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Roots are a Good Source of Cuttings in Propagation of Apple Rootstock M9 'Lancep'

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Summary

Alternative methods for propagation of apple rootstocks are needed where the climate is not optimal for stool bed multiplication and where *in vitro* culture is not an option. This study examined production of cuttings from roots and rooting of these cuttings to explore whether efficient propagation of apple rootstock M9 'Lancep' is possible by this method. Hypotheses for the study were: i) Root quality (length and thickness) and soil conditions affect the production of cuttings from root tissues; and ii) the rooting success of cuttings is promoted by an external supply of hormones. Cuttings were taken from roots of six qualities in three growing media and at two root depths (3.5 and 7.0 cm) and the production of cuttings and the rooting process were monitored. Medium to long roots of large diameter (5–10 mm) produced the highest number of cuttings. A thin soil cover (3.5 cm) on top of the root mass was better than a thick layer (7.0 cm) for high production efficiency and cutting quality. The etiolated base of such cuttings did not need to be treated with plant growth regulators in order to obtain high rooting efficiency, probably because of the juvenile state of cuttings produced from these roots. Compact cuttings of high quality rooted well and gave plantlets of good quality. These results contribute to the establishment of an efficient propagation procedure for apple rootstock M9 'Lancep'.

Key words. apple trees – auxin – multiplication – root cuttings – root quality – soil temperature

Introduction

Dwarf apple rootstocks of M9 and sub-clones of these are most commonly used in commercial apple production because of their ability to decrease tree size and increase yield and fruit quality, thus positively affecting the economics of fruit production (Di Vaio et al. 2009; Gjamowski and Kiprijanovski 2011). The propagation of rootstocks must support genetic stability and high plant quality, and for the nurseryman the production methods must be economically and practically feasible. As the physiological age of trees increases from juvenile seedling to adult/flowering plant, tree rooting ability tends to decrease (Hartmann et al. 1997). However, *in vitro* multiplication and shoot tip grafting are tools that can induce rejuvenation (Chabukswar and Deodhar 2006). Apple cultivars, when propagated *in vitro*, may also perform well on their own roots but each cultivar may respond differently and should be studied separately (Zimmermann and Steffens 1996). Those authors found that only one clone showed inter-clonal variation in growth and yield among 20 micro propagated scions. However, for the future regulation of growth and development of apple scions in commercial orchards, there will continue to be a great need for rootstocks.

Apple rootstocks have traditionally been, and still are, propagated by layering from stool beds or by cuttings from mother plants in nurseries (Hansen 1990; Hartmann et al. 1997). Production of cuttings from roots is not a new method (Hartmann et al. 1997), although not much in use. Species and cultivars differ with respect to potential for shoot regeneration from roots, which may not be the best source of cuttings in all species and cultivars. However, production of cuttings originating from roots may have two advantages. 1) The production method does not need investment in cost-intensive technology and the production costs may thus be low. 2) Production of cuttings from roots may yield juvenile plants, which probably root more easily than cuttings from physiologically older mother plant tissues (Osterc et al. 2009). The best rooting in hybrid aspen has been observed in cuttings originating from thin root segments (Stenvall et al. 2006).

The action of the plant hormone seems to occur in the initial phase of rooting (the first five days) in apple rootstock mm106 and exposure to auxin (IBA) for more than five days in the rooting process produces unwanted callus

and leaf necrosis (Naija et al. 2008). The need for exogenous applied auxin may be unnecessary for some plants, as shown for tissue culture-propagated *Rosa hybrida* (Ibrahim and Debergh 2001).

The present study explored the use of roots as a source of cuttings and rooting of green cuttings for the propagation of M9 'Lancep'. The starting hypotheses for the study were: i) Root quality and soil conditions affect the production of cuttings from root tissues; and ii) the rooting success of cuttings is promoted by temperature conditions and an external supply of hormones.

