DBH squared $\times$ height (m³). Tree form measurements were made by counting the number of dominant stems arising from each cutting or each rooted, containerized plant. Based on this assessment, the relative proportion of single-stem trees of each treatment was calculated.

Development of biomass equations

We utilized data from destructive harvests of cottonwood grown in a USDA Forest Service test established at the Savannah River Site (SRS), a National Environmental Research Park, located near Aiken, South Carolina, USA (33°23'N, 81°40'W) (Coleman et al. 2004). This test is located 51 km northwest of the MWV test. In the SRS test, 23 individual trees of cottonwood clone ST66 and 22 trees of clone S7-C15 were destructively harvested near the end of the summer of their second growing season (18 months following planting). During the destructive harvest at the SRS test, trees were measured to determine height, basal diameter, and DBH. Subsamples of foliage were collected from each tree and returned to the lab to determine specific leaf area (SLA) on a Licor 3100 leaf area meter. This subsample of leaves was then oven-dried at a temperature of 65.0 °C. All the remaining leaves on trees in the field were collected and these leaves were also oven-dried at 65.0 °C. Based on total dry leaf mass and SLA data, total leaf area per individual tree was calculated. Branch and boles were harvested and these in turn were oven dried. Once completed, these measurements allowed total aboveground woody biomass (stem biomass plus branch biomass), total aboveground biomass (stem biomass plus branch biomass plus leaf biomass), and total leaf area to be calculated for each destructive harvest tree.

We were unable to utilize biomass equations presented in Coleman et al. (2004) since they presented only biomass equations for one-year old cottonwood based on basal diameters. At the MWV test, we only measured DBH, not basal diameters. In addition, 7-month-old trees in the MWV test were substantially larger than one-year-old trees at the SRS test. Eighteen-month-old trees at the SRS test were comparable in size to the 7-month-old trees MWV study. Destructively harvested, 18-month-old trees in the SRS test ranged from 1.74 to 5.01 m tall (mean = 3.40 m) while trees in the MWV test ranged from 0.33 to 4.36 m (mean = 3.08 m). We independently developed regression equations to predict these parameters for each tree in the current study. These equations were simple linear regression equations based on DBH² $\times$ height relationships. Total aboveground biomass equations did not differ by clone, therefore a single, uniform equation was developed for all clones. Our analyses indicated that biomass equations based on DBH² $\times$ height relationships more accurately predicted biomass components compared to biomass equations based only on basal diameter² or DBH² relationships (Kaczmarek et al., unpublished data). The equation developed by this method accurately predicted total aboveground woody biomass dry weights ($R^2 = 0.917$), total aboveground biomass dry weight ($R^2 = 0.893$), and total leaf area ($R^2 = 0.788$). Full equations for each component are listed below.

- Stem biomass (g) = $199.54 + 199,496.89 \times \text{Volume Index}$ ($R^2 = 0.962$)
- Total branch biomass (g) = $101.58 + 115,357.48 \times \text{Volume Index}$ ($R^2 = 0.776$)
- Total leaf biomass (g) = $351.00 + 152,703.28 \times \text{Volume Index}$ ($R^2 = 0.764$)

- Total aboveground woody biomass (g, includes stem + branches) = $301.12 + 314,854.37 \times \text{Volume Index}$ ($R^2 = 0.917$)
- Total leaf area (cm^2) = $34,339.02 + 16,351,626 \times \text{Volume Index}$ ($R^2 = 0.788$)
- Total aboveground biomass (g, includes stem + branches + leaves) = $652.12 + 467,557.64 \times \text{Volume Index}$ ($R^2 = 0.893$)

We estimated leaf areas since this parameter has often been closely associated with early growth potential of *Populus* clones (Ridge et al. 1986; Barigah et al. 1994; Monclus et al. 2005). We realize that site and clonal specific biomass equations and leaf area equations are preferred over generalized equations. It is true that these sites were separated by approximately 51 km and that this test was established in a different year than the test in which the biomass equations were derived. These seem to be relatively minor differences that would influence specific leaf area measurements. We believe that the state of canopy development and clonal components would be most likely to influence specific leaf area measurements. Trees in the current test closely match the size of the harvested trees and canopy development was similar. Clone ST 66 is included in both tests and the three remaining clones in the cutting test are all pure *P. deltoides* clones. While we do not have specific leaf area measurements in this test, results from other internal MWV tests demonstrated little difference in specific leaf area measurements at similar stages of canopy development among the four clones (Kaczmarek et al. unpublished data). Projected leaf area on a per hectare basis was calculated by multiplying the predicted total leaf area of the mean tree of each clone and cutting treatment multiplied by the mean number of surviving trees per hectare (Survival percentage divided by $100 \times 1,346$ trees per hectare) for that treatment combination.

