The Effect of Root Segment Origin, Size, and Orientation in Aspen Rootling Propagation

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Abstract: A vegetative propagation trial was conducted at two Alberta nurseries using juvenile root segments taken from potted plants of six clones of aspen (*Populus tremuloides* Michx.). The purpose of this study was to investigate the effect of origin and orientation of root segments on new plant development, and to determine a minimum size of root segment that will still result in acceptable propagation success rates. Five donor plants of each clone were grown at Woodmere Forest Nursery in Fairview, Alberta in 2003. In the fall, stems were removed and the root masses were cold stored over the winter. In the spring of 2004 half of the root masses were shipped to Smoky Lake Forest Nursery. In early June, 2004, root segments were excised and inserted vertically into styroblock cells. Treatment variables were clone, origin (proximal or distal to donor plant stem), root segment length, and orientation (proximal or distal end upwards). Propagation success was assessed at 10 weeks.

There were large differences in propagation success between the two locations; survival was 53% at Woodmere Forest Nursery and only 20% at Smoky Lake Forest Nursery. This difference is believed to have arisen because of heat stress damage in the root donor plants during shipment and setup at Smoky Lake. Due to the resulting large imbalance at Smoky Lake, it was decided to limit further interpretation of the results to the Woodmere location only. Segment length had a large and significant effect on rooting success on both sites. The shortest segments achieved 4% success and the longest 39% at Smoky Lake. The corresponding figures are 5% and 85% at Woodmere. Segment length was by far the most important factor, explaining 42.5% of the total variation at Woodmere. Rooting success appears to be directly proportional to segment length.

The root segments expressed clear polarity, with proximal orientation (root segment planted right way up) showing greater success than distal orientation (root segment planted upside down). There were significant differences in propagation success between clones, although clones explain only 1.3% of the total variation. Root segment origin effects were not significant. Rootling propagation appears to be a viable method for mass clonal propagation of aspen. Root segment length and orientation are critical in ensuring high success rates.

Keywords: Rootling, propagation, aspen, root size, origin and orientation

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INTRODUCTION

The increased harvest of aspen (*Populus tremuloides* Michaux) in northern Alberta has prompted Canadian forest companies to develop methods for plantation silviculture of this dominant species of the Boreal forest. An efficient, effective and economical propagation method is needed as a pre-requisite for any plantation silviculture.

The Western Boreal Aspen Corporation (WBAC) is a cooperative tree improvement venture involving four Alberta forest companies that harvest aspen for pulp and oriented-strand board (OSB) production. WBAC has been developing a mass vegetative propagation method based on juvenile root segments termed "rootling propagation" (Niemi *et al.* 2003). This method uses small root segments that generate both stem suckers and fine roots to produce an autonomous rootling that can be used as planting stock (Figure 1). Rootling propagation is much easier and cheaper than tissue culture or greenwood cuttings as a means of mass vegetative propagation.

Aspens produce suckers from mature roots and rely on this method for natural regeneration following browse, fire, or other stand disturbance. Sucker stems can be excised from mature roots and induced to form roots under laboratory conditions. Libby (1986) coined the term "steckling" to describe a "plantable rooted cutting". This term indicates the stem cutting origins of this type of propagule. In a similar vein, Hall *et al.* 1990 coined the term "rootling" to describe plants of root cutting origin. They planted root segments directly into nursery beds to produce bare-root stock. Dreeson and Harrington (1999) developed this method further using potted stock plants grown to provide juvenile root masses for containerized stock production. Dreeson (2001) termed this vegetative propagation method the "root cutting" method.

Given the necessity of growing potted stock plants as root donors, this study was initiated to test the effect of root segment size on propagation success. The origin of the roots and their orientation when planted vertically was also investigated.

MATERIALS AND METHODS

This experiment was replicated at two commercial forest nurseries: Woodmere Forest Nursery at Fairview, and at Smoky Lake Forest Nursery (Coast to Coast Reforestation) at Smoky Lake, Alberta. Six local clones of aspen were used: these are numbered 1176, 1177, 1221, 1223, 1229 and 1230 in WBAC's clone testing program. Five donor pots were grown at Woodmere in 2003. In the fall, the stems were cut off and the root masses cold stored over the winter. In June 2004, half of the pots were shipped to Smoky Lake. Unfortunately, these were delayed in transit and are believed to have been subjected to excessively hot conditions for a few days. The pots remaining at Woodmere were used directly without transshipment.

