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Influence of Cutting Type and Fertilization in Production of Containerized Poplar Plants

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Abstract: Most poplar plantations are planted on marginal agricultural land, but poplar plantations also hold the potential for increased profits compared to plantations of other species on non-agricultural, previously forested land. To date, the establishment of poplar plantations on previously forested land is limited by the production of suitable containerized poplar stock for planting. The objective of this study is to investigate how different cutting quality and fertilizer treatments influence height, diameter, and root biomass growth and root-to-shoot ratio, all important variables for plant establishment. Our results show that fertilization increases plant growth and that single-bud and two-bud cuttings with cutting diameters of 5 to 10 mm can be used in the production of containerized plants. Root biomass was similar between these plant types but the number of roots per plant was higher if two-bud cuttings were used. In contrast to fertilized plants, only one cutting type (two-bud 10 mm) grew to a sufficient height and diameter for use in poplar plantation establishment. Interestingly, the root-to-shoot ratio for this cutting type was 0.16 while the ratio for the same cutting type is 0.11 if fertilized. Together, these results suggest that most types of poplar cuttings can be used to establish poplar plantations if fertilizer is used and that the largest cutting type (two-bud 10 mm) might be more suitable to establish poplar plantations at harsh sites, thus reducing the cost of poplar plant production.

Keywords: *Populus trichocarpa*; cutting type; production of containerized plants; plant development; fertilization

1. Introduction

Poplar (*Populus* spp.) plantations are an important source of wood, pulp and biomass in much of the temperate northern hemisphere. Poplar plantations are established mainly on marginal agricultural land [1–8]. Poplar is less commonly planted on forest land [9–11]; such lands hold much potential for the expansion of poplar production. However, poplar plantings on forest land are not always successful. Poplar plantations may fail due to poor vegetation control or soil management as young poplars are sensitive to competing vegetation [7,10,12]. Poplars are fast growing, with a rotation period of 10 to 25 years depending on planting density and location [11,13]. To reduce the cost and enhance the profit from such plantations, the establishment phase of poplar plants needs to be better understood.

Plant establishment depends on two factors: seedling quality at the time of planting and environmental conditions [14]. While environmental conditions are hard to govern, plant quality can be checked before planting. Plant quality factors that promote high initial growth and low mortality include high root-to-shoot ratio, large root volume, root size root biomass, height and stem diameter [15,16]. Newly-planted seedlings often have limited root system permeability or root–soil contact and restricted root placement that limit water uptake from the soil [17–19]. Quick post-planting root growth enables plants to reach soil water, avoid drought stress, grow taller and shorten the period when they are sensitive to stress factors such as competing vegetation, drought, and browsing [20].

Establishing poplar plantations on previously forested land is more challenging than on previous agricultural land due to lower nutrients and water availability on forested lands which typically lack a long history of cultivation and fertilization. The choice between agricultural or forest land also dictates soil preparation and vegetation control techniques and choice of seedling type. It was recently demonstrated that poplar containerized plants outperformed bare-rooted plants and cuttings during the first two years of growth on forest land [21–25]. Poplars can grow adventitious roots from their stems, making cuttings easy to transplant. This works well on agricultural land [26,27] but has not been so successful on forest land [25]. Instead, containerized plants have shown the fastest growth on forest land.

Optimal plant nursery practices are important to the success of poplar cuttings in the production of containerized plants, where cutting length and diameter are key factors in determining planting success [28–30]. If different lengths and diameters of cuttings (especially thin and short cuttings) can be successfully established, this will maximize the number of cuttings from the stool bed and reduce per-plant costs. Short cuttings may have an additional advantage: the soil within their entire container has roots, reducing soil–root connection loss during planting. In the nursery, watering and fertilization practices can be tailored to optimize plant growth. Poplar clones also differ in rooting capacity and growth rates. Therefore, growth responses of different poplar clones, cutting types and fertilization regimes must be analyzed jointly. In this study, we report growth differences in a full factorial experiment on three poplar clones, two cutting diameters, and two cutting lengths, both with and without fertilization. Our results provide crucial information on the improvements of containerized poplar production including (1) the importance of fertilization; and (2) differences in relative growth between cutting types and allocation with fertilization.