Statistical analyses

Standard Analysis of Variance (ANOVA) was utilized to determine if main effect terms (block, clone, and cutting treatment) and clone $\times$ cutting treatment interaction terms were significant for the measured or estimated variables at the 5 % level. For each variable, normality assumptions were examined before ANOVA tests were performed. All variables with the exception of mean number of stems per cutting, single-stem percentage, and survival percentage were normally distributed. For these three variables, logarithmic transformations were performed before ANOVA tests were conducted. If the main effect or interaction terms were significant, mean separation tests were then performed using Tukey's HSD test at the significance 5 % level. All statistical analyses were completed using the JMP statistical analysis software package (SAS Institute, Cary, NC).

We assessed potential stand uniformity by calculating Gini coefficients (G_u) for each clone and cutting treatment combination. The G_u measures inequality as a function of the sum of the absolute differences between all pairs of observations (Weiner and Thomas 1986; Thomas and Weiner 1989; Knox et al. 1989; Lexerød and Eid 2006; Metsaranta and Lieffers 2008). Gini coefficients vary from 0 to 1.0 with a G_u of zero indicating perfect equality, where all values are the same while a G_u of one expresses maximal inequality among values. Separate G_u were calculated for height, DBH, volume, total aboveground woody biomass, and total leaf area. Algorithms to calculate G_u for each variable were developed in JMP and all calculations were completed using these derived algorithms.

Results

Clonal selection and the five cutting treatments each significantly influenced most growth parameters and stem form assessments, but with the exception of single stem percentage, interactions between clone and cutting treatments were generally negligible (Table 2). Clonal selection significantly influenced all individual tree growth parameters (height, DBH, volume, aboveground woody biomass, and total leaf area) and stem form assessments (number of stems and single stem percentage) (Table 2). Survival was not significantly different among the four test clones (Table 2). Clone ST66 was generally the slowest-growing while clones 5 and 6 exhibited more rapid growth. Clone 4 generally had lower initial growth rates than clones 5 and 6, but more rapid growth than clone ST66. Clone 4 had significantly greater DBH growth than Clone ST66. Although all other growth parameters were slightly larger, none were significantly greater than ST66 (Table 3).

Stem form assessments (number of stems, single stem percentage) suggest that initial form of clone ST66 was equal to or superior to clones 4, 5, or 6. Clone ST66 had significantly lower number of stems than clone 6 (1.06 vs. 1.21) and significantly greater single-stem percentages (96 vs. 83 %) (Table 3). Stem form for clones 4 and 5 was intermediate between clones ST66 and clone 6 and did not differ significantly from either clone (Table 3). Mean overall survival ranged from 90.4 % for clone 6 to 95.2 % for clone ST66 and did not differ significantly by clone.

All growth, stem form parameters, and survival rates were influenced by cutting treatment (Table 2). Cutting treatments comparing different cuttings lengths with 2.5 cm of the cutting remaining above ground (Treatments 2, 3, and 4) demonstrated a trend for longer cuttings to have greater first-year growth than shorter cuttings (Table 3). This was demonstrated by increasing height, DBH, volume, aboveground biomass, and increased leaf areas of longer cuttings compared to shorter cuttings (Table 3). These differences were not always statistically significant, but in no case did shorter cuttings outperform longer cuttings (Treatment 2). Among these 3 treatments, stem form as assessed by the number of stems per cutting or the single-stem percentage were unaltered by differing cuttings lengths if the same absolute amount of the cutting remained aboveground. Survival of the shortest cuttings (Treatment 4) was approximately 7–8 percentage points lower than survival of longer cuttings (Treatments 2 and 3), but this difference was not statistically significant at the 5 % level. Among these cutting lengths, the trend was for the longest cuttings to exhibit

Table 2 Analysis of variance table (ANOVA) for main effect terms and clone $\times$ cutting treatment interactions for selected plant parameters