Materials and Methods

Plant material

The root mass used in this experiment originated from tissue culture-propagated rootstocks of apple M9 'Lancep'. After the tissue culture stage, the micro-cuttings were rooted and established in plug trays (Vefi 96, Vefi A/S, Drammen, Norway) in a greenhouse during autumn 2006. The rooted micro-cuttings were stored at 1.5 °C until spring, when they were planted in the field and grown into rootstocks with diameter about 0.8 cm (at 10 cm above ground) during summer 2007. Root mass was cut and then placed in plastic bags and stored in a cooling room at 1.5 °C and 85 % RH during the winter. In March 2008, the roots were prepared for a multifactorial experiment with root quality, growing medium and root temperature as treatments.

Root qualities

The roots were sorted into three thickness classes (Table 1): 'Thin', < 3 mm in diameter; 'Medium', 3–5 mm diameter; and 'Thick', 5–10 mm diameter. Within each of the thickness classes, the roots were cut into pieces of different lengths (Table 1): 'Short', 3–5 cm; 'Medium' 8–12 cm; and 'Long' (uncut roots) (> 20 cm). A 160 g mass of each root quality class was put in each box. Based on measurements on 10 g sub-samples of roots with different qualities, total root length and dry matter content of the 160 g fresh weight sample were calculated (Table 2).

Table 1. Dry matter content (%) of roots of different thickness and root length. Estimates are based on measurements of 10 g fresh weight and re-calculated for 160 g fresh weight per box. Means ± SD.

Characteristics of roots of different thickness			
Root thickness		Dry matter %	Total root length box^{-1} (m)
Thin	< 3 mm	35.1 ± 2.1	75.6 ± 16.7
Medium	3–5 mm	40.2 ± 0.8	40.5 ± 0.9
Thick	5–10 mm	42.1 ± 0.9	14.3 ± 2.4
Characteristics of roots of different lengths			
Root length		Dry matter %	Total root length box^{-1} (m)
Short	3–5 cm	38.1 ± 3.7	46.2 ± 28.3
Medium	8–12 cm	39.6 ± 1.5	36.9 ± 14.8
Long	> 20 cm	39.6 ± 3.8	47.3 ± 33.1

Table 2. Quality of cuttings harvested from roots. Ten cuttings of each quality were harvested and weighed (fresh weight and dry matter), but each value shown represents the mean (g) for one cutting within each class. Ratio = (dry matter/fresh weight) × 100, i.e. dry matter percentage. Means ± SD.

Size	Fresh weight (FW) (g cutting^{-1})	Dry matter (DM) (g cutting^{-1})	Ratio DM/FW
Short thin	0.046 ± 0.006	0.025 ± 0.011	55.9 ± 29.6
Short medium	0.096 ± 0.030	0.031 ± 0.004	32.7 ± 7.5
Short thick	0.259 ± 0.037	0.082 ± 0.008	33.0 ± 5.1
Long thin	0.096 ± 0.039	0.030 ± 0.011	29.0 ± 3.9
Long medium	0.166 ± 0.037	0.047 ± 0.008	31.8 ± 1.7
Long thick	0.274 ± 0.051	0.085 ± 0.015	31.5 ± 5.5

Containers and growing medium

A 10 cm layer of peat was placed in free-draining plastic boxes (47 cm long, 26 cm wide and 24 cm deep) and the roots of different quality classes were spread evenly on top. An additional layer of medium, the same as that used in the bottom, was then added to a depth of either about 3.5 cm or 7 cm on top of the roots. Three growing media were tested: a) Commercial peat that had been fertilised and limed (Go' torv Degernes peat, Degernes Torvstrøfabrikk, Degernes, Norway). b) The same peat quality with 25 vol.% Perlite (Agra Vermiculite, Pull Rhenen, Rhenen, Netherlands) mixed homogenously into the peat. c) Natural peat (Degernes peat, Degernes Torvstrøfabrikk, Degernes, Norway) without fertilisers but with lime added. The fertilised medium contained 200 mg l^{-1} nitrogen (NO_3 + NH_4), 50 mg l^{-1} phosphorus, 300 mg l^{-1} potassium and 1500 mg l^{-1} calcium and the pH was 5.5. The peat without added fertiliser was of the same origin and quality and the pH was adjusted to 5.5 by addition of 1500 mg l^{-1} lime in the form of $Ca(CO_3)_2$. Water and nutrients were added as needed, with approximately equal rates to all treatments.