2. Materials and Methods

2.1. Plant Material, Cutting Preparation, Soil Substrates, Growth Containers and Planting

Three poplar clones were used in this study: OP42 (*Populus trichocarpa* Torrey & Gray × *P. maximowiczii* Henry), Rochester (*P. nigra* Linnaeus × *P. maximowiczii*) and clone 15 (*P. trichocarpa*), all of which are used in commercial poplar plantations. The cuttings were harvested in mid-January from one-year-old sprouts from stool beds. After harvesting, the cuttings were stored at +4 °C until planting in early February. Cuttings with two target diameters (Ø 5 mm and Ø 10 mm) and two lengths (one and two buds) were used for a total of four different diameter–length combinations (Figure 1). Actual diameters and lengths of the different cutting types of each clone are shown in Table 1. No significant differences were found among clones for the one- and two-bud cuttings. All cuttings were planted in homogenized soil up to the first bud (Figure 1) in 475 mL cylindrical plastic pots (diameter 67 mm and height 202 mm). The soil consisted of Sphagnum moss (block peat 45%, harrowed peat 25%), peat humus (15%) and perlite (15%). It was also supplemented with limestone (2 kg/m^3), dolomite (2 kg/m^3), a solid nitrogen-phosphorus-potassium (N-P-K) fertilizer (14-7-15, 1 kg/m^3) and micronutrients (FTE 36, 0.05 kg/m^3). The soil pH was 5.7.

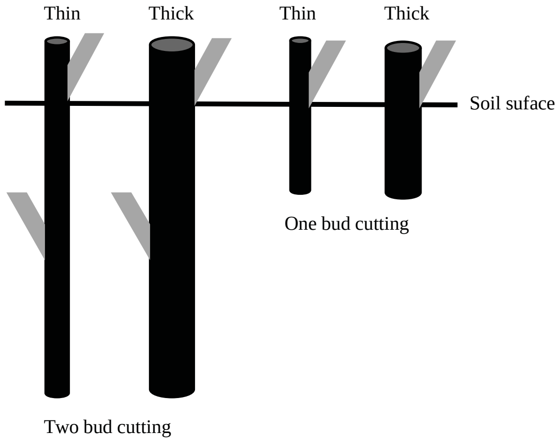

Figure 1. A schematic view of the cutting types and their planting positions.

Table 1. Lengths and diameters of different cutting types and clones at the time of planting.

Poplar Clone	Cutting Type	Diamenter			Length		
		Mean		**SE**	**mean**		**SE**
Rochester	Two-bud Ø 5 mm	4.1	±	0.8	44	±	5
	Two-bud Ø 10 mm	9.8	±	1.3	53	±	6
	One-bud Ø 5 mm	4.2	±	0.7	96	±	15
	One-bud Ø 10 mm	9.4	±	1.4	106	±	11
Clone 15	Two-bud Ø 5 mm	3.9	±	0.7	47	±	5
	Two-bud Ø 10 mm	9.2	±	0.8	55	±	6
	One-bud Ø 5 mm	4.3	±	0.8	97	±	18
	One-bud Ø 10 mm	10.2	±	1.8	105	±	11
OP42	Two-bud Ø 5 mm	4.2	±	0.6	49	±	6
	Two-bud Ø 10 mm	10.5	±	1.6	59	±	5
	One-bud Ø 5 mm	4.3	±	0.7	93	±	13
	One-bud Ø 10 mm	10.6	±	1.5	117	±	12

Note: The Table includes mean length and diameter for three poplar clones: Rochester (*P. nigra* × *P. maximowiczii)*, Clone 15 (*P. trichocarpa*), and OP42 (*P. trichocarpa* × *P. maximowiczii)*. Length and diameter are shown in mm (n = 30). SE: standard error.

