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# Early root development of poplars (*Populus* spp.) in relation to moist and saturated soil conditions

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**ABSTRACT**

Poplars (*Populus* spp.) are among the fastest growing trees raised in temperate regions of the world. Testing of newly developed cultivars informs assessment of potential planting stock for local environments. Initial rooting by nine poplar clones was tested in moist and saturated soil conditions during an 18-day greenhouse experiment. Clones responded differently to soil moisture, particularly in number of roots, root distribution, and root dry mass accumulation. About 73% of cuttings planted in moist soil produced roots from callus tissue, whereas only 1% of cuttings planted in saturated soil developed such roots. This drove root distribution towards the basal section of cuttings in moist soil, while in saturated soil roots were more evenly distributed among all three below-ground sections of cuttings. Roots originating from the basal section of cuttings planted in moist soil were longer than roots originating from apical and middle sections. Conversely, roots from the apical and middle sections of cuttings planted in saturated soil were longer than those originating from the basal section. Initial rooting among poplar clones established under two soil moisture regimes has implications for genotype deployment in the field, but long-term effects in the field are still unknown.

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**KEYWORDS**
Flooding; genotype; greenhouse experiment; regeneration; unrooted cutting

## Introduction

Poplars (*Populus* spp.) are among the fastest growing tree species in the world and they are raised for various wood products, e.g. pulp and paper, high quality timber, and bioenergy (Stanturf & van Oosten 2014). The interest in cultivating poplars is large and has resulted in numerous breeding programs where hybrids are common, and these breeding programs have been successful in developing cultivars with broad suitability outside the natural range of poplar (Dickmann 2006; Stanton et al. 2014). Controlled testing of newly developed cultivars is a critical component of assessing suitability of potential planting stock for local environments.

The establishment phase is crucial for the development of a poplar stand and if this phase fails additional expenses and delays will jeopardize economic sustainability. In general, poplars need fertile soils that are well-drained and aerated with good water availability to support fast early growth (Stanturf & van Oosten 2014). In northern Europe, however, soils may hold high moisture and even experience periods of waterlogging after forest harvesting. This necessitates implementation of silvicultural practices to improve site conditions for successful regeneration (e.g. Czapowskyj & Safford 1993; Bilodeau-Gauthier et al. 2011; Löf et al. 2012; Stanturf & van Oosten 2014). While judiciously applied silvicultural practices can mitigate certain detrimental site conditions, plantation sustainability ultimately hinges on deployment of plant material well-adapted to various moisture conditions.

Stem cuttings are typically used for poplar planting stock. Quick initiation of shoot and root growth by the stem cutting is essential, since this ensures acquisition of water and nutrients for production of carbohydrates needed for survival and growth (Zalesny & Zalesny 2009). Poplar species generally root adventitiously from stem cuttings. For most species, root growth is initiated by preformed root primordia in the stem tissue (Haissig 1974b; Zalesny & Zalesny 2009). But, roots can also develop from callus tissue that forms at the base of the stem cutting after planting (Haissig 1974b; Zalesny & Zalesny 2009). Root initiation differs between species and hybrids, but also between clones (Dickmann et al. 2001; DesRochers & Thomas 2003; Zalesny & Zalesny 2009). Water and carbohydrate status of the stem cutting, as well as origin of the cutting on the parent shoot also play a vital role in root initiation (Haissig 1974a; Okoro & Grace 1976; Tschaplinski & Blake 1989; DesRochers & Thomas 2003; Zalesny et al. 2003). Additionally, environmental conditions, such as soil moisture, nutrient availability (Pregitzer & Friend 1996; Zalesny & Zalesny 2009), soil temperature (Zalesny et al. 2005), and bulk density of the soil (McIvor et al. 2014) can impact rooting of poplar stem cuttings.

Some poplars, particularly riparian species, are tolerant of waterlogged soil conditions, e.g. *P. deltoides* and *P. trichocarpa* (Allen & McComb 1956; Dickmann et al. 2001); while others, e.g. *P. simonii*, do not grow well under flooded conditions (Du et al. 2012; Peng et al. 2013). Flood-tolerance in poplars

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is genetically controlled, and hence genotypes within the same species or hybrid exhibit differing abilities to handle saturated soil conditions (Cao & Conner 1999). A lower root and leaf biomass in young flooded hybrid poplars have been observed in several studies (Liu & Dickmann 1992; Guo et al. 2011; Du et al. 2012; Peng et al. 2013).

Most of our knowledge regarding poplar flood-tolerance and response to saturated soil conditions has been developed from plants that had already initiated root and shoot growth (cf. Liu & Dickmann 1992, 1996; Cao & Conner 1999). Little is known about the rooting response of poplar stem cuttings during periods of soil saturation or flooding, though field conditions are often very wet in the spring when root formation is initiated. The aim of this study was to investigate initial root development by stem cuttings from different poplar clones when planted under saturated soil conditions. Species and hybrids used in the experiment were from the sections *Aigeiros* (cottonwoods and black poplar) and *Tacamahaca* (balsam poplars), with the majority of clones being D × N hybrid poplars (*P. deltoides* (Marshall) × *P. nigra* (Linnaeus)).