Soil temperature

The boxes with root mass were placed on tables with two different temperature conditions. One table had elevated temperature achieved by running warm water pipes under the table, which gave a soil temperature of 25 ± 1 °C, while the other table had no extra heating except that of the greenhouse and the soil temperature was 20 ± 1 °C.

Climate and growing conditions

In the greenhouse, the photoperiod was maintained at 16 h per day with supplemental lighting of 12000 lux at plant canopy level using 400 W SON/T lamps (Phillips). Supplemental light was cut when the natural radiation exceeded 250 W m^{-2}. Set point for the temperature was 20/18 °C during day/night. Ventilation was activated when temperature was 2 °C above the set temperature. RH was affected by the outdoor conditions and was 56.6 ± 11.6. Irrigation was carried out when needed.

Two-year production

If the root mass that produces cuttings in one year is to be able to continue production in the following year, overwintering roots must be supplied with carbohydrates from photosynthesising shoots before winter storage. In order to study the significance of supply of resources from shoots to roots, in a separate experiment the harvest of cuttings was terminated on three different dates, 1 June, 1 July and 1 August. After these dates, three boxes per termination date were left for regrowth of shoots from the roots. In mid-December, when the plants had shed their leaves, boxes with plants were placed in cold storage at a temperature of 2 ± 1 °C. In March 2009 the boxes were returned to the greenhouse, with the same conditions as described for the first year. The shoots were cut back to the surface of the soil medium and cuttings were harvested and counted as already described.

Rooting of cuttings

The green cuttings were harvested by tearing them from the medium. In this way an approximated 3.5 cm or 7 cm proportion of the stem with etiolated tissue accompanied the cutting, depending on the depth of cutting-producing roots. The cuttings (Table 2), which had 4–5 leaves, were then transferred to the rooting stage. Long and short cuttings were separated and monitored in the rooting stage. The cuttings were rooted in plug trays (Vefi 96, Vefi A/S, Drammen, Norway) with a medium consisting of fertilised and limed peat with 25 % Perlite. The plug trays were placed on a greenhouse table with white plastic sheeting (tent). Half the cuttings were treated with a commercial rooting hormone mix (Floramon A with 1 % auxin NAA, Novotrade, Herlev, Denmark), while the other half were planted in the rooting medium without any treatment. A maximum of 50 % of cutting stem in the peat medium was treated with hormone powder, according to the manufacturer's instructions for the product. After two weeks, the cuttings were harvested and examined for rooting percentage.

Experimental design and statistics

Factorial combinations of soil type, soil depth and soil warming were used in a completely randomized design. The experimental units, three per treatment, were randomly distributed on the greenhouse tables and moved to new positions on the tables once a week. Cuttings were harvested and counted separately for each box every three days. The effects of soil type, soil depth and soil warming as fixed factors in the statistical model, were tested in 3-way ANOVA, using PROC GLM in SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA). Multiple comparisons among species were performed with the Ryan-Einot-Gabriel-Welsch Q (REGWQ) multiple comparison test.

Results

The overall statistical responses are shown in Table 3. Two-way interactions were not detected between the different treatments. There was a significant three-way interaction between soil type, soil depth and heating on time until cutting emergence but not on the number of cuttings produced. However, in the following the responses are treated as main responses for the three treatments.

Table 3. ANOVA of the effects of soil depth, soil type and heating on the total number of cuttings and the time until harvest.