2.2. Experimental Design

A total of 15 experimental blocks were established. Each block contained one potted cutting of each combination of three clones (Rochester, Clone 15 and OP42), four cutting types (one-bud Ø 5 mm, Ø 10 mm, two-bud Ø 5 mm and Ø 10 mm) and two fertilization treatments (fertilized and un-fertilized). Within each block, the 24 pots were randomly relocated each week.

2.3. Growth Conditions and Fertilizers

The experiment was performed in a greenhouse at 20 °C with 16 h of additional light supplied from fluorescent lamps with a photon flux density of 130 µmol m^{-2} s^{-1}. Fertilizer was applied every week beginning four weeks after planting. The fertilizer used (liquid fertilizer NPK solution, from Plantagen nursery, Malmö, Sweden) was selected based on its commercial availability. This fertilizer contains 10% nitrogen, 3.9% phosphorus, 5.8% potassium, 0.01% boron, 0.009% copper, 0.034% iron, 0.016 manganese, 0.01% molybdenum and 0.018 zinc.

2.4. Measurements

Survival, height, diameter, number of roots and biomass of roots, stems and leaves were recorded ten weeks after planting. Stem and leaf biomasses were separated from roots that were washed and together with stem and leaves dried at 70 °C for two days before weighing.

2.5. Statistical Analysis

To test the effects of fertilization treatments and cutting types and interactions on the outcome variables of height, diameter, biomasses of root, stem and leaf, root-to-shoot ratio and number of roots per plant, we used a mixed model (lme package) procedure implemented in R version 3.1.1 [31]. To evaluate differences among treatments, we used Tukey´s HSD (honest significant difference) as a post-hoc test, implemented in the 'glht' R package. A *p*-value of 0.05 was used as the cutoff for statistical significance. Response variables (height, diameter, leaf, stem and root biomasses and root-to-shoot ratio and roots per plant, clone and cutting type) were fixed effects and block was included as a random effect in the model. For survival, a Chi-squared (χ^2) test was used. For all variables, residuals were inspected and showed normal distributions with no signs of outliers.

3. Results

3.1. Survival of Transplanted Cuttings

Across all clones, cutting types and fertilizer treatments, 79% of transplanted cuttings resulted in surviving plants. The only significant survival difference among clone–cutting combinations was for fertilized plants. For one-bud Ø 5 mm cuttings, clone Rochester cuttings had a lower survival (40%) than clone 15 (93%) (χ^2 = 4.8, p = *0.03*). Similar results were found for two-bud Ø 5 mm cuttings with survival of 40% for Rochester cuttings and 100% for clone 15 (χ^2 = 4.6, p = *0.03*). For the other clones and cutting types, survival varied between 52% and 93% with $p > 0.05$ for all χ^2 tests.

3.2. Growth of Fertilized and Un-Fertilized Plants, Interactions between Clone and Cutting Phenotype

Fertilization increased height, diameter, leaf, and stem growth, as well as root biomass across clone and cutting types ($p \leq 0.001$). However, the number of roots and root-to-shoot ratios responded differently to fertilization (Section 3.5). In general, few statistically significant interactions were found between clone and other treatment characteristics (cutting length, diameter) (Table 2). A total of 24 out of 28 interaction terms tested were non-significant (Table 2). As most interaction terms were non-significant, we analyze all three clones together in the following sections to give a more easily interpreted overview of cutting type and fertilization impacts on plant growth.

Table 2. *P*-values for the interaction between clone and cutting attributes (length and diameter) as shown by plant growth and morphology for fertilized and un-fertilized plants.

Interactions	Fertilization	Height	Diameter	Biomasses			Root to Shot Ratio	Number of Roots
				Stem	Leaf	Root		
Clone: cutting length	Yes	0.5	0.94	**0.02**	0.06	0.21	0.8	0.83
Clone: cutting diameter	Yes	0.82	0.19	0.18	0.09	0.26	0.06	0.21
Clone: cutting length	No	0.07	0.34	**0.003**	**0.002**	0.51	0.81	0.6
Clone: cutting diameter	No	0.35	**0.0001**	0.21	0.36	0.09	0.64	0.11

Note: Table 2 shows *p*-values from a type 3 ANOVA after a linear mixed model. The response variables—height, diameter, leaf, stem and root biomasses, roots per plant, clone and cutting type—were fixed effects and experimental block was included as a random effect in the model. Bold types highlight significant (<0.05) *p*-values. Fertilized and un-fertilized plants are analyzed separately.