We established two hypotheses. (1) Poplar root initiation and growth will, in general, be limited by soil saturation, but clonal responses will illustrate variation among genotypes in the ability to root under conditions of soil saturation. (2) Soil saturation will constrain poplar root initiation and growth to near the soil surface. Under this moisture condition the relative distribution of roots will differ between clones.

## Materials and methods

### Plant material and experimental conditions

One-year-old shoot material was obtained from stools of nine poplar clones (Table 1) located at the University of Minnesota's experimental research nursery (The North Central Research and Outreach Center (UM NCROC)), Grand Rapids, MN, USA. The experimental set includes two commercially available clones, one open-pollinated clone from Wisconsin, and six clones being field tested in Europe (Table 1). No more than two clones were chosen from a full-sib family. Cutting material was collected in the winter of 2013/2014 and kept frozen until use in this experiment.

**Table 1.** List of poplar clones used in the experiment.

| Clone | Clone ID | Species or hybrid[a] | Comment | Diam. (mm) | DM (mg) |
|---|---|---|---|---|---|
| 1[b] | MN1 | D × N | | 11.3 ± 0.4 | 6505 ± 447 |
| 2[b] | MN2 | D × N | Half-sib with clones 3 and 4 | 10.1 ± 0.2 | 5534 ± 161 |
| 3[b] | MN3 | D × N | Half-sib with clones 2 and 4 | 9.6 ± 0.2 | 4660 ± 288 |
| 4[b] | MN4 | D × N | Half-sib with clones 2 and 3 | 9.8 ± 0.2 | 5774 ± 158 |
| 5[b] | MN5 | D × N | Full-sib with clone 6 | 10.2 ± 0.3 | 5261 ± 192 |
| 6[b] | MN6 | D × N | Full-sib with clone 5 | 11.3 ± 0.3 | 6256 ± 150 |
| 7[b] | D110 | D | Open-pollinated, Osceola, WI, USA | 9.0 ± 0.1 | 3725 ± 56 |
| 8 | DN5 | D × N | Commercial in USA, Canada | 11.3 ± 0.3 | 7468 ± 614 |
| 9 | NM6 (MAX-5) | N × M | Commercial in USA, Canada, Europe | 11.0 ± 0.5 | 7438 ± 432 |

Notes: Clone represents the identifier used in this study, Clone ID is the commercial identity or the identity assigned by the University of Minnesota's experimental research nursery (UM NCROC), Grand Rapids, MN, USA. Cutting size before planting is shown to the right as mean top diameter (Diam.) and mean dry mass (DM) ± standard error.

[a]D × N hybrids: *P. deltoides* (Marshall) × *P. nigra* (Linnaeus), D species: *P. deltoides*, N × M hybird: *P. nigra* × *P. maximowiczii* (Henry).

[b]Currently tested in Germany, Lithuania, Poland, Russia, Sweden and Ukraine.

The study was conducted in October 2014 in a greenhouse located at the USDA Forest Service's Center for Bottomland Hardwoods Research in Stoneville, Mississippi, USA. The greenhouse was equipped with high pressure sodium lamps that supplemented ambient radiation to maintain a 14-hr diurnal period. Photosynthetically active radiation (PAR) during the diurnal period averaged 377 µmol $m^{-2}$ $s^{-1}$, and the maximum PAR during the diurnal period averaged 874 µmol $m^{-2}$ $s^{-1}$. Mean air temperature during the experimental period was 21°C and ranged from an average low of 20°C during the nocturnal period to an average high of 22°C during the diurnal period.

### Experimental design

The experiment included two treatment levels that targeted natural soil moisture conditions of moist, well-aerated soil (referred to as moist), and completely saturated soil (referred to as saturated) (Figure 1(a)). These soil moisture levels were imposed by placing sand-filled containers in racks within 190-liter plastic tanks that held an assigned level of 0.5 strength modified Hoagland's solution (Hoagland Modified Basal Salt Mixture, PhytoTechnology Laboratories, Shawnee Mission, KS, USA). The concentration of nutrient solution used in this study demonstrated to provide suitable nutrient availability for poplar root and shoot growth in a pilot study. The six 190-liter plastic tanks were arranged in the greenhouse to create three replicates (blocks) of the two soil moisture treatment levels. Replicates were blocked based on location in the greenhouse (Figure 1(b)). Four cuttings for each of the nine clones (36 cuttings) were planted in sand-filled containers randomly assigned within each tank (experimental unit). Nutrient solution levels were monitored and maintained daily. In the nutrient solution, dissolved oxygen content averaged 7.25 mg $l^{-1}$, temperature averaged 23°C, and pH averaged 6.3 during the study period.