Plant parameter	Block	Clone	Cutting treatment	Clone $\times$ cutting treatment
Height (m)	<0.0001	<0.0001	<0.0001	0.4989
DBH (cm)	<0.0001	<0.0001	<0.0001	0.5753
Volume	<0.0001	<0.0001	<0.0001	0.3009
Total aboveground woody biomass	<0.0001	<0.0001	<0.0001	0.3009
Leaf area	<0.0001	<0.0001	<0.0001	0.3009
Number of stems	0.7530	0.0191	0.0391	0.1838
Single stem percentage	0.3159	0.0254	0.0040	0.0250
Survival percentage	0.8337	0.4471	0.0194	0.2579

For each growth parameter, *P* values are indicated. *P* values <0.05 (5 % level) are considered statistically significant in this study

Table 3 Mean values for selected plant parameters

Clone, cutting treatment combinations	Height (m)	DBH (cm)	Volume Index (m ³)	Aboveground woody biomass (kg)	Mean number of stems	Single stem percentage	Survival percentage	Total individual tree leaf area (m ²)	Projected stand leaf area
Clone									
ST66	2.85b	1.73c	0.00117b	0.668b	1.06b	95.8a	95.2a	5.34b	0.68
Clone 4	2.88b	2.02b	0.00160b	0.803b	1.18ab	87.3ab	94.4a	6.04b	0.77
Clone 5	3.22a	2.29ab	0.00212a	0.969a	1.11ab	90.6ab	92.8a	6.90a	0.86
Clone 6	3.36a	2.43a	0.00238a	1.051a	1.21a	83.2b	90.4a	7.33a	0.89
Cutting treatment									
Treatment 1	3.25a	2.42a	0.00239a	1.053a	1.07b	95.8a	96.0a	7.34a	0.95
Treatment 2	3.06abc	2.08bc	0.00174bc	0.848bc	1.13ab	90.3ab	93.0ab	6.27bc	0.79
Treatment 3	3.01bc	1.97cd	0.00150c	0.773c	1.13ab	91.5a	94.0ab	5.88c	0.74
Treatment 4	2.82c	1.72d	0.00121c	0.683c	1.14ab	89.7ab	86.0b	5.42c	0.63
Treatment 5	3.21ab	2.33ab	0.00213ab	0.972ab	1.23a	79.4b	97.0a	6.92ab	0.90

For each growth parameter clone or cutting treatments followed by the same letter do not differ significantly from one another at the 5 % level

superior growth compared to the moderate-length cuttings and these cuttings general exhibited superior growth to the shortest cuttings. Stem form assessments were not altered by cutting length (Table 3).

Comparisons of the same cuttings lengths with different cuttings lengths remaining aboveground (Treatment 2 vs. Treatment 5) suggest that leaving 20 cm of 51 cm long cuttings aboveground has the potential to increase growth (Table 3). While not statistically significant, treatment 5 showed increased height, DBH, Volume Index, aboveground biomass, and increased leaf areas compared to cuttings with only 3 cm of their cutting length remaining aboveground (Treatment 2). Survival was not altered by these treatments, but there was a tendency for cuttings with increased cutting lengths remaining aboveground to produce a greater number of stems per cutting and have a reduced percentage of resulting trees to be assessed as single-stem (Table 3). Stem form assessments exhibited significant clone $\times$ treatment interactions. Clone ST66 exhibited relatively small decreases in single-stem plants arising from shallower planting (approximately 4 percentage point decreases) while advanced clones 4, 5, and 6 exhibited slightly greater decreases in single-stem plants (approximately 12, 13, and 13 percentage points for clones 4, 5, and 6, respectively) (Table 4).

Comparisons of rooted cuttings to standard, unrooted, 51 cm long cuttings planted 48 cm deep suggest that first-year growth of the rooted cuttings was superior to the unrooted cuttings (Table 3). Diameter at Breast Height, volumes, aboveground biomass, and leaf area development were significantly greater for the rooted cuttings compared to the unrooted cuttings (Treatment 1 vs. 2). Growth of the rooted cuttings exceeded the standard treatment (treatment 2) by approximately 20–25 % (Table 3). Neither stem form assessments nor survival varied between these two treatments suggesting that rooted, containerized cuttings provided an equivalent, but slightly larger tree than standard, unrooted cuttings at the end of the first growing season.