3.3. Height and Diameter Growth

After ten weeks, unfertilized plant heights ranged from 54 to 275 mm, with a mean of 136 mm. Mean diameter growth was 2.3 mm, with extremes from 1.2 to 3.6 mm (Figure 2A,B). Thick cuttings

(Ø 10 mm) resulted in longer and thicker plants than thin cuttings (Ø 5 mm) regardless of cutting length. However, thin cuttings' (Ø 5mm) responses depended on length; longer cuttings resulted in both taller and thicker plants.

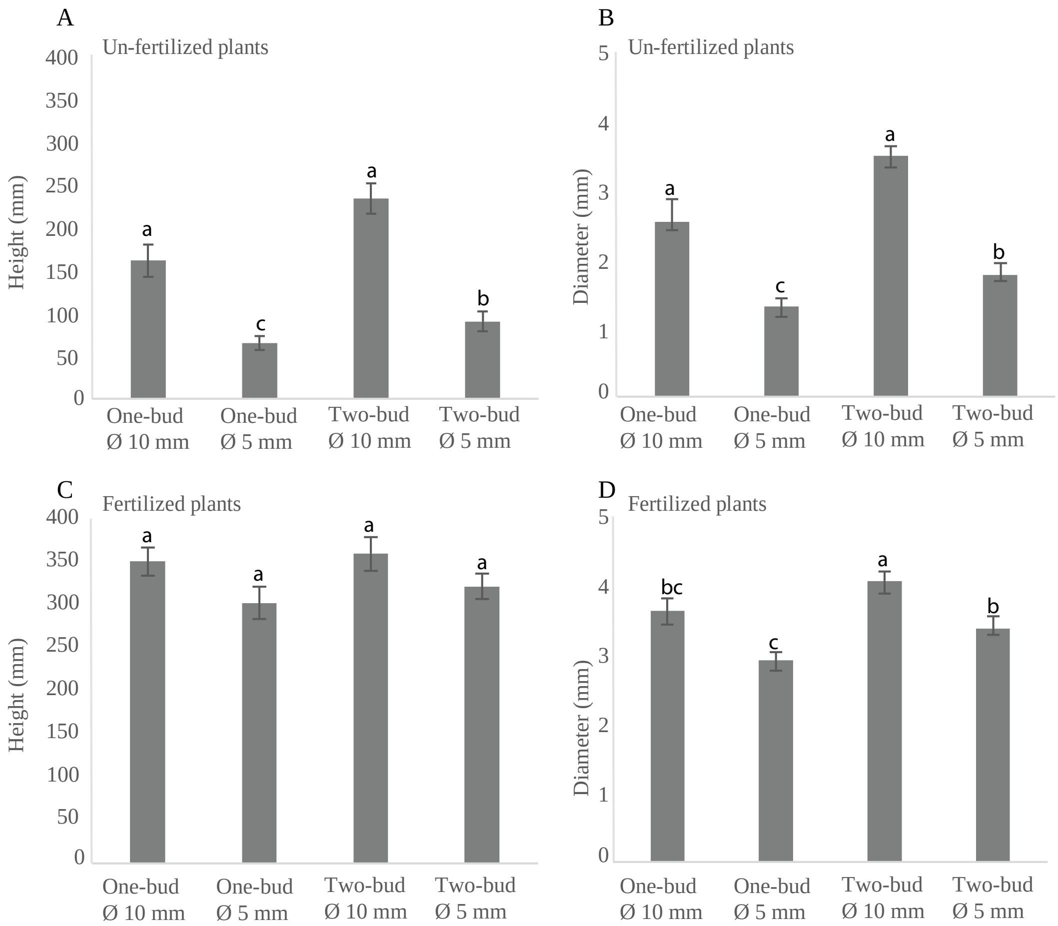

Figure 2. Height and diameter as a function of cutting type and fertilization. Un-fertilized plants' (**A**) height and (**B**) diameter. Fertilized plants' (**C**) height and (**D**) diameter. Error bars indicate standard errors (n = 45). Different letters indicate significant differences at p = 0.05 using Tukey's HSD (honest significant difference).