Figure 1. A rootling at 3 weeks after sowing

At each site, root segments from the six clones were extracted and cut to 13-cm lengths. Each length was cut into two equal halves one proximal to the stem axis and the other distal. Root segments of the following lengths were cut from each half: 3 cm, 2 cm, 1 cm and 0.5 cm (see Figure 2). The root segments were 'sown' into styroblock cells (Beaver Plastics, Styroblock 410A – 112 cells / 80 ml/cell. The medium was 2.5:1 peat/vermiculite at Woodmere and peat/perlite at SLFN. Irrigation, lime, gypsum, macronutrients and micronutrients were applied as part of the nurseries standard growing protocol for hardwood seedlings. Segments were 'sown' vertically in the styroblock cells according to the pattern in Figure 3.

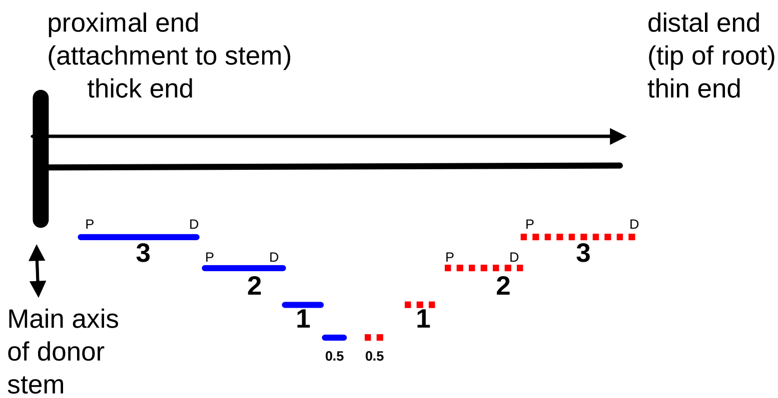

Figure 2. Cutting pattern

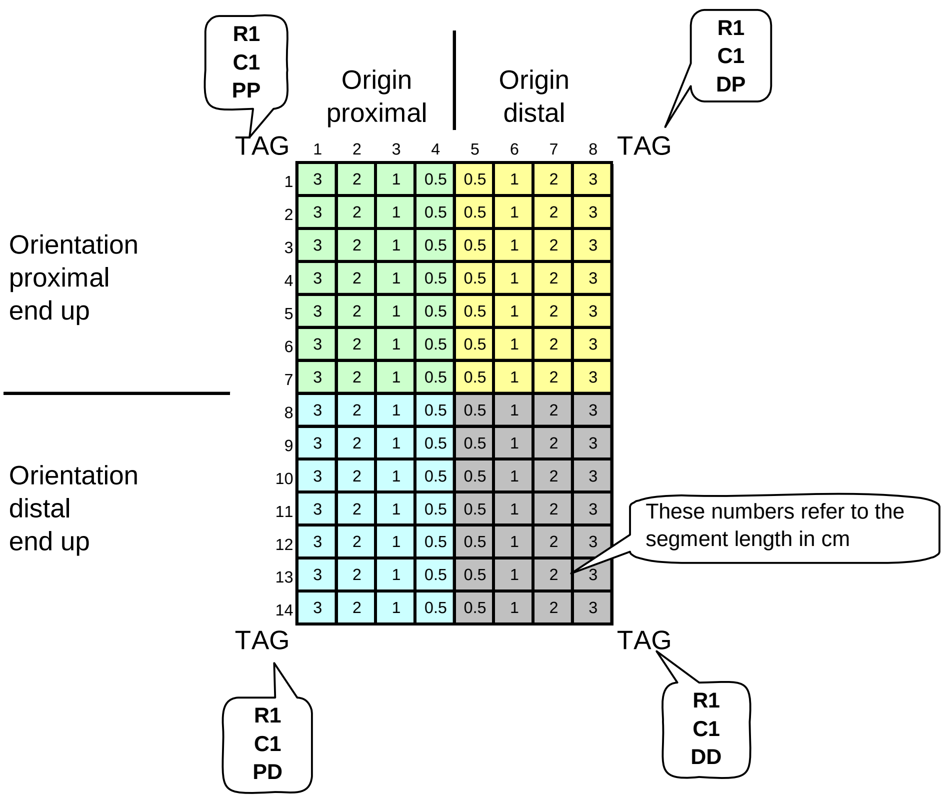

Figure 3. Arrangement of root segments in styroblocks

The experimental design was a split-plot factorial with 2 replicates of 6 clones in whole styroblocks as main plots, and segment length with four levels, and origin and orientation as sub-plot factors each with two levels. Each plot consisted of seven cells arranged systematically within the styroblock so that the long segments were on the outside and the short segments in the center of the block.