Gini coefficients (G_u) were calculated for each clone and cutting treatment combination (Table 5). Within a cutting treatment, the four clones have similar, low G_u values indicating relatively high uniformity of growth. When G_u values for each individual clonal cutting treatment are examined, no clear trends are present. This suggests that the five cutting treatments have not altered stand uniformity of surviving trees. These results suggest that stands regenerated from whip-tip material on similar sites and under similar cultural conditions would have similar stand uniformity patterns to those derived from traditional unrooted cuttings.

Discussion

The clones selected for this study represent a range of clonal performance with the advanced clonal selections exhibiting increased first-year growth compared to clone ST66. While it is questionable to assess growth based on age-one heights or diameters, longer-term tests of these clones do suggest that there are pronounced differences in growth potential (Rousseau, unpublished data). Individual tree leaf area assessments are approximately 13, 29, and 37 % greater for clones 4, 5, and 6 respectively compared to the individual tree leaf areas of clone ST66 (Table 3). Woody biomass also shows relatively large increases for the advanced clones relative to clone ST66. Aboveground biomass for clones 4, 5, and 6 exceeds that of clone ST66 by 9, 31, and 57 %, respectively. Despite the relatively large range of performance, all clones respond in similar ways to the treatments. The only measured parameter that exhibited significant clone $\times$ treatment interactions was

Table 4 Mean values for selected plant parameters by clone and cutting treatment combinations

Clone, cutting treatment combinations	Height (m)	DBH (cm)	Volume Index (m ³)	Aboveground woody biomass (kg)	Mean number of stems	Single stem percentage	Survival percentage	Total individual tree leaf area (m ²)	Projected stand leaf area
Clone ST66									
Treatment 1	2.97	1.99	0.00150	0.773	1.00	100.0a	100.0	5.88	0.79
Treatment 2	2.80	1.70	0.00108	0.732	1.00	100.0a	96.0	5.20	0.67
Treatment 3	2.91	1.68	0.00110	0.649	1.26	82.6ab	92.0	5.24	0.65
Treatment 4	2.60	1.40	0.00077	0.545	1.00	100.0a	96.0	4.70	0.61
Treatment 5	2.99	1.89	0.00137	0.736	1.04	95.8a	96.0	5.67	0.73
Clone 4									
Treatment 1	3.08	2.28	0.00199	0.928	1.04	95.6a	92.0	6.69	0.83
Treatment 2	2.91	2.03	0.00201	0.815	1.17	87.5ab	96.0	6.10	0.79
Treatment 3	2.87	2.02	0.00156	0.792	1.13	91.3ab	92.0	5.98	0.74
Treatment 4	2.45	1.41	0.00075	0.537	1.26	87.0ab	92.0	4.66	0.58
Treatment 5	3.09	2.33	0.00201	0.934	1.28	76.0ab	100.0	6.72	0.90
Clone 5									
Treatment 1	3.40	2.71	0.00315	1.282	1.08	96.0a	100.0	8.58	1.15
Treatment 2	3.11	2.15	0.00183	1.119	1.08	95.8a	96.0	6.43	0.83
Treatment 3	3.06	2.01	0.00148	0.766	1.00	100.0a	96.0	5.85	0.76
Treatment 4	3.15	1.91	0.00141	0.745	1.20	76.2ab	80.0	5.74	0.62
Treatment 5	3.38	2.58	0.00260	1.119	1.22	82.6ab	92.0	7.68	0.95
Clone 6									
Treatment 1	3.56	2.68	0.00290	1.214	1.17	91.3ab	92.0	8.17	1.01
Treatment 2	3.44	2.47	0.00244	1.069	1.27	77.3ab	88.0	7.42	0.88
Treatment 3	3.18	2.15	0.00183	0.879	1.13	91.7ab	96.0	6.43	0.83
Treatment 4	3.22	2.30	0.00212	0.968	1.11	94.7ab	76.0	6.90	0.71
Treatment 5	3.38	2.53	0.00256	1.106	1.36	64.0b	100.0	7.62	1.03

For variables where the clone × cutting treatment interaction is significant, treatment combinations followed by the same letter do not differ significantly from one another at the 5 % level