Source/F and P-values	DF	No. of cuttings		Time until cutting harvest	
		F	P	F	P
Soil type	2	6.25	0.007	0.16	0.851
Soil depth	1	22.51	0.001	6.57	0.017
Soil heating	1	0.09	0.770	0.42	0.524
Soil type*depth	2	3.31	0.055	0.21	0.811
Soil type*heating	2	0.57	0.573	1.69	0.207
Soil depth*heating	1	0.44	0.513	0.54	0.469
Soil type*depth*heating	2	0.23	0.794	4.81	0.018

Root quality

The number of cuttings per box increased with increasing root diameter, with 3.8-fold more cuttings from the thickest roots compared with the thinnest (Fig. 1A). It was possible to harvest the cuttings 37 days after the start of forcing of thin roots, while only half this period (18 days) was required before harvest of cuttings from thick roots (Fig. 1A). A significant positive relationship between root length and the number of cuttings produced was observed. It was possible to harvest the cuttings sooner after the start of the experiment when the root pieces were long as opposed to short (Fig. 1B). The thickest and medium longest roots tended to be the most efficient in cutting production during the whole experimental period, from spring until summer (Fig. 2).

Thickness of covering layer and medium type

In comparison with the thinner layer (3.5 cm) of medium covering the roots, the thicker layer (7.0 cm) decreased the number of cuttings by 69 % (Table 4) and increased the time until the cuttings were large enough for harvest by 8.7 days. The difference between treatments also yielded cuttings of different qualities (data not shown). Having a thicker layer of medium covering the roots gave longer cuttings and a larger part of the cutting was etiolated.

Limed medium with no fertilizers (peat 3) gave 52 % more cuttings than average of both fertilized mediums (peat 1 and 2, Fig. 3). Adding 25 % Perlite to the fertilised peat medium increased the number of cuttings by 36 % in the case of 3.5 cm covering layer. However, the type of soil did not affect the number of days until harvest of cuttings.

Soil temperature

The higher soil temperature in the growing medium (25 °C) increased cutting quality, with 6.6 % more cuttings rated as being of high quality (data not shown) compared with cuttings produced at the lower temperature (20 °C, data not shown).

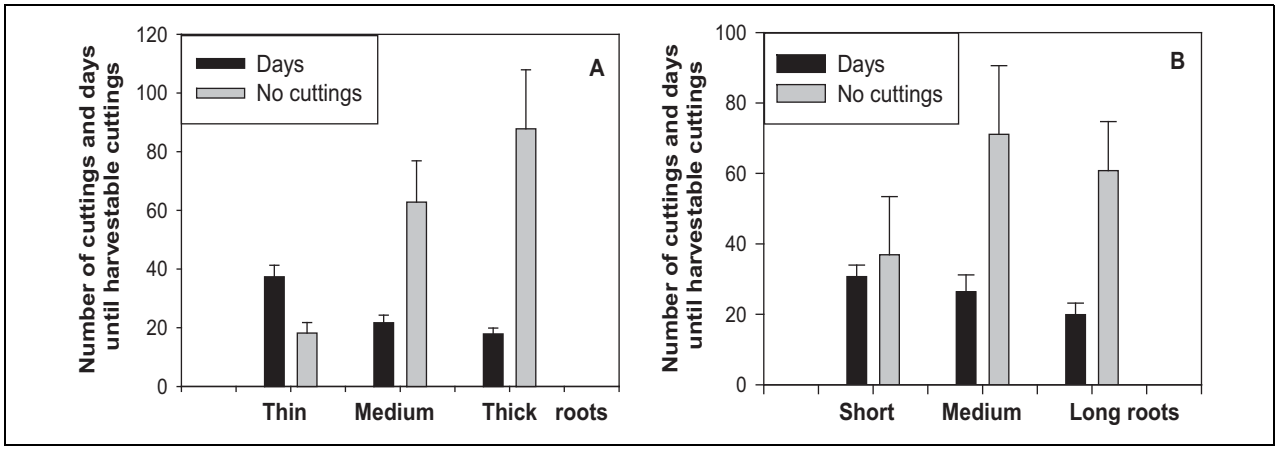


Fig. 1. Number of cuttings and days until harvestable cuttings as affected by (A) root thicknesses (thin, medium, thick) and (B) root lenghts (short, medium, long). Means ± SD.