Cuttings, with a mean top diameter of 10.4 mm (range = 6.75–15.3 mm), were prepared for planting by trimming their length to 20 cm, with at least 1 cm removed from each end. Trimming was done such that the highest bud was located 1–4 cm from the top of the cutting, and all other buds were removed. All cuttings were soaked in water at room temperature for 16–24 h prior to potting in individual 0.95 l (6 cm top diameter and 34 cm tall) plastic seedling containers filled with washed sand. Precise potting to a depth of 15 cm ensured all plants had an equal amount of cutting tissue above (5 cm) and below (15 cm) the surface of the sand (Figure 1(a)). Following planting, the containers were immediately placed in the experimental units described above. Aluminum foil placed on the top of each seedling container prevented algae growth in the sand medium. In total,

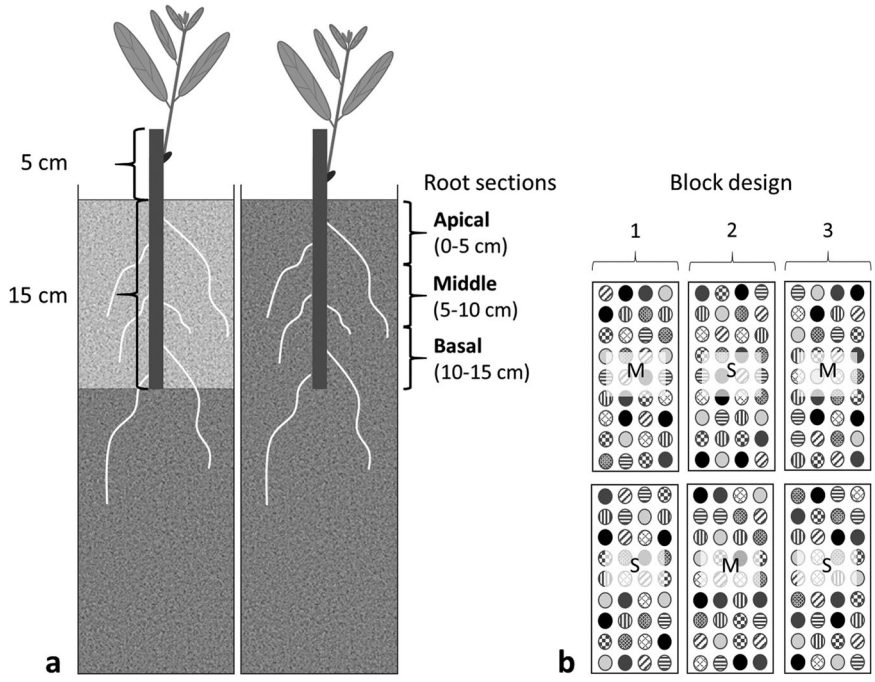


**Figure 1.** (a) Illustration of the two soil moisture treatment levels within sand-filled containers indicating the depth to saturation with nutrient solution (dark grey) relative to the "below-ground" portion of the cutting. The moist (M) treatment level is illustrated on the left, and the saturated (S) treatment level is illustrated on the right. The block design is illustrated in part b of the figure with randomly placed treatments (M and S) and 9 clones (circles with each pattern representing one clone) repeated four times randomly within each tank.

216 cuttings were planted in containers for use in the experiment.

The moist treatment was imposed by maintaining nutrient solution 15 cm below the surface of the sand medium at the bottom of the cutting (Figure 1(a)). Capillary movement of nutrient solution upward through the sand medium provided for well-aerated, moist sand along the 15 cm "below-ground" portion of cuttings. The saturated treatment was imposed by maintaining nutrient solution at the surface of the sand medium. This level of nutrient solution ensured complete inundation of the sand medium along the 15 cm, "below-ground" portion of cuttings. Sand in the moist treatment averaged 8% (gravimetric) moisture content from the surface down to 15 cm, while sand in the saturated treatment averaged 14% moisture content from the surface down to 15 cm.

### *Measurements*

Prior to planting in the containers, cutting diameter (mm) was measured at the top with two perpendicular measurements with a caliper. After planting, cuttings were observed daily to record time of bud break, i.e. when leaves emerged from the bud. All plants were harvested 18 days after planting. At this time, the shoots (new growth comprised of the stem plus leaves) were separated from the original cuttings, and leaves were separated from stems. We measured the length (cm) of stems after they were removed from cuttings. Cuttings and all roots were carefully washed in water to remove sand. The leaves and cuttings with roots were then placed in refrigerated storage for laboratory processing.

Root location, measured as distance (cm) from the top of the cutting, and individual root length (cm) were recorded for each plant. It was registered if the root origin was from a root primordia or callus tissue, and if the cutting had callus formation at the bottom. Leaf area ($cm^2$) of each plant was quantified with a Li-3100 Area Meter (Li-COR, Lincoln, Nebraska, USA). Roots, stems, leaves, and cuttings were then dried at 65°C until constant weights were achieved, and dry mass (DM) (g) was recorded.

### *Calculations and statistical analyses*

All analyses were conducted on treatment combination means that included plants scored to be vital. Vitality was determined to be a living plant that developed at least one root, and a single shoot that was free of defect. While 99% of all cuttings survived the experimental period, 94% of cuttings planted in the moist treatment level and 88% of cuttings planted in the saturated treatment level were scored vital. Root analyses were conducted for the total plant and by three sections of the cutting. The sections were: apical (0–5 cm below sand surface), middle (5–10 cm below sand surface), and basal (10–15 cm below sand surface). Mean root length per cutting was calculated as total root length divided by number of roots. A similar method was used to calculate mean root length for the apical, middle, and basal sections of cuttings. The relative number of roots per cutting section was calculated as the number of roots for a section divided by the total for the plant (in percentage). Presence of callus roots (roots originating from callus tissue) and growth of callus tissue at the bottom of the cutting was calculated as the count of cuttings with presence of callus roots or callus divided by total number of cuttings (as percentage).