Table 5 Calculated Gini coefficients (G_u) for selected plant parameters

Clone, cutting treatment combination	Height (m)	DBH (cm)	Volume Index (m ³)	Aboveground woody biomass (kg)	Total leaf area (cm ²)
Clone ST66					
Treatment 1	0.106	0.217	0.428	0.261	0.222
Treatment 2	0.129	0.225	0.438	0.232	0.191
Treatment 3	0.132	0.229	0.486	0.272	0.215
Treatment 4	0.164	0.271	0.549	0.246	0.196
Treatment 5	0.105	0.222	0.445	0.262	0.220
Clone 4					
Treatment 1	0.096	0.205	0.416	0.281	0.245
Treatment 2	0.128	0.178	0.441	0.278	0.238
Treatment 3	0.130	0.211	0.386	0.239	0.204
Treatment 4	0.149	0.300	0.515	0.226	0.180
Treatment 5	0.091	0.176	0.320	0.217	0.189
Clone 5					
Treatment 1	0.096	0.217	0.399	0.306	0.277
Treatment 2	0.085	0.224	0.473	0.311	0.269
Treatment 3	0.074	0.201	0.391	0.237	0.201
Treatment 4	0.077	0.202	0.437	0.260	0.220
Treatment 5	0.072	0.164	0.331	0.242	0.215
Clone 6					
Treatment 1	0.073	0.152	0.322	0.242	0.218
Treatment 2	0.081	0.166	0.351	0.251	0.223
Treatment 3	0.104	0.201	0.383	0.252	0.216
Treatment 4	0.101	0.187	0.366	0.252	0.226
Treatment 5	0.082	0.173	0.364	0.265	0.236

Gini coefficients vary from 0 to 1.0 with A Gini coefficient of zero expresses perfect equality, where all values are the same while a Gini coefficient of one expresses maximal inequality among values

the proportion of trees with single stems (Table 2). For all other variables, clone $\times$ treatment interactions were not significant. This suggests that, for clones examined, there were consistent clonal responses.

The effects of changing cutting lengths are consistent with previous studies indicating the potential for increased survival and growth with increased cutting lengths (Desrochers and Thomas 2003; Verwijst et al. 2012) (Table 2). This suggests that the primary mechanism altering survival and growth was increased stored carbohydrate reserves of the longer cuttings. All cuttings were within the same diameter range thus avoiding confounding cutting diameter changes with changing cutting lengths. The various cutting lengths did have different rooting depths in the field, but it is unlikely rooting environment differed by depth. At the study site, the surface 60 cm to approximately 90 cm consisted of soils of fine-sand to loamy-sand textures so cuttings in treatments 2, 3, and 4 would be rooting in the soil to depths of 48, 38, and 28 cm (Table 1). The irrigation system at this site was engineered to produce a continuous, wetted soil zone approximately 75 cm wide at depths of 20 cm or greater. This would be expected to reduce potential growth differences due to differences in soil water availability at the different depths (Puri and Thompson

2003). The magnitude of the growth responses in treatments 2, 3 and 4 suggests a gradual growth gradient exists resulting from changing cutting lengths rather than a sharp threshold being reached. Other studies, have shown increased growth or survival with increased carbohydrate reserves as a function of increased cutting lengths (Desrochers and Thomas 2003; Verwijst et al. 2012) or diameters (Dickmann et al. 1980; Hansen and Tolsted 1981; Robison and Raffa 1998; Zalesny et al. 2003).

Leaf area development was approximately 16 % greater for treatment 2 as compared to treatment 4 while treatment 3 had approximately 11 % greater leaf area than treatment 4. Survival did not differ significantly for cutting treatments 2, 3 or 4, but survival for the shortest cuttings was reduced by approximately 7–8 % compared to the longer cuttings (Table 3). Survival and growth measures in the current study occurred under irrigated conditions with almost complete competition control. Cottonwood plantations can be subject to poor rooting leading to non-uniform stands and lower rotation yields. The high survival rates of all four clones are a result of multiple trait selection that included both rooting and growth characteristics. The location of the study, the specific timing of establishment, and the ability to irrigate the site contributed to excellent establishment success. The early-May establishment period in west-central South Carolina provided the cuttings with favorable soil temperatures for rapid rooting and a drip irrigation system provided needed soil moisture. Landhausser (2003) found that rooting and early growth of *Populus* cuttings were greater at soil temperatures of 25 °C compared to 15 or 5 °C. Zalesny et al. (2004) demonstrated that rooting of *P. deltoides* clones increased with increased belowground growing degree days and the threshold for effective rooting of *P. deltoides* exceeded that of *Populus* hybrids. We did not measure soil temperatures in the current study, but measurements in other studies at this site suggest that soil temperatures in the 0–15 cm depth at this site in May and June generally range from approximately 22–30 °C (Kaczmarek, unpublished data). These conditions may represent best-case scenarios for rooting and growth. Under more stressful conditions, larger differences between cutting length treatments 2, 3 and 4 could be evident. There was no indication that stem form was altered by changing cuttings lengths. Cutting treatments 2, 3, and 4 had similar numbers of stems and the relative proportion of single-stem trees was unaffected by changes in cutting length.