The trials were established June 2, 2004 and assessed on August 4 after 65 days. The following traits were assessed: number of sprouts per cell, survival (i.e. presence or absence of sucker sprouts) termed 'rooting success', height of tallest sprout in dm, and the diameter of the original root segments.

RESULTS

1. Nursery effects

Overall rooting success was 20% at Smoky Lake and 53% at Woodmere (Table 1). This difference is believed to be due to heat stress to the donor pots being delayed in transshipment from Woodmere to Smoky Lake. The Woodmere materials did not suffer from any heat stress.

2. Clone differences

There were significant differences in clone performance as measured by rooting success (Table 1). There were rank order changes across the two sites (Figure 4).

Table 1. Rooting success by clone and nursery

		Success%	
Freq	**Clone**	**SLFN**	**Woodmere**
244	1176	24.6	41.5
244	1177	23.4	50.0
244	1221	13.8	65.7
244	1223	17.9	50.9
244	1229	13.4	55.0
244	1230	21.2	52.2

3. Segment length

There were distinct size effects. For all clones and all treatments, the longer the root segment, the greater was the propagation success (Table 2 and Figure 5). The optimal segment length is probably around 4 to 5 cm, but these lengths were not tested, since the objective of this experiment was to test how small a segment could be used.

Table 2. The effect of root segment length on propagation success

Freq	Length cm	#Sprouts	Success%
336	0.5	0.05	0.05
336	1	0.47	0.43
336	2	0.96	0.77
336	3	1.13	0.85