Treatment effects were analyzed with a split-plot mixed model (clone within treatment (Equation 1), or section within treatment (Equation 2)) fitted with reduced

maximum likelihood (REML). An alpha level of 0.05 was used for all analyses and mean separations. The following models were used:

$$Y_{ijk} = \mu + Treat_i + Clone_j + b_k + (Treat \times Clone)_{ij} + e_{ijk} \quad (1)$$

$$Y_{lik} = \mu + Section_l + Treat_i + b_k + (Section \times Treat)_{li} + e_{lik} \quad (2)$$

where $Y_{ijk}$ and $Y_{lik}$ are the observed characteristics for treatment $i$, $\mu$ is the overall mean, $Treat_i$ is the fixed effect of the treatment, $Clone_j$ the fixed clonal effect, $Section_l$ is the fixed effect of cutting section. $b_k$ is the random block effect and $e_{ijk}$ and $e_{lik}$ are the random error terms, assumed to be normally distributed with mean 0 and with constant variance. When significant main effects or interacting effects were found, separation of treatment means was conducted according to Tukey's method.

## Results

### Aboveground growth

Ninety-seven percent of all cuttings exhibited bud break 3–4 days after planting, and this phenology was not driven by any treatment effect. At the conclusion of the 18-day study period, treatment effects were observed on several aboveground growth characteristics (Table 2). Many responses were determined by interacting effects of soil moisture and clone. Establishment in saturated soil decreased shoot DM, total leaf area, and leaf DM ($p = .0076$, $p = .0010$ and $p = .0061$, respectively) for clones 1, 4, and 9. Soil saturation did not influence stem length or stem DM, but slightly reduced the number of leaves produced by plants ($p = .0209$). The effect of clone was significant for all aboveground response variables (all variables showed $p \leq .02$), but clone ranking was not consistent among variables (Table 2).

### Root growth

#### General root growth

The number of roots we observed on poplar cuttings varied according to the interacting effects of soil moisture and clone (Tables 3 and 4). Rooting by clones 1 and 3 was most severely inhibited by saturated soil as they had 6–10 roots fewer than in moist soil. Root DM also showed interactions between treatment and clone (Table 3). As compared to moist soil, saturated soil decreased root DM 30% and 50%, respectively, for clones 1 and 9. Total root length and mean root length were not influenced by soil moisture or the interacting effects of soil moisture and clone (Table 3). Clone was a significant but weak source of variation for total root length and mean root length (Table 3). Clonal differences in total root length could not be distinguished with the post hoc test we employed (Table 4).

For several clones, the number of roots originating from primordia increased when cuttings were planted in saturated soil ($p = .0075$) (Table 4). Total length of primordia roots increased for clone 7 when planted in saturated soil ($p = .0090$); most of the other clones showed a similar trend which contributed to the significant main effect for this variable (Table 4).

Ninety percent of all cuttings showed callus formation when planted in moist soil, while only 11% of all cuttings showed callus formation in saturated soil (Table 4). In moist soil, all clones developed callus-origin roots (73% of all cuttings produced an average of six roots from callus tissue) (Table 4). Clones 2 and 9 only developed callus roots on 17% and 25% of their cuttings, respectively. Additionally, the number of callus roots produced relative to the number of primordia roots produced appeared to vary among clones in moist soil. For example, callus roots comprised 5% of all roots for clone 9, and about 56% of all roots for clone 7. Callus root length accounted for about 2% of total root length for clone 9, and about 34% of total root length for clone 4 in moist soil. In contrast to our observations from moist soil, only 1% of all cuttings produced callus-origin roots when grown in saturated soil, and these roots were only observed on clone 6.

#### Distribution of roots

Soil moisture level and clone clearly influenced root distribution among apical, middle and basal sections of poplar cuttings (Figure 2). The relative number of roots (%) originating in each of the three sections of the cutting differed by soil moisture level ($p < .0001$, data not shown). In moist soil, 11%

**Table 2.** Shoot characteristics of 9 poplar clones grown for 18 days under moist (M) and saturated (S) soil conditions.