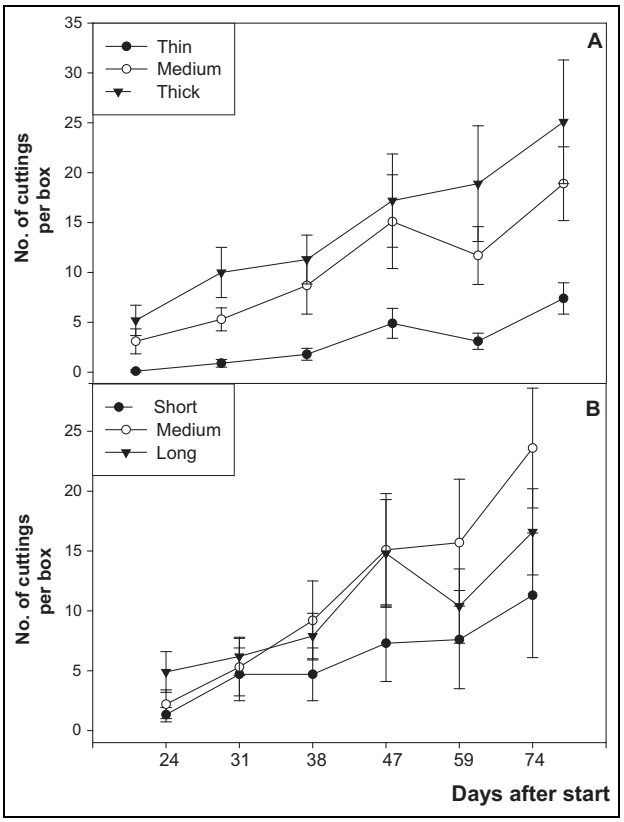


Fig. 2. Accumulated number of cuttings harvested at 6 dates after start of forcing of root mass sorted by quality. (A) thin, medium and thick roots and (B) short, medium and long roots. Bars on means are SD.

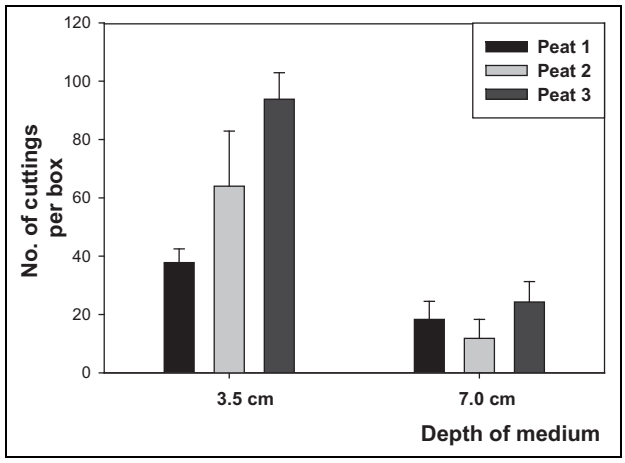


Fig. 3. Number of cuttings produced from the root mass in three types of medium. Peat 1 was fertilised and limed, Peat 2 was fertilised, limed and contained 25 % Perlite. Peat 3 was limed but without added fertilizers. Medium depth covering the roots was 1: 3.5 cm and 2: 7.0 cm. Means ± SD.

Table 4. Influence of soil cover thickness on number of cuttings produced and days until cuttings emerged from roots. Means ± SD.

Soil depth	No. of cuttings	Days to emergence
3.5 cm	65.2 ± 42.1	16.1 ± 3.8
7.0 cm	20.4 ± 19.2	27.9 ± 13.6
Statistics	6.20/0.0010	4.11/0.0092

Re-use of the same roots for cutting production in two seasons

When the production of cuttings was continued based on the same roots as in the preceding year, the number of cuttings was only 13.0, 10.3 and 2.8 per box when the harvest of cuttings in the preceding year continued until 1 June, 1 July and 1 August, respectively (data not shown).

