



THE EFFECT OF THE FERTILISATION SCHEDULE DURING THE PROPAGATION PERIOD OF THE WITCH-HAZEL (*HAMAMELIS MOLLIS* OLIV. EX FORB. & HEMSL.) ON THE SUCROSE PROFILE: THE RELATION WITH HARDENING

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Abstract

The problem of the first overwintering of freshly rooted cuttings is a serious one in many woody species, and in various witch-hazel species. The fertilisation strategy which affects the hardening of cuttings is very important. Cuttings of *Hamamelis mollis* Oliv. ex Forb. & Hemsl. were cut in the middle of June 2009 in Ljubljana on 20 years old stockplants and were immediately inserted in the peat : sand mixture (1:1; v/v). Different fertiliser strategies were conducted. Beside the control variant (no fertiliser was added), additionally three fertilised variants were studied. Cuttings were inserted in four replicates in each fertiliser variant. The experiment was set in an unheated plastic house with the fog system. The sucrose accumulation in cuttings and leaves was different during the propagation period. The lowest sucrose amounts, between 0.2 and 0.3 mg g⁻¹ fresh weight (FW) were measured in cuttings and leaves in August and the highest, between 0.4 and 0.85 mg g⁻¹ FW in cuttings and leaves toward the end of the propagation season, in September and October. There were no significant differences in sucrose concentration among fertiliser variants. Rooting success before and after the winter period differed considerably, between 50 and 95% especially in the control variant; it was more uniform in all fertilised variants, reaching average values around 80%.

Key words: leafy cuttings, substrate, winter surviving

INTRODUCTION

Witch-hazel (*Hamamelis*) is a well known genus of shrubs, the ornamental value of which is especially pronounced in spring when the plants begin to flower, from the end of January to the end of March. Production of these plants follows the strategy of other groups of shrubs. This holds true for all production phases, including the propagation phase. We can propagate these plants using different methods developed for woody species, like cuttings, layers, mound-layering, and grafting. Leafy cuttings are often used to propagate different witch-hazel species. Plietzsch (1993) demonstrated that among different species *Hamamelis virginiana* cuttings rooted better if they had been treated with auxin before insertion into the substrate. Jacob et al. (1991) succeeded to improve rooting in *Hamamelis virginiana*, with adding *Bacillus subtilis* to the substrate mixture.

One of the main problems posed to successful plant production is the overwintering period of the rooted plants, which is very problematic in witch-hazel, regard-

less of the propagation method used. There are frequent reports about witch-hazel species, which can be rooted very well but the rooted cuttings survive only the first winter with great losses (Spethmann 1997). Nair et al. (2008) showed with just rooted cuttings of *Stewartia pseudocamellia* that they survived winter most successfully when the growing conditions (substrate mixture) during rooting were optimal. A woody plant is not able to acquire tolerance to lower temperatures at every phase of its phenological cycle but the process of hardening has to be finished before winter starts. The hardening is characterised by accumulation of sugars and other protective substances, which make cells to become less turgid (Larcher 2003).

Sugar synthesis and their accumulation during the propagation process are important for carbohydrate synthesis. Carbohydrates play an important role in the process of adventitious rooting (AR). As primary metabolites they are important for cutting growth during the propagation season and, based on their involvement

in the hardening of cuttings, are consequently also important for cutting survival during the winter period. Numerous reports (Spellerberg 1986, Veierskov 1988, Druege 2009) have discussed the relationships between the carbohydrate status in cuttings and different sub-phases during AR. They all emphasized that the formation of roots and their growth is tightly dependent on the carbohydrate status. Carbohydrates are carriers of the assimilated C, which is used for the root formation and for forming the structural compounds that are necessary in the process of building up the cell wall during the formation of roots (Druege 2009). Moreover, some reports also recognise carbohydrates as the potentially necessary factor in the root initiation phase as energy and C providers (Veierskov 1988).

Carbohydrates are not only important as providers of energy and C-skeletons for other essential organic compounds formation needed for AR but also have a crucial role to properly prepare plants for the winter period (Veierskov 1988, Larcher 2003, Druege 2009). Therefore, the propagation strategy may not be directed only to root formation but has to enable the rooted plant to form enough essential sugars as well. In the case of cuttings, their surrounding has to minimise stress after their severance to enable photosynthesis also during the propagation process. This can be achieved only by including an appropriate irrigation system (Štefanič et al. 2008). Optimal photosynthesis results in higher concentration of primary metabolites (carbohydrates), which enhance stronger cutting growth during the propagation season. As Spellerberg (1986) has already postulated with different plant species, stronger growth of cuttings reduced losses of cuttings during the subsequent winter. The fertilisation during the propagation period is also crucial for the appropriate growth of cuttings and sugar synthesis. Numerous experiments cover the fertilisation topic in cuttings. MacCarthaigh and Eble (1989) described the importance of adding a slow release fertiliser to the rooting substrate to reduce the problems caused by the loss of nutrients as a result of the leaching effect. Carney and Whitcomb (1983) also emphasised the importance of adding a slow release fertiliser to the rooting substrate to enhance the growth of cuttings, except the potassium fertilisers which did not cause stronger growth. They also showed that stronger fertilisation of the rooting substrate resulted in higher concentrations of added nutrients (N, P_2O_5 , K_2O) in the cutting tissues. In terms of winter survival rates, this is especially important in the case of potassium, which strengthens cellular membranes (Larcher 2003).