| | Shoot DM (mg) | | | | | Leaf area ($cm^2$) | | Leaf DM (mg) | |
|---|---|---|---|---|---|---|---|---|---|
| Source | M | S | Stem length (cm) | Stem DM (mg) | No. of leaves | M | S | M | S |
| **Clone** | | | | | | | | | |
| 1 | $867^{ab}$ | $676^{ab*}$ | $20.5^{a}$ | $238^{a}$ | $15.7^{a}$ | $173^{ab}$ | $145^{ab*}$ | $606^{b}$ | $461^{abc*}$ |
| 2 | $559^{cde}$ | $472^{cd}$ | $13.1^{c}$ | $137^{cd}$ | $11.5^{cd}$ | $119^{cd}$ | $119^{ab}$ | $411^{de}$ | $347^{cd}$ |
| 3 | $648^{cde}$ | $547^{bcd}$ | $18.2^{ab}$ | $189^{bc}$ | $12.2^{bd}$ | $131^{cd}$ | $119^{ab}$ | $450^{cde}$ | $366^{bcd}$ |
| 4 | $706^{bcd}$ | $557^{bcd*}$ | $16.2^{ab}$ | $182^{bc}$ | $12.8^{bc}$ | $140^{bc}$ | $112^{bc*}$ | $507^{bcd}$ | $393^{bcd*}$ |
| 5 | $739^{bcd}$ | $689^{ab}$ | $18.3^{ab}$ | $212^{ab}$ | $13.1^{bc}$ | $144^{bc}$ | $145^{ab}$ | $518^{bcd}$ | $486^{ab}$ |
| 6 | $738^{bcd}$ | $621^{abc}$ | $16.0^{bc}$ | $209^{ab}$ | $12.4^{bd}$ | $151^{bc}$ | $136^{ab}$ | $514^{bcd}$ | $428^{abc}$ |
| 7 | $470^{e}$ | $377^{d}$ | $15.0^{bc}$ | $113^{d}$ | $11.5^{cd}$ | $95^{d}$ | $80^{c}$ | $353^{e}$ | $268^{d}$ |
| 8 | $775^{bc}$ | $759^{a}$ | $17.8^{ab}$ | $223^{ab}$ | $13.5^{b}$ | $145^{bc}$ | $151^{a}$ | $549^{bc}$ | $540^{a}$ |
| 9 | $1042^{a}$ | $668^{ab*}$ | $19.7^{a}$ | $212^{ab}$ | $10.6^{d}$ | $200^{a}$ | $136^{ab*}$ | $799^{a}$ | $488^{ab*}$ |
| **Treatment** | | | | | | | | | |
| M | 727* | | 18.0 ns | 204 ns | $12.8^{a}$ | 144* | | 523* | |
| S | 596* | | 16.4 ns | 177 ns | $12.3^{b}$ | 127* | | 419* | |

Notes: Shoot dry mass (shoot DM) is the stem plus leaves ($n = 3$). Treatment effects and means are shown at the bottom (ns = no significant treatment effect). Letters within column represent differences between clones or treatments (bottom) according to Tukey's test, asterisks (*) represent treatment effects within clone (row) according to interacting effects of the mixed model (Equation 1).

**Table 3.** Analysis of variance statistics for root variables measured on 9 poplar clones grown for 18 days under moist or saturated soil treatment levels.

| Source of variation | df[a] | *p*-value[b] |
|---|---|---|
| **Number of roots** | | |
| Treatment | 1 | **0.0391** |
| Clone | 8 | **<0.0001** |
| Treatment × Clone | 8 | **0.0078** |
| **Total root length** | | |
| Treatment | 1 | 0.2505 |
| Clone | 8 | **0.0499** |
| Treatment × Clone | 8 | 0.0889 |
| **Mean root length** | | |
| Treatment | 1 | 0.2586 |
| Clone | 8 | **0.0306** |
| Treatment × Clone | 8 | 0.0826 |
| **Root DM** | | |
| Treatment | 1 | 0.1772 |
| Clone | 8 | **<0.0001** |
| Treatment × Clone | 8 | **0.0116** |

[a]df = degrees of freedom.
[b]Significant *p*-values are bold at an *α*-level of .05.

originated from the apical section, 28% originated from the middle section, and 61% originated from the basal section of cuttings. In saturated soil, roots were more evenly distributed with apical, middle, and basal sections accounting for 27%, 40%, and 33% of all roots, respectively. The number of roots increased with depth in moist soil—this response was not as strong in saturated soil as the middle section of cuttings developed a similar number of roots to apical and basal sections ($p < .0001$) (Figure 2(a)). Total root length increased with depth of section in moist soil, but in saturated soil total root length was greatest in the apical and middle section ($p < .0001$) (Figure 2(b)). Mean root length decreased with section depth in saturated soil, but showed no difference among sections in moist soil ($p < .0001$) (Figure 2(c)).

Clones also exhibited differing root responses relative to soil moisture and section of the cutting. For most clones, more roots were produced in the apical section of cuttings established in saturated soil than in moist soil ($p = .0231$) (Figure 2(a)). The same can be said for mean root length. With the exception of clone 9, mean root length in the apical section of cuttings was longer when clones were established in saturated soil ($p = .0361$) (Figure 2(c)). For the middle section of cuttings, clone 7 was unique in that total root length in saturated soil was substantially greater than in moist soil (12 cm in moist soil versus 61 cm in saturated soil) ($p = .0026$) (Figure 2(b)). In the basal section of cuttings, mean root length of clone 9 decrease from 8 cm in moist soil to 3 cm in saturated soil ($p = .0310$) (Figure 2(c)).