Differences in stored carbohydrate reserves alone do not appear to completely control growth and development of unrooted cottonwood cuttings. First-year growth parameters of 51 cm long cuttings planted 30.5 cm deep (Treatment 5) were consistently greater than growth parameters of 51 cm long cuttings planted 48 cm deep (Treatment 2). Cuttings with greater shoot length remaining aboveground had individual tree leaf area increases of 10 % and aboveground biomass increases of 15 % over treatment 2. This occurs despite equal carbohydrate reserves of the identical cuttings lengths. The greater length of the cutting remaining aboveground led to greater aboveground budbreak and more rapid leaf area development. This increased leaf area provides greater photosynthetic capacity allowing the tree to grow on sugars produced rather than relying on the initial carbohydrate reserves of the cutting. In treatment 2, it was most common for either 1 or 2 shoots to arise from the cutting. In contrast, in treatment 5, it was common for two or more buds to elongate from the aboveground portion of the cutting. These multiple shoots gave the visual appearance of having greater total tree leaf area. By the end of the first growing season, many of these resulting stems assumed broad branch angles and were clearly subordinate to the dominant stem. Cuttings of treatment 5 had slightly greater number of stems than treatment 2 (1.23 vs. 1.13, Table 3) and a lower percentage of single stems trees (79.4 vs. 90.3 %). These differences were not significant, but do suggest that potential changes in stem form should

be considered. These results are consistent with those obtained by Hansen et al. (1991), which found that the number of shoots arising from hybrid poplar cuttings increased as the length of shoot remaining aboveground increased. The practical significance of these potential stem form changes may depend upon intended end uses. If plantations are established at close spacings with bioenergy production as the intended end product, greater resulting stems may be a neutral or even positive effect. If solid wood products with conventional harvesting regimes are used, increasing numbers of stems may be less desirable. The significant clone $\times$ cutting treatment interaction for single-stem percentage suggests that these effects could vary by clone. This suggests that the genetic propensity of individual clones to produce multiple stems will influence responses to varying planting methods. Potential growth advantages of shallower planting allowing more rapid budbreak and leaf area development may need to be tested for individual clones of interest and weighed against potential stem form changes in the resulting stand.

Rooted, containerized cuttings (Treatment 1) demonstrated growth advantages over standard 51 cm long unrooted cottonwood cuttings planted 48 cm deep (Treatment 2, Table 3). Across all clones, leaf area and aboveground biomass of treatment 1 exceeded those of treatment 2 by 17 % and 24 %. Aboveground biomass differences between these two treatments varied from approximately 6 % for clone ST66 to 15 % for clone 5. These growth advantages may be due to the initial size advantages of the rooting cuttings. At the time of planting, all containerized plants selected for inclusion in the test were 25–35 cm tall. At the end of the first growing season, plants in treatment 1 were approximately 21 cm taller than treatment 2. These trees maintained height advantages but did not actually exhibit greater height growth through the first growing season. Trees in treatment 1 also had the advantage of an established, actively-growing root system. All unrooted cutting treatments (treatments 2–5) had to initiate root growth at the time of planting. This may have been an initial disadvantage for the unrooted cuttings, but their growth for the remainder of the first growing season was similar to the rooted cuttings. Unrooted cuttings are carrying on two processes simultaneously, with the production of roots and initiating leaf development. The drain on food reserves in the cutting plays a critical role in these two processes. Studies have shown that there are two types of root systems initiated on *Populus* cuttings. The first of these root systems are basal roots initiated at the base of the cutting. Roots are also initiated along the length of the cutting. The relative importance of each of these root systems can vary by species and clone. Heilman et al. (1994) demonstrated that basal root development was most closely associated with first-year growth and development while roots developed along the length of the cutting contribute relatively little to first-year growth.