Rooting of cuttings

Large cuttings yielded a higher proportion of plantlets rated as being of undesirable quality (Table 5). It was more difficult to work with the long cuttings, which broke easily during preparation for the propagation stage. The rooting percentage was not affected by hormone treatment of the cuttings (F = 3.03 and P = 0.1427), with only 6 % higher rooting in hormone-treated than non-treated cuttings. In general (for all cuttings), the higher growing temperature (25 °C) gave only 5 % higher rooting than the lower temperature (20 °C). However, for the largest cuttings, a lower rooting percentage was observed unless the cuttings were kept at the higher temperature during rooting. The difference in rooting percentage among the largest cuttings between the high and low temperatures was 15 % (F = 147.2, P = 0.0067).

Discussion

Root quality

The thickest roots gave the largest number of cuttings. Greater thickness of roots also promoted the production of apple cuttings in a study by Skogerbø and Måge (1986), but they used old trees and considerably thicker roots as the source of root mass than in the present study. In a study of hybrid aspen, root pieces close to the root collar produced sprouts faster, but with fewer cuttings than from thinner roots at larger distance from the root collar (Stenvall et al. 2006). Thus, there may be an optimal root thickness for the production of cuttings and for the rooting ability of the cuttings. In our research, the mother plants were propagated by tissue culture and thereafter grown *in vivo* for only one season before roots were harvested for cutting production. Under the climate of Nor-

Table 5. Proportion of cuttings rated to be of undesirable quality and rooting percentage, within each size class of the harvested cuttings.

Cutting size	Proportion low quality (%)	Rooting (%)
Small; 2–3 leaves, fresh weight about 0.07 g	8.2 ab	91.8 ab
Medium; 4–6 leaves, fresh weight about 0.13 g	6.2 b	93.8 a
Large: more than 6 leaves, fresh weight about 0.27 g	13.6 a	86.4 b

Values followed by different letters are significantly different ($P < 0.05$).

way, it is not possible to produce much thicker roots than those tested here. However, the use of polytunnels could increase growth and root thickness. The age and size of the roots must be optimised in terms of i) efficiency of production and ii) good health status (pests and diseases) of offspring plants. Therefore, we suggest that roots should not be taken from old trees. Tissue culture propagation can restore at least some plant juvenility (Chabukswar and Deodhar 2006). In another study, *in vitro* efficiency of proliferation of a *Prunus* rootstock was 70 % higher when mother plants were tissue culture-propagated compared with mother plants that were propagated from traditional stem cuttings (Andreau and Marín 2005). Thus, choice of mother plant origin and tissues used for cutting production are crucial in the achievement of better efficiency in the vegetative propagation of plants. The use of either tissue-cultured mother plants or plants of the first generation after tissue culture would probably help maintain a high rooting percentage in cutting offspring. Thus, the use of roots from first generation plants after tissue culture may be ideal for cutting production. However, in experiments with *Prunus microcarpa*, Nas et al. (2010) did not obtain *in vitro* shoots originating from explants of roots, indicating the difference between species and perhaps also between closely related cultivars within species. Our results show that cutting the roots into small pieces does not stimulate auxiliary or adventitious buds on the roots to break and produce more root cuttings. However, removing thin and unproductive roots can make space for more productive roots, yielding more cuttings per unit greenhouse area.

Medium and soil temperature

The highest number of cuttings was produced by roots with only a thin layer of soil on top of the cutting-producing roots, presumably since the cuttings spent less time penetrating 3.5 rather than 7.0 cm of soil. However, in order to maintain moisture and avoid drought stress in the sensitive roots, the layer must not be too thin. A thickness of 3.5 cm was sufficient under the conditions tested here. The experiments showed that a thinner layer of medium on top of the roots also gave the shortest and most compact cuttings. These cuttings were easy to handle and was less prone to damage than the longer cuttings.