Fertilized plants reached a mean height of 331 mm, ranging from 300 to 357 mm after ten weeks. Mean diameter growth was 3.5 mm, ranging from 3 to 4 mm (Figure 2C,D). For these plants, no significant difference in height was found among the four cutting types (Figure 2C). For diameter growth, the thick and long cuttings (two-bud Ø 10 mm) resulted in the thickest plants while the thin and short cutting (one-bud Ø 5 mm) resulted in the thinnest plants.

3.4. Analysis of Leaf Stem, and Root Biomasses

The largest cuttings (two-bud Ø 10 mm) resulted in the highest leaf, stem and root biomasses among unfertilized plants (Figure 3A–C). Biomasses (leaf, stem and root) for the other cutting types were similar. The only significant difference found was leaf biomass between one-bud Ø 10 mm and one-bud Ø 5 mm (Figure 3A). For fertilized plants, the smallest cuttings (one-bud Ø 5 mm) resulted in the least leaf, stem and root growth (Figure 3D–F). Thicker cuttings grew more leaf and stem biomass regardless of cutting length, but one-bud Ø 10 mm and two-bud Ø 5mm cuttings showed similar growth. The smallest cuttings (one-bud Ø 5 mm) grew the least root biomass (Figure 3F), while the other cutting types produced similar root biomasses.