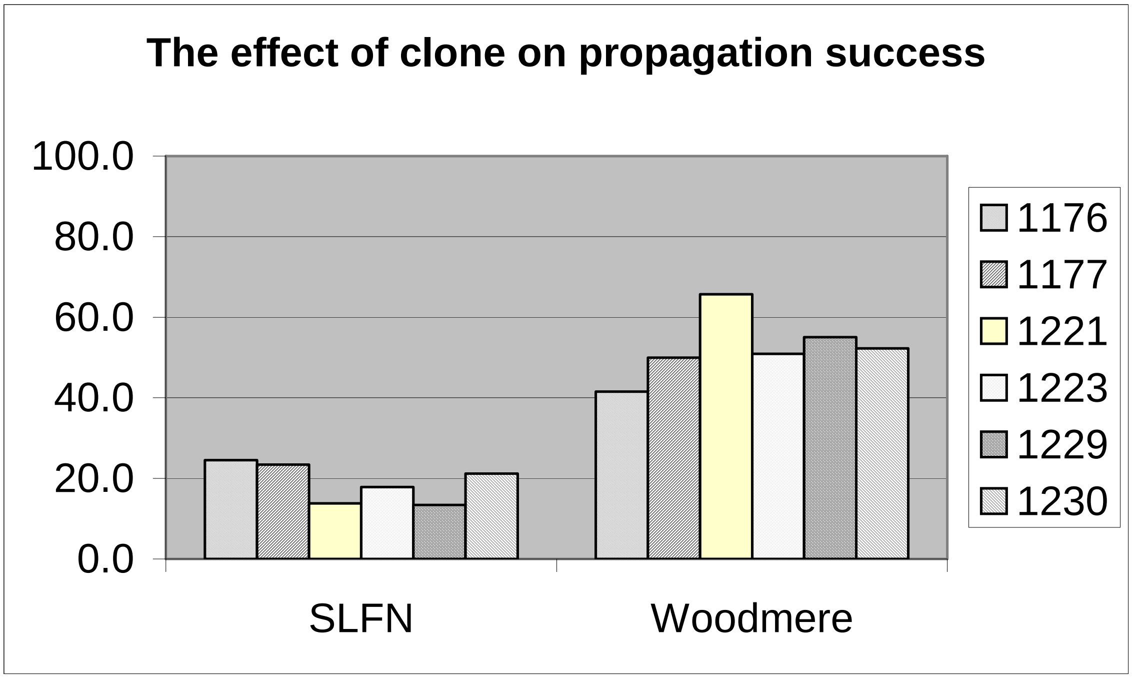

Figure 4. The effect of clone and nursery on propagation success

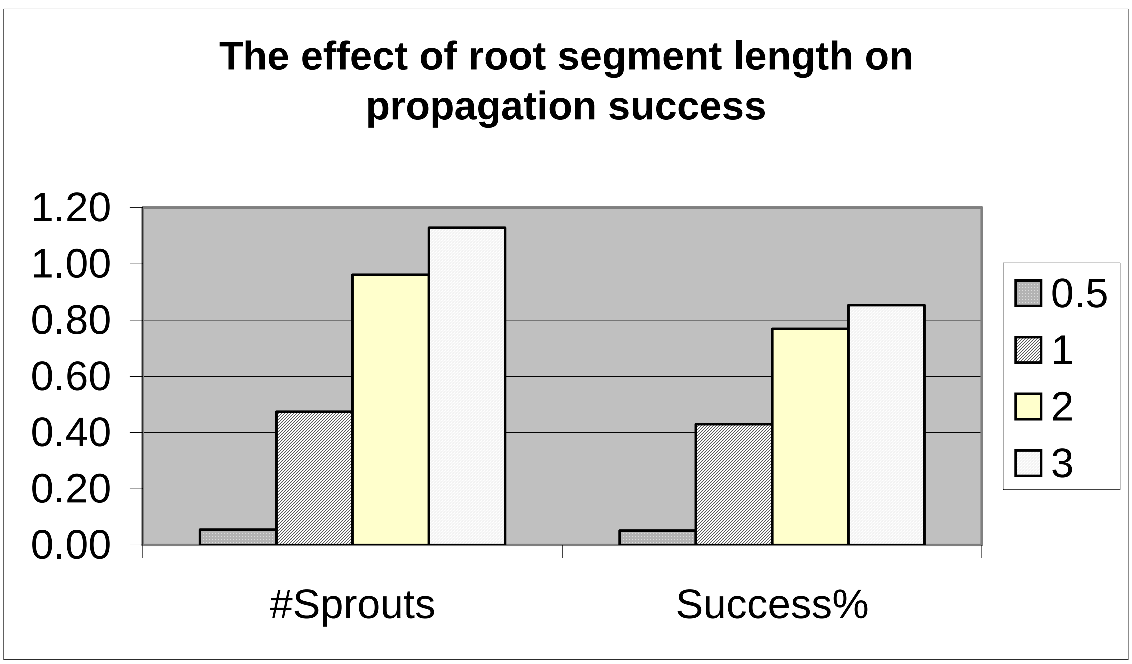

Figure 5. Propagation success and number of sprouts as a function of segment length

4. Segment origin

Proximal origins had marginally better propagation success than distal ones (proximal = 54% versus distal = 52%). These small differences were not significant (Table 3 & Figure 6).