These measured growth advantages for cuttings of treatment 1 occurred under irrigated conditions and deployment of this type of planting stock may not be possible under all conditions. Potential water use of in-leaf, containerized cottonwood cuttings planted in May in the Southeast could be high and without irrigation or significant precipitation, this stock type could result in considerable mortality. The use of this stock type in the construction of cottonwood cutting orchards under irrigation is quite feasible. There are other scenarios that could utilize these containerized plants. Containerized plants could be produced using frozen, stored cottonwood whip-tips. MeadWestvaco used various production schedules to produce containerized cottonwood cuttings which were allowed to go dormant and then planted throughout the fall, winter, and early spring. Given reasonable soil moisture conditions, these plants can be successfully established without irrigation. The economic feasibility would need to be determined by each individual grower, but full utilization of whip-tip material would offer the potential for rapid bulk-up of new or

limited clones. For MWV, the cost of producing rooted containerized stock from whip-tip material was very similar to production of unrooted cuttings.

Another potential use of plants propagated via whip-tip cuttings may be in clonal comparison tests. When a range of *Populus* clones are being tested from a variety of sources, researchers are often faced with choosing between establishing “common garden” cutting orchards or using cuttings from a variety of different sources (Kaczmarek et al. 2013). Establishment of a common cutting orchard, can delay testing for several years. If tests are established with cuttings from a range of sources then variation in cutting sizes (Desrochers and Thomas 2003) or source (Zalesny et al. 2003) can result in increased variability in both survival and growth. Use of containerized cottonwood whip-tip material could eliminate time delays associated with new cutting orchard establishment.

One of the goals was to determine if plants propagated as containerized, rooted whip-tip cuttings were equivalent to plants derived from standard, unrooted cuttings. This was a potential area of concern since these plants were propagated using vegetation material that is usually discarded. The resulting stem form measures suggest that no alteration occurred compared to standard operational practices (Treatment 2). This suggests that whip-tip material is fully capable of producing similar, high-quality plants as those that normally originate from standard, unrooted cottonwood cuttings. The potential advantage of this method is far greater production capacity from a cutting orchard. Fege (1983) estimated that mature, *Populus* cutting orchards with spacing of 1 m² per stool could yield 4–10 stems per stool. Each stem could yield 2–3 standard, 51 cm cuttings. Actual cutting yields from newly planted cutting orchards could be much lower. First-year cutting orchards can yield less than one standard operational 51 cm long cottonwood cutting and in some cases these first-year orchards may yield no cuttings. When new clones are selected, these low bulk-up rates can limit large-scale deployment of new clones. Internal MWV research demonstrated that each individual stool of cottonwood clones ST66 and clone six had the potential to produce 5.9 branches to 8.3 branches each year. Each branch has the potential to produce 6–12 individual single or double-node cuttings. Terminal ends of each stool with diameters less than 16 mm would produce additional whip- tip cuttings. Based on these calculations, one individual cottonwood ramet could produce 8–30, standard 51 cm cuttings from ages two through eight. Utilization of whip-tip cuttings from the same stools could produce an additional 144–400 single or double-node cuttings. This dramatically increases productivity from cutting orchards (MWV Forest research, internal publication).

Projecting longer-term growth based on first-year growth data can be debated. Our experiences with these sites and cultural conditions suggest that cottonwood plants that are not affected by leaf or stem diseases during the first growing season or have not been stressed due to herbaceous competition will exhibit substantial growth in subsequent years. It may be more useful to try to understand if various treatments would affect stand uniformity and how this in turn could alter stand productivity. Increasing emphasis has been placed on quantifying and understanding the practical significance of stand uniformity on growth of individual trees and overall stand-level productivity. Binkley et al. (2002) and Binkley (2004) presented the hypotheses that stated that declines in stand-level growth were due to differences in the efficiency of resource use between dominant and non-dominant trees. As competition for resources increases, dominant trees gradually become more efficient in obtaining resources at the expense of nondominant trees. This leads to greater stand differentiation. Increased productivity of dominant trees is insufficient to offset decreased growth of nondominant trees. This leads to reductions in productivity. According to this hypothesis, more uniform stands should be more productive than highly heterogeneous stands.