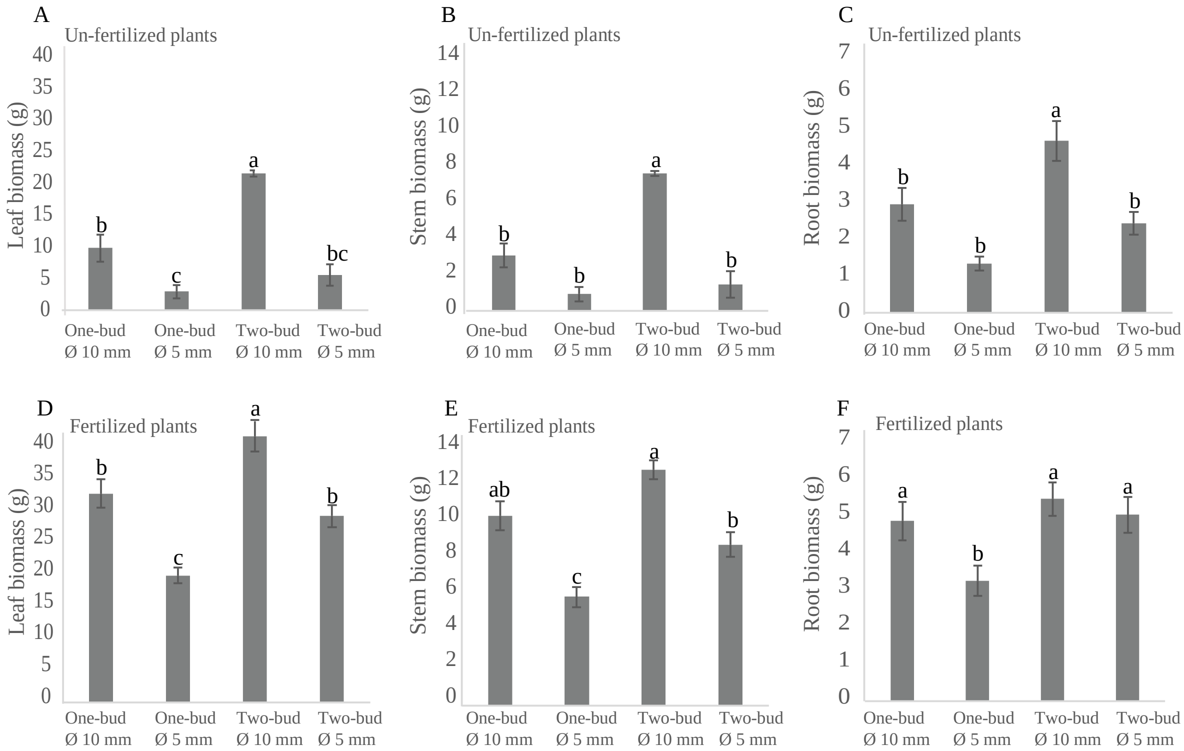

Figure 3. Leaf, stem and root biomasses of plants from different cutting types and fertilization treatments. Data shown are biomasses for un-fertilized plants' (**A**) leaf, (**B**) stem and root (**C**) and fertilized plants' (**D**) leaf, (**E**) stem and (**F**) root. Data shown are means (n = 45); error bars indicate standard errors. Different letters indicate significant differences at p = 0.05 using Tukey's HSD.

3.5. Root Number and Root-To-Shoot Ratios

Across clones and cutting types, fertilization had no significant effect on the root numbers (p = 0.06) with 5.9 mean roots per cutting for fertilized and unfertilized plants. The number of roots per plant (fertilized and un-fertilized plants) was higher if long cuttings were used (Figure 4A,B). This was significant for both cutting diameters for two-bud cuttings (fertilized) and two-bud Ø 10 mm (un-fertilized). For both fertilized and un-fertilized plants, two-bud cuttings with thicker stems resulted in more roots than thin cuttings (Figure 4A,B). For one-bud cuttings, diameter did not influence root numbers. Across clones and cutting types, the root-to-shot ratio was higher for un-fertilized plants than for fertilized plants ($p \leq 0.001$). For fertilized plants, no differences among root-to-shoot ratios could be found (Figure 4D), but for un-fertilized plants there were differences between cutting types (Figure 4C). Three of the cutting types' (one-bud Ø 5 mm and 10 mm and two-bud Ø 5 mm) root-to-shoot ratios were similar. Plants with the largest above-ground biomass (two-bud Ø 10 mm cutting) had a lower root-to-shoot ratio compared to one-bud Ø 5 mm and two-bud Ø 10 mm.