Table 3. The effect of root segment origin on propagation success

Freq	Origin	#Sprouts	Success%
672	Distal	0.6171	0.52
672	Proximal	0.6952	0.54

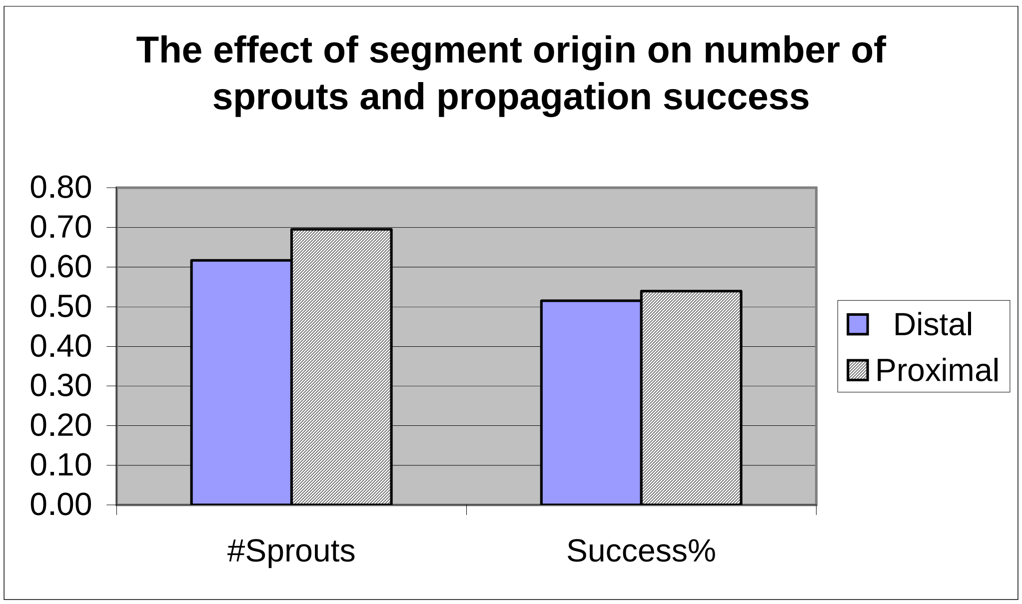

Figure 6. Propagation success and number of sprouts as a function of segment origin

5. Segment orientation

Segments planted proximal-end up produced more shoots and had higher success than distal orientations. These differences were larger than those for origin, and were significant (Table 4 & Figure 7).

Table 4. Effect of orientation on propagation success at Woodmere

Freq	Orient	#Sprouts	Success%	Height dm
672	Distal	0.60	0.48	1.77
672	Proximal	0.71	0.57	1.74

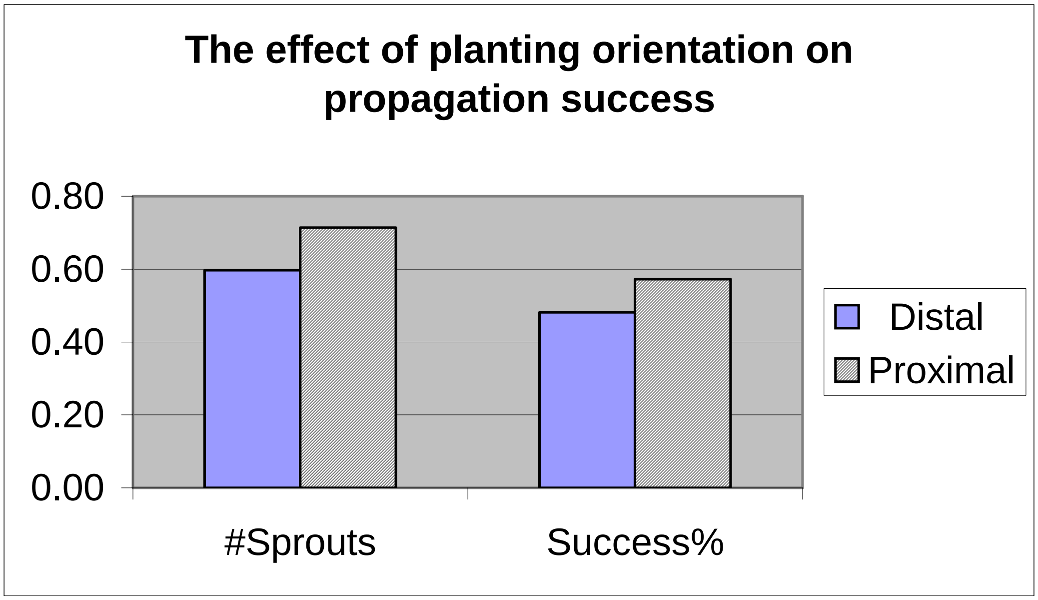

Figure 7. The effects of root segment origin

6. Combined effects of origin, orientation and length

The effect of segment length was striking and consistent across all combinations of origin and orientation. (Table 5 & Figure 8). It is clear that orientation is more important than origin. Proximal orientation with distal origin has higher success than distal orientation with proximal origin.