## Discussion

At the whole plant level, rooting characteristics among genotypes (clones) was generally affected by soil moisture (Hypothesis 1). The importance of root system genotype to plant tolerance of soil saturation has been demonstrated by Peng et al. (2013); and several others have also documented how genotype influences rooting under various soil conditions (e.g. Liu & Dickmann 1992; Cao & Conner 1999; Zalesny & Zalesny 2009; Guo et al. 2011; McIvor et al. 2014). Further, flood-tolerance of trees has been linked to the

**Table 4.** Root characteristics of 9 poplar clones grown for 18 days under moist (M) or saturated (S) soil conditions.

| Source | All roots | | | | | | Roots from primordia | | | | Roots from callus | | | | | | Callus tissue (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of roots | | TRL (cm) | | Root DM (mg) | | No. of roots | | TRL (cm) | | No. of roots | | TRL (cm) | | Presence (%) | | | |
| | M | S | M | S | M | S | M | S | M | S | M | S | M | S | M | S | M | S |
| **Clone** | | | | | | | | | | | | | | | | | | |
| 1 | 30.3[a] | 20.7[ab]* | 81.9 | 116.1 | 114[a] | 80[ab]* | 17.8[a] | 20.7[ab] | 65.5[ab] | 116.1[ab] | 13.2 | 0 | 17.4 | 0 | 91 | 0 | 90 | 0 |
| 2 | 15.3[c] | 17.2[ab] | 54.7 | 99.2 | 61[b] | 56[ab] | 15.1[abc] | 17.2[bc] | 53.7[b] | 99.2[ab] | 1.5 | 0 | 6.3 | 0 | 17 | 0 | 67 | 0 |
| 3 | 24.1[ab] | 17.7[ab]* | 81.9 | 111.1 | 83[b] | 63[ab] | 17.5[ab] | 17.7[abc] | 69.4[ab] | 111.1[ab] | 6.9 | 0 | 13.7 | 0 | 91 | 0 | 100 | 0 |
| 4 | 17.6[bc] | 12.8[b] | 92.6 | 84.2 | 72[b] | 59[ab] | 11.0[bc] | 12.8[c] | 69.6[ab] | 84.2[b] | 8.8 | 0 | 31.1 | 0 | 78 | 0 | 100 | 10 |
| 5 | 13.5[c] | 13.7[b] | 66.8 | 97.9 | 61[b] | 61[ab] | 8.9[c] | 13.7[c]* | 55.6[b] | 97.9[ab] | 5.0 | 0 | 12.5 | 0 | 92 | 0 | 100 | 50 |
| 6 | 17.1[bc] | 17.5[ab] | 108.0 | 124.9 | 59[b] | 53[b] | 12.0[abc] | 17.1[bc]* | 87.9[ab] | 124.5[ab] | 5.5 | 3.0 | 21.1 | 3.3 | 92 | 10 | 100 | 20 |
| 7 | 17.0[bc] | 17.8[ab] | 83.0 | 152.3 | 59[b] | 54[ab] | 8.8[c] | 17.8[abc]* | 49.5[b] | 152.3[a]* | 9.6 | 0 | 39.5 | 0 | 92 | 0 | 92 | 0 |
| 8 | 20.1[bc] | 24.1[a] | 102.1 | 136.6 | 80[b] | 80[a] | 15.3[abc] | 24.1[a]* | 92.4[ab] | 136.6[ab] | 5.7 | 0 | 11.9 | 0 | 82 | 0 | 73 | 10 |
| 9 | 18.2[bc] | 16.0[ab] | 121.5 | 90.9 | 81[b] | 42[b]* | 17.9[a] | 16.0[bc] | 120.8[a] | 90.9[ab] | 1.0 | 0 | 2.4 | 0 | 25 | 0 | 89 | 0 |
| **Treatment** | | | | | | | | | | | | | | | | | | |
| M | 19.2 | * | 88.0 | ns | 74 | * | 13.8 | * | 73.8 | * | 6.4 | | 17.3 | | 73 | | 90 | |
| S | 17.5 | * | 112.6 | ns | 61 | * | 17.4 | * | 112.5 | * | 3.0 | | 3.3 | | 1 | | 11 | |

Notes: The three first columns represent mean values of all roots, which are divided into roots originating from primordia and callus further to the right. Treatment effects and means are shown at the bottom (ns = no significant treatment effect). Letters within column represent differences between clones or treatments (bottom) according to Tukey's test, asterisks (*) represent treatment effects within clone (row) according to interacting effects of the mixed model (Equation 1).
No. = number, TRL = total root length ($n = 3$), DM = dry mass ($n = 3$), Presence = percentage of the cuttings with callus roots ($n = 12$), and Callus tissue = percentage of the cuttings with callus tissue formation at the bottom of the cutting ($n = 12$).