Stand structure has been manipulated by changing the level of genetic control of the plants deployed or altering silvicultural treatments. Increasing productivity of more homogenous versus more heterogeneous *Eucalyptus* stands has been demonstrated by Ryan et al. (2008, 2010), Stape et al. (2010), and Luu et al. (2013). In loblolly pine, more intensive silvicultural treatments resulted in greater stand uniformity measures compared to less intensive cultural treatments (Nilsson and Allen 2003). Formal studies in mixed, clonal cottonwood plantings have examined productivity and stand dynamics (Knowe et al. 1994; Foster et al. 1998). These studies have shown that stand level productivity was not always a simple function of the percentage of each individual clone in the planting. Rather, complex interactions existed between clones with certain clones either over or underperforming in specific clonal combinations. These results suggest that specific morphological or physiological characteristics can affect growth of individual clones in either positive or negative ways. Additional research with *P. trichocarpa* and *P. trichocarpa* × *P. deltoides* clones examined stand-level productivity with different clonal combinations and plantation spacings (DeBell and Harrington 1997). This research also demonstrated complex stand dynamics that varied with changes in density and clonal composition. Devine et al. (2010) examined effects of clonal composition and density effects and in this research, differential patterns of survival were influenced by both these factors.

Studies with loblolly pine have examined the role of changing genetic control on stand uniformity and these results also suggest complex interactions between changing genetic sources, stand uniformity, and subsequent productivity. Aspinwall et al. (2011) examined stand level uniformity and growth of open-pollinated, full-sib families, and clonal loblolly pine test plantings. They found no clear and consistent trend for increasing uniformity with increasing genetic control but two open-pollinated and full-sib families had stand uniformity that exceeded the clonal stands tested. Adams and Roberts (2013) found greater productivity in pure family plantings of loblolly pine families that closely resembled crop ideotypes compared to mixed family plantings. These findings suggest that increased uniformity of trees in plantations can lead to overall greater stand-level productivity.

In this study, we utilized cottonwood clones which had similar levels of variation in growth within a given cutting treatment (Table 5). We also utilized a fairly uniform study site and effectively blocked the study to limit inherent site variation. This allowed us to isolate the variation arising from the five different cutting treatments. When these variation patterns are examined, similar levels of variation in growth of surviving cuttings is present (Table 5). This occurs even though cuttings in treatments 3 and 4 generally exhibited lower first-year growth than the remaining treatments. A second source of variation in these resulting stands can occur if differential survival patterns exist among the five cutting treatments. There is a trend for lower survival in treatment 4. These were the shortest cuttings. Survival reductions were most prevalent for clones 5 and 6 which had survival percentages that were 12–24 % points lower than the other cutting treatments (Tables 3 and 4). Lower survival rates would be expected to induce large growth differences among trees in the resulting stands. Surviving trees adjacent to planting locations with dead trees would be expected to have increased growth relative to trees which were surrounded by all surviving trees. Given the uniform sites with little micro-site variation, and similar levels of first-year growth variability and survival percentages, we would expect stands established using cutting treatments 1, 2, 3, or 5 to have similar levels of stand uniformity throughout the stand development process. Given reduced survival in treatment 4, higher stand variability in this treatment would be expected due to enhanced growth of surviving trees adjacent to planting locations where cuttings died.

Conclusions

Among the four clones, a gradient of growth potential exists and the advanced clones exhibited moderate first-year growth increases compared to standard operational clone ST66. Generally, clone $\times$ cutting treatment interactions were insignificant. The effects of changing cutting lengths are consistent with previous studies which indicated the potential for increased plant survival or growth with increased cutting lengths. Survival did not differ significantly for cutting treatments 2, 3 or 4, but survival for the shortest cuttings was reduced by approximately 7–8 % points compared to longer cuttings. The difference in stored carbohydrate reserves alone does not appear to completely control first-year growth development of unrooted cottonwood cuttings. First-year growth parameters of 51 cm long cuttings planted 30.5 cm deep (treatment 5) were consistently greater than growth parameters of 51 cm long cuttings planted 48 cm deep (treatment 2). In-leaf rooted, containerized cottonwood cuttings had first year growth advantages over standard 51 cm long unrooted cottonwood cuttings planted 48 cm deep. Across all clones, leaf area and total aboveground biomass of treatment 1 exceeded those of treatment 2. Stem form of plants propagated as containerized, rooted whip-tip cuttings suggest that no alteration occurred compared to standard operational practices. Whip-tip material that is normally discarded from cottonwood cutting orchards is fully capable of high-quality plants. The potential advantage of this method is far greater production capacity from a cutting orchard. Stand uniformity assessments suggest that surviving trees of each individual cutting treatment exhibit similar levels of growth variation, but there is the potential for survival differences among treatments to induce greater levels of stand variation as stand development continues.

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