Table 5. Combined effects of origin, orientation and length at Woodmere

		Origin and Orientation			
FREQ	Segment Length-cm	Distal Distal	Distal Proximal	Proximal Distal	Proximal Proximal
84	0.5	0.04	0.06	0.05	0.06
84	1	0.39	0.48	0.30	0.55
84	2	0.66	0.82	0.76	0.83
84	3	0.80	0.87	0.84	0.90

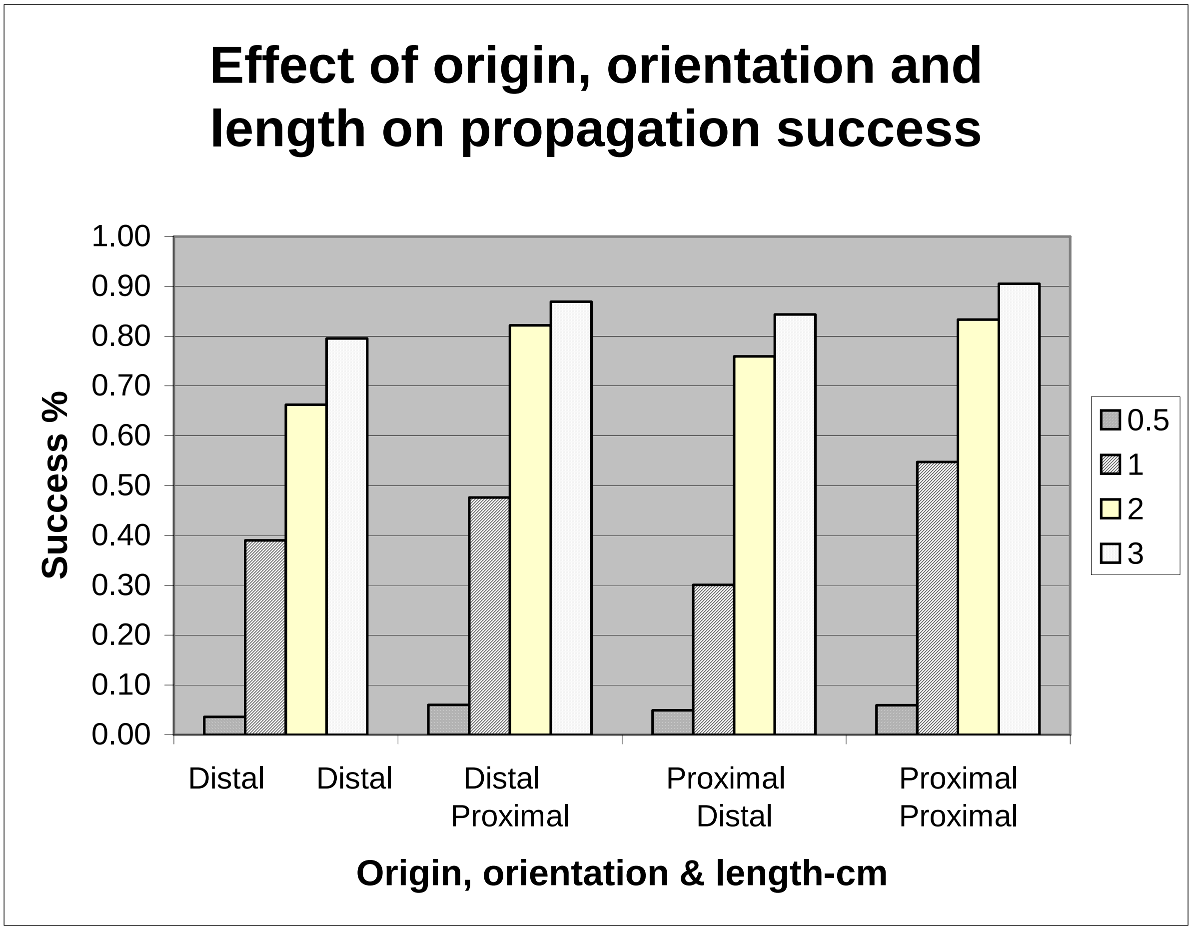

Figure 8. Combined effects of origin, orientation and length on propagation success at Woodmere

7. Variance components

The variance components for a full split plot model were calculated using SAS PROC VARCOMP (SAS Institute 1985).