The unfertilised peat gave the highest number of cuttings. The higher salt concentrations in the fertilised peat, compared to the unfertilised, may have inhibited shoot formation, possibly through damaging the sensitive new tissues of shoots emerging from buds on the roots. The addition of 25 % Perlite increased the number of shoots produced, suggesting that the dilution of nutrients and/or the effect on soil physical factors was positive. No interaction was observed between the medium and the thickness of the peat layer covering the roots during cutting production. However a thick layer of fertilised peat is obviously not favourable for cutting production.

Soil temperature did not affect the production of cuttings significantly, except for a small decrease in cutting quality at the lowest temperature. However, both the low (20 °C) and the high (25 °C) temperatures are probably close to the optimum temperatures used for plant growth and plant propagation of many temperate zone plants.

The re-use of roots for a second season could have saved costs in cutting production. The results confirmed our hypothesis that translocation of resources from shoots to roots before overwintering is important for cutting production in the following season. However, this method yielded far too few cuttings and is therefore not of interest.

Rooting of cuttings

Since the highest soil temperature (25 °C) only gave 5 % higher rooting percentage than the lower (20 °C) temperature (data not shown), this factor does not seem to be very important for propagation of cuttings from roots. The largest cuttings rooted in a lower percentage and decreased plantlet quality after rooting compared with smaller cuttings (Table 5). The reason could be related to the larger mass of tissue, which may respire more than the more compact cuttings with the same number of leaves. Furthermore, the long cuttings broke easily on handling. The results suggest that cuttings of high quality will root well even if the environment changes, whereas lower quality cuttings need more optimal conditions. Short and compact cuttings are most suitable in the propagation of apple rootstock M9.

When applying growth regulator (NAA) to the cuttings, the mean increase in rooting was about 10 % (not statistically significant). However, other studies show that especially the first stage of the rooting process is promoted by auxin and that root development may actually be inhibited by the continued presence of auxin in the medium after the initial induction phase has been passed (Nalja et al. 2008). In our experiment, we used an auxin powder that may have been active even after the induction of rooting,

thus inhibiting root development. Rooting of apple rootstock MM 106 from tissue cultures was not initiated unless the cuttings were exposed to the auxin IBA (Naija et al. 2008), but for tissue culture-propagated cuttings of *Rosa hybrida,* the rooting did not depend on an exogenous supply of auxin (Ibrahim and Debergh 2001).

Our results indicate that the juvenile plant material we used did not need an external supply of auxin for the rooting process. The reason for the small or non-existent effect of an external supply of auxin was not studied, but could be related to enough auxin being present in the plant tissues. The emerging roots may also have been inhibited by high salt concentrations. Optimising the use of the growth regulator and a better suited growth medium may have yielded even better results than those reported here. However, the rather rough method we used in this part of the study is close to that used in practice. Robustness of the propagation system is an important factor, indicating that the method is likely to succeed.

Conclusions

This study examined whether roots of apple rootstock M9 'Lancep' were good sources of cuttings. The starting hypothesis that root quality and soil conditions affect the production of cuttings from root tissues was confirmed, but the hypothesis that the success of rooting cuttings is promoted by an external supply of hormones and soil temperature conditions was only partly confirmed. Roots of M9 'Lancep' yielded a high number of cuttings, and thick (5–10 mm) and relatively long (uncut) roots produced more cuttings than thinner roots cut into short pieces. A high percentage of cuttings rooted easily in peat with 25 % Perlite, but cuttings should be of the best quality, *i.e.* short and compact. We concluded that easily rooted cuttings can be produced efficiently from maternal roots of apple rootstock M9 'Lancep'. Further work should focus on optimisation of root production, either in the field or, if climate is a limiting factor, in polytunnels. The treatment with the highest number of cuttings yielded about 85 cuttings per box and 8 boxes can be accommodated per m^2. By fine-tuning the choice of root materials, growing medium and conditions during the rooting stage, even higher cutting production rates may be achieved in the propagation of M9 'Lancep'.

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