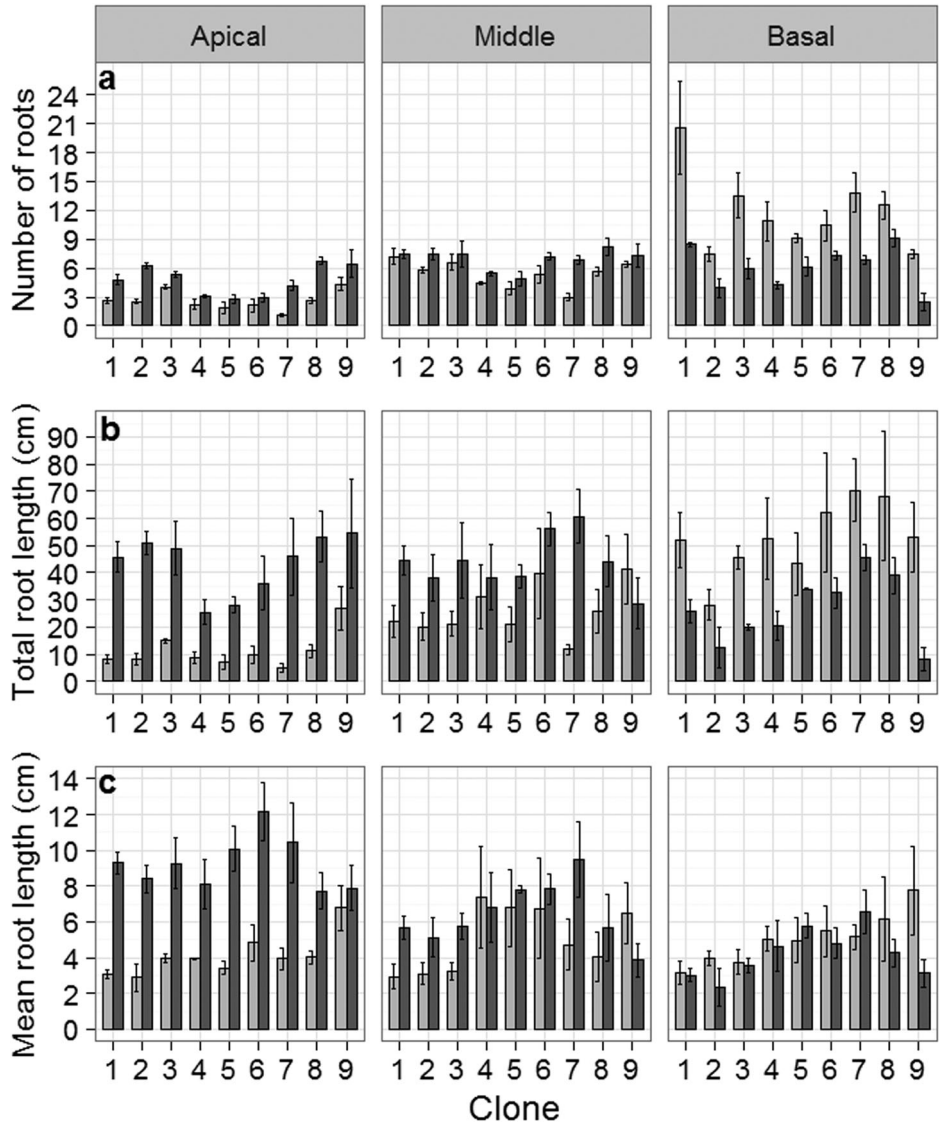


**Figure 2.** Root characteristics of nine poplar clones by three sections of the cutting in two soil moisture treatments, (a) Number of roots, (b) total root length (cm), and (c) mean root length (cm). The three sections below soil surface of the cutting were; apical (0–5 cm), middle (5–10 cm), and basal (10–15 cm). Moist treatment = light grey, saturated treatment = dark grey, and bars represent ± standard error.

production of adventitious roots (e.g. Kozlowski 1986), which are the type of roots initiated by cuttings. Our findings indicate that some genotypes, namely clone 1 and 9 (NM6), showed restricted development of root biomass when established in saturated soil. This is in contrast to other genotypes, such as clone 8 (DN5), which were able to maintain a relatively large root system, in terms of root number and DM, when grown in saturated soil. These clonal differences in root initiation and short-term root system development could translate to significant field performance implications in poplar plantings.

The commercially available clone 9 (NM6) appeared to exhibit rooting behavior that contrasted with many of the other tested genotypes. In moist soil, this clone developed a root system of comparable size to most other clones, but saturated soil restricted biomass accumulation relative to that observed in moist soil. This clone is of different origin compared to the other clones due to its *P. maximowiczii* parent from Asia. It has a high rooting success from cuttings under the conditions it has been selected – an important issue for commercialization. However, its ability to accumulate a substantial root biomass appears limited under saturated soil conditions as demonstrated in this study.

The importance of root origin to field performance remains a topic of debate among workers studying early poplar establishment. Bloomberg (1963) suggested that roots originating from primordia are of greater importance to early survival and growth than are roots of callus-origin. In their study, callus roots were produced up to two months later than primordia roots. Heilman et al. (1994a) noted that primordia roots may also develop a larger share of fine roots because of their more horizontal orientation. In our study, the relative importance of callus roots appeared to differ among clones, as we observed a wide range in the contribution of callus roots to total root number and total root length. All clones studied in this experiment produced roots from primordia, and soil saturation even appeared to increase root initiation from primordia for several clones. We suggest that this is

likely a matter of resource allocation important for early survival. Cuttings in moist soil put more energy into producing callus roots and leaves, while cuttings in saturated soil put more energy in producing primordia roots closer to soil surface to avoid oxygen deficit. This is in line with what Smit and Stachowiak (1988) suggested; that internal aeration (i.e. through aerenchyma tissue) and development of adventitious roots is a strategy poplar species may use to maintain water uptake and survival during hypoxic soil conditions.