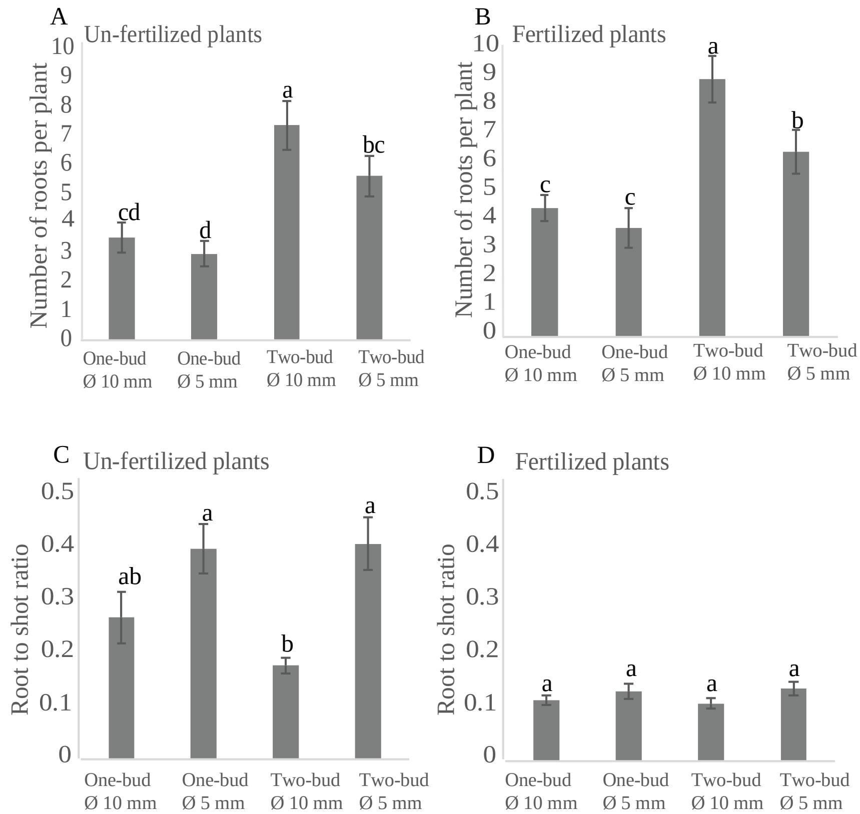

Figure 4. Number of roots and root-to-shot ratio as a function of cutting type and fertilization. Panel (**A** and **C**) shows un-fertilized and (**B** and **D**) shows fertilized plants. **A** and **B** number of roots, and **C** and **D** root-to-shot ratio. Error bars indicate standard errors (n = 45). Small letters indicate significant differences at p = 0.05 using Tukey's HSD.

4. Discussion

We investigated the morphology of containerized poplar plants produced from cuttings of different thicknesses and lengths. Our main findings are that if plants are fertilized, initial cutting morphology has little impact on the vigor of the resulting plants, meaning that more cutting types can be used to produce containerized plants (Figures 2–4). If plants are not fertilized, only one cutting type (two-bud Ø 10 mm) resulted in satisfactory plants (Figures 2–4). However, the number of roots is clearly influenced by the cutting length (Figure 4).

An earlier report found that longer cuttings (10 cm) had higher rooting success than short cuttings and that cutting diameter had no effect on survival [30]. Our results do not agree with these findings as survival was similar within a clone, regardless of cutting length or diameter. Instead, we found a clonal effect on survival within a specific cutting type (one-bud and two-bud Ø 5 mm) but only between two clones. Interestingly, when planted under field conditions, cutting diameters (bigger or smaller than 1 cm) influenced plant survival [29,32].

Our results bear on several important considerations when contemplating the use of one-bud cuttings in containerized plant production. One-bud cuttings are probably more sensitive to dry conditions as they do not reach the deeper soil of the container. Moreover, if large-scale production of containerized poplar plants is desired, the cultivation is likely done outdoors with little control of climate conditions. In this case, a longer cutting (two-bud) would be a safer choice as it extends deeper into the soil. By using one-bud cuttings, more plants can be produced from each stool bed, but the rooting capacity and survival need to be tested for the chosen clones.

Several reports demonstrate that longer cuttings grow faster, both in height and diameter [28,30,33]. However, in our experiment, fertilized plants showed little clear response to initial morphology. For cuttings with similar diameters but different lengths, plant height, stem and root biomass were similar (Figure 3C–F), but diameter and leaf biomass were different. Several of these publications [28,30,33] are from field experiments where longer cuttings would likely have had better access to soil water content if planted deeper. In our experiment, temperature and moisture are kept at optional levels for poplar growth and therefore cutting length may be less important. Instead, we found that cutting diameter, especially of one-bud cuttings, is important for plant growth (Figure 2B–E). In contrast to [30], we found differences in root number (Figure 4A,B) between 10 cm (two-bud) cuttings and 5 cm cuttings (one-bud). However, similarly to [30], root biomass was the same for one-bud Ø 10 mm, two-bud Ø 5 and two-bud Ø 10 mm cuttings (Figure 3F).

In a typical forest site in southern Sweden, the competition from other vegetation is low in the first two years after planting [22]. Therefore, establishment of poplar should be performed at this time as poplar is sensitive to competing vegetation [7,10,12]. A newly transplanted plant needs time to recover from the stress of handling and moving and establish roots to uptake soil water and nutrients. Site environmental conditions are impossible to control but plants can be chosen to maximize stress tolerance.