The following model was used:

Y_{ijklmn} =Mean + Rep + Clone + Rep*Clone + L + L*Rep + L*Clone + L*Rep*Clone + Orig + Orig*Rep + Orig*Clone + Orig*Rep*Clone + Orig*L + Orig*L*Rep + Orig*L*Clone + Orig*L*Rep*Clone + Orient + Orient*Rep + Orient*Clone + Orient*Rep*Clone + Orient*L + Orient*L*Rep + Orient*L*Clone + Orient*L*Rep*Clone + Orient*Orig*Rep + Orient*Orig*Clone + Orient*Orig*Rep*Clone + Orient*Orig*L + Orient*Orig*L*Rep + Orient*Orig*L*Clone + Orient*Orig*L*Rep*Clone + e_{ijklmn}

where there are i reps, j clones, k lengths, l origins, m orientations, and n trees per plot. All effects and interactions were considered random. Subscripts have been omitted in the model statement for clarity. The percentage contributions to total variance are presented in table 6.

Table 6 Variance components and percentage contribution to total variance Woodmere site

Source	Var comp.	%
Rep	0.000000	0
Clone	0.003909	1.3
Rep*Clone	0.000400	0.1
L	0.123000	42.5
Rep*L	0.001086	0.4
Clone*L	0.001265	0.4
Rep*Clone*L	0.000000	0.0
orig	0.000497	0.2
Rep*orig	0.000000	0.0
Clone*orig	0.000780	0.3
Rep*Clone*orig	0.000000	0.0
L*orig	0.000000	0.0
Rep*L*orig	0.000000	0.0
Clone*L*orig	0.000000	0.0
Rep*Clone*L*orig	0.004148	1.4
orient	0.003410	1.2
Rep*orient	0.000499	0.2
Clone*orient	0.000000	0.0
Rep*Clone*orient	0.000368	0.1
L*orient	0.000000	0.0
Rep*L*orient	0.000000	0.0
Clone*L*orient	0.007900	2.7
Rep*Clone*L*orient	0.007433	2.6
orig*orient	0.000000	0.0
Rep*orig*orient	0.000177	0.1
Clone*orig*orient	0.000480	0.2
Rep*Clone*orig*orien	0.001704	0.6
L*orig*orient	0.001478	0.5
Rep*L*orig*orient	0.000647	0.2
Clone*L*orig*orient	0.001216	0.4
Rep*Clon*L*orig*orie	0.000000	0.0
Residual	0.129200	44.6
	0.289597	

DISCUSSION

Segment length was by far the most important factor in explaining rooting success. This was likely due to a combination of factors such as availability of stored carbohydrate reserves, potential sites for new root or sucker primordia, or even availability of residual water reserves to tide over the segment and developing suckers while new fine roots are developing. The optimum length is probably greater than 3 cm but this size was not tested here since we were trying to maximize root segment yield per donor plant.

Origin had little effect on rooting success. The small advantage of proximal over distal origins may best be explained by differences in root diameter close to and away from the stem axis, i.e.

segments of proximal origin are larger than those of distal origin if they are of equal length and from the same root.

The effect of planting orientation might best be explained by hormone gradients in the root segments. On inspecting dug up rootlings, the majority of suckers appear to originate at the proximal end, and the majority of fine roots appear at the distal end regardless of planting orientation.

In many cases, where multiple suckers were observed from a single root segment there was a tendency for one sucker to suppress the others. Usually the first sucker to expose its leaves to the light won this competition and ended up being the dominant or single stem. While horizontal placement of root segments does result in successful rootling production, the authors believe that vertical orientation with proximal side up is the optimal method. Horizontally placed segments are unstable and liable to topple whereas vertically placed segments are better anchored. There may also be more intense competition between suckers originating from horizontal roots with overall lower propagation success. This hypothesis should be tested in some further trials.

The different clones tested here had different rootling success. Such genetic differences in propagation ability have been commonly observed in other species for steckling production as well as tissue culture.

The large nursery effects are believed to have been accentuated by the heat stress that the donor root systems were subjected to in transit. Nonetheless, it can be expected that differences in cultural practices could explain some fairly large differences of response at different nurseries – critical factors could include ambient temperature, moisture availability and light quality and intensity.

With optimum treatment (3-5 cm root segments planted proximal-end up) we can expect 80-90 rooting success. It appears that rootling propagation is a promising method for mass vegetative propagation of aspen.

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