Though roots originating from primordia may initiate early growth of poplar cuttings, Heilman et al. (1994b) found that callus roots dominated root growth after the first growing season. In this respect, the absence of callus root initiation we observed in saturated soil identifies a significant limitation to root system development by poplar cuttings planted in waterlogged soils. A potential detriment could arise later in the growing season as waterlogged soils begin to dry. Inhibition of callus root development has also been observed on cuttings planted in dry soil (Bloomberg 1963), and this has also been suggested as a hindrance to survival and growth under these conditions (Heilman et al. 1994b). Therefore, callus root development may be important for responding to transitioning soil moisture conditions. Additionally, deep growing roots, from either origin, are vital for maintaining plant water relations, and could affect tree resistance to windthrow (Kozlowski 1986; Zalesny & Zalesny 2009).

Formation of callus tissue at the bottom of the cutting is a reaction to wounding and has also been associated with disease resistance (Bier 1961). Though it is common for roots to emerge from this tissue, presence of callus tissue is no guarantee that callus roots will emerge (Haissig 1974b). In our study, formation of callus tissue at the base of the cutting was more common than callus root formation. Environmental conditions favorable to callus formation are reportedly similar to conditions favorable to adventitious root production (Bloomberg 1964). Bloomberg (1964) reported differences between clones in the ability to produce callus tissue and suggested that poplar clones exhibiting limited callus production may be less reliable producers of callus roots in field.

In addition to root origin, we observed clear differences in poplar root distribution in moist soil versus saturated soil (Hypothesis 2). Roots were distributed more towards the basal section of cuttings planted in moist soil, while they tended to be more evenly distributed among sections in saturated soil. Treatment differences observed for root initiation within the basal section of cuttings strongly related to the presence or absence of callus roots. Development of a shallow root system is a common rooting strategy of other plants growing in flooded soils (e.g. Kozlowski 1986; Justin & Armstrong 1987; Pezeshki et al. 1998). For example, Pezeshki et al. (1998), who studied *Salix nigra* (Marshall) cuttings that were 1.8 m in length, observed an inhibition of root formation and growth that was associated with depth in saturated soil.

It has also been shown that poplar root morphology is characteristically plastic to a range of environmental conditions (Liu & Dickmann 1992; Neuman et al. 1996; McIvor et al. 2014). Curiously, the total length of roots originating from primordia was shorter when cuttings were planted in moist soil as compared to saturated soil. This may be because soil moisture, aeration, and nutrients were optimal in the rooting zone of the moist soil treatment level. Liu and Dickmann (1992) observed similar results when poplar was grown under well-watered soil conditions with supplemental nitrogen. Pregitzer et al. (1993) reported an increase of new roots within nitrogen enriched soil patches, but also reported a higher density of lateral roots and root hairs. In our experiment, roots that developed from primordia, especially for some clones, appeared to branch more in moist soil than in saturated soil. In saturated soil, roots appeared less branched and more elongated – perhaps this is a morphology indicative of low soil oxygen (Kozlowski 1986). However, we note that our results were obtained for a relatively short growing period, and Heilman et al. (1994b) reported that up to 50% of first-order roots died during the first growing season thereby reducing their reliability as an indicator of root structure in later years. Hence, it is not known if the initial rooting we observed will correspond to lasting effects relative to soil moisture conditions.

Root growth by poplar is highly affected by soil temperature (Hansen 1986; Landhäusser 2003; Zalesny et al. 2005). The temperature in the rooting zone for this study (average = 23°C) was close to the 25°C that Landhäusser (2003) found to promote relatively high root growth from cuttings. But, these are temperatures that are substantially greater than soil temperatures representative of field sites for which these clones were developed. Therefore, care should be taken before extending our results to field locations at higher latitudes. Nevertheless, controlled experiments such as ours are necessary for the study of root initiation, early root development, and clonal differences in suitability to certain soil conditions such as waterlogging (e.g. Branislav et al. 2009; Krabel et al. 2015). Clones that rooted well in the saturated soil of our study should be prioritized for field testing on wet sites. Additionally, clones showing better performance in saturated soil may possess the ability to establish quicker under these soil conditions.

In conclusion, all clones examined in this study rooted when established in water-saturated soil, but the length of roots originating from primordia and root mass differed more than 50% between the highest and lowest ranking clones (Hypothesis 1). This finding emphasizes the importance of selecting genotypes well suited to site conditions. In moist soil, roots were disproportionately distributed towards the basal section of cuttings, and this is partially attributed to the initiation of callus roots at the base of cuttings. Saturated soil conditions limited callus root formation, and roots were more evenly distributed among apical, middle and basal sections of cuttings (Hypothesis 2). The long-term effect of this response (limited callus root formation) is uncertain, but maladaptation to changes in soil moisture and poor wind-firmness are potential concerns. Mechanical site preparation resulting in elevated planting positions, e.g. mounding, is currently recommended when planting poplars on soils showing a high water table; but, it is not known how this practice impacts root system, particularly callus root, development. We recognize the need for additional investigation into the long-term effects of early

root initiation and development by poplar cuttings planted in saturated soil, particularly in operational plantings.

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