Could it be that these differences in plant growth are not important after transplantation and that differences in cutting morphology are unimportant? Before conducting extensive field trials, other studies can give indications. On agricultural sites, the choice of plant type (bare-root, cuttings and containerized plants) or container size were not important for plant growth [25]. Thus, it is most likely that all of our fertilized plant types would establish and grow satisfactorily and perhaps un-fertilized plants from the largest cutting (two-bud Ø 10 mm) could be used. On forest sites, however, different cutting types could result in early growth differences as container-grown plants resulted in faster growth (leaf, stem and root biomass) in the first year after planting [25]. This is in line with [15] that recommends using shoot height, stem diameter, root mass and root-to-shoot ratio as indicators of plant quality. Other authors [34,35] suggest that root collar diameter is the best measurement for seedling size and has the best relation to future survival. Interestingly, stem diameter correlates with water absorption in the roots and water transport in the stem [36]. A low root-to-shoot ratio can be a warning sign for lower survival, especially in drier conditions, since the larger above-ground biomass has a higher transpiration. Therefore, in harsh conditions (forest sites), the ideal plant would be large (tall), have a large root collar (stem diameter), high root-to-shoot ratios, and a large number of roots. The largest cuttings of the unfertilized plants may be the best at harsh, forest sites, given their high root-to-shoot ratios and similar number of roots. These plants (largest unfertilized cutting) are of similar size (height and diameter of 233 mm and 3.5 mm) to the fertilized plants and the root-to-shoot ratios are lower. The use of the thin cuttings produced shorter, thinner plants with less biomass in all organs (Figure 2A,B and Figure 3A–C), and one-bud Ø 10 mm cuttings have only a height of 150 mm and a stem diameter of 2.5 mm. Therefore, these cutting types might not be useful for containerized poplar plant production.

Application of fertilizers increased plant growth, height, diameter and biomasses for all cutting types. Although there were significant differences between the cutting types (Figure 1D, Figure 2C, and Figure 3E,F), these were not as pronounced as among un-fertilized plants (Figures 2 and 3). Fertilization enables the use of a greater variety of cutting types when producing containerized poplar plants, thereby increasing the yield of each stool bed. However, our experiment lasted only for 10 weeks and continuation would increase growth of both un-fertilized and fertilized plants. Nevertheless, differences between un-fertilized and fertilized plants would be maintained, resulting in fertilized plants that are still better to use as transplants. If height, root biomass and root-to-shoot ratio are considered as indications of plant quality in our experiment, one-bud Ø 10 mm, two-bud Ø 5 and two-bud Ø 10 mm would be of similar quality. However, stem diameters are different between the cuttings (two-bud Ø 10 mm cuttings had the highest diameter) but this difference is only 0.6 mm. Therefore, this might not influence future plant growth. Although the root biomasses are similar for Ø 10 mm cuttings with one or two buds, the number of roots per plant is higher if a two-bud cutting is used. This would suggest that a two-bud cutting might be a better choice than a one-bud cutting for the production of plants used at harsh forest sites. Moreover, the fact that more roots are found on two-bud cuttings, even though their biomass is similar, could suggest that fine roots, also known as feeder roots, are more abundant. These roots are small in diameter and therefore, difference in these might not influence root biomass. These potential differences in fine roots might influence the establishment of these plants as they are thought to be important for taking up water and nutrients [36].

5. Conclusions and Practical Implications

If poplar plantations on forest and agricultural land are to be introduced on a large scale, a reliable source of planting material is needed. As poplar is clonally-propagated, available cutting material could be a limiting factor. If fertilizers are not used, the choices are limited to the largest cutting type. This would disqualify most of the cutting material produced in stool beds and probably increase the per-unit cost of the transplants. If fertilizer is used in the production of containerized plants, our results suggest that most of the material from cutting stool beds can be used to produce material for establishing polar plantations. Cuttings with the highest quality (>Ø 10 mm and 30 cm length) could be transplanted directly to agricultural plantation sites. Cuttings with intermediate quality (Ø 10 mm) could be used directly at plantation sites but also as one- or two-bud cuttings for the production of containerized plants. If one-bud cuttings are used in containerized plant production, each clone used should be tested for initial rooting capacity. The thin (Ø 5 mm) two-bud cuttings can produce acceptable containerized plants while one-bud cuttings might not be suitable. If these recommendations are followed in the production of transplant material, the establishment of poplar plantations will likely be cost efficient.

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Conflicts of Interest: The authors declare no conflict of interest.

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