Although the cutting propagation method has been improved substantially in the past years, successful rooting and overwintering of the witch-hazel are still an open issue. The aim of the research was to study the overwintering success based on different fertilisation schedules of cuttings during the propagation period.

MATERIALS AND METHODS

Plant material

The cuttings of *Hamamelis mollis* were harvested from 20-years old stockplants in the University Botanic Gardens in Ljubljana, Slovenia. The harvest took place on 18th of June 2009 and the material was adjusted to 12 cm long leafy cuttings with apical meristems. On average, cuttings had three to four fully developed leaves.

Further they were treated with 0.5% indole-3-butyric acid (IBA) + 10% Euparen on talcum basis prior insertion into the peat/sand (1:1; v/v) substrate mixture. Before insertion of the cuttings, rooting substrate was fertilised using a slow-release fertiliser. The effect of different N concentrations and different N/K ratios were studied, resulting in four different variants:

Variant 1: no added fertiliser (control variant)

Variant 2: 0.2 g pure N l⁻¹ substrate (1.25 g l⁻¹ 3-4M Osmocote® Exact 16+11+11+3 Mg+Te)

Variant 3: 0.2 g pure N l⁻¹ substrate (1.80 g l⁻¹ 3-4M Osmocote® Exact 11+11+18+3 Mg+Te)

Variant 4: 0.4 g pure N l⁻¹ substrate (3.60 g l⁻¹ 3-4M Osmocote® Exact 11+11+18+3 Mg+Te)

The pH of substrate mixture was adjusted to 4.0 with lime. All fertiliser treatments were replicated 4 times with 19 cuttings per plot: 15 cuttings for rooting evaluation + 4 cuttings for sucrose analyses.

The experiment was carried out in an unheated plastic house under a fogging system (Plantfog-Befeuchtungsanlagen Nebelsysteme, Fishamend, Austria). The daily air temperature in the house fluctuated substantially (up to 50°C during daytime and between 18 and 20°C during the night). The substrate temperature (rooting zone) did not show such oscillations (between 20 and 24°C), mainly due to the qualitative fogging system (oscillations of air humidity were reduced). Fogging was regulated manually to obtain a 90-95% relative humidity, on average. Fogging intervals lasted approx. 30 s, with a 60 s pause. Fogging was not carried out during the night (19.00 - 07.00 h). The pause intervals were extended to late August and fogging was stopped completely in late September.

In winter, cuttings were left without heating in the place of rooting in plastic house. The rooting of cuttings was evaluated on three different dates, which were recognised as sampling dates. Experiment evaluation was done on the pre-dormancy phase (November 26, 161 days after severance), on the true dormancy phase (January 12, 208 days after severance), and on the post dormancy phase (February 25, 252 days after severance) to evaluate the whole winter effect (Larcher 2003). On the first two dates three cuttings per replicate (4 replicates, 12 cuttings per variant) were measured, whereas the rest of cuttings (9 cuttings in 4 replicates, 36 cuttings per variant) were measured on the spring evaluation date. The rooting rate (%), number of first order roots, and the root system length were measured.

Extraction and analysis of sucrose

Sucrose was analysed in cuttings five times during the propagation period (18 June - the severance date, July 16, August 13, September 10, and October 08) to evaluate the flux of primary metabolites in the cuttings. Samples, one cutting per fertiliser variant, were analysed for their sucrose content levels. Two different tissues of each cutting, leaves and stems, were included into analyses. Between 0.3 g and 2.0 g of frozen material was ground to powder using mortar and pestle. Plant material was immersed in 3 to 20 ml of double distilled water (depending on the amount of plant material at the beginning). Samples were left for extraction for half an hour at room temperature with frequent stirring. Afterwards, the extracted samples were centrifuged at 10.000 g for 7 min at 10°C (Eppendorf Centrifuge 5810R, Hamburg, Germany). The supernatants were filtered through a 0.45 µm filter (Macherey-Nagel), transferred to a vial and stored at -20°C until analysis using high-performance liquid chromatography (HPLC; Thermo Scientific, Finnigan Spectra System, Waltham, MA, USA). For each analysis, 20 µl of sample was used. The analysis of sugars was carried out using a Rezex-RCM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, CA) with a flow rate of 0.6 ml min⁻¹ and with column temperature maintained at 65°C. For the mobile phase, in the HPLC-system double distilled water was used, and a refractive index (RI) detector for identification. The concentrations of carbohydrates were calculated with the help of corresponding external standards.

Statistical analysis

Statistical analysis was carried out with the Statgraphics Plus statistical program (version 4.0). The experiment was analysed using two-factor design of ANOVA with fertiliser variant and sampling date as factors. In the case of sugar measurements, the sampling date was the date of sampling the cuttings from the propagation bed. Statistically significant differences among treatments were tested with the Duncan test at $\alpha = 0.05$.

RESULTS

Average rooting success in all fertilisation variants was very high, reaching values between 50 and 100% (Fig. 1). It did not differ considerably among the different sampling dates. In cuttings where no fertiliser was applied, rooting success varied substantially, ranging between 50 and 95% at different sampling dates. Cuttings rooted and grown in fertilised substrate rooted more equally but the differences were not significant. Fertilised variants showed regarding root quality (number and length of roots) better results again and cuttings fertilised with 0.2 g N l⁻¹ substrate (using 11-11-18 fertiliser) formed longer roots than those in the control variant and fertilised with 0.2 g N l⁻¹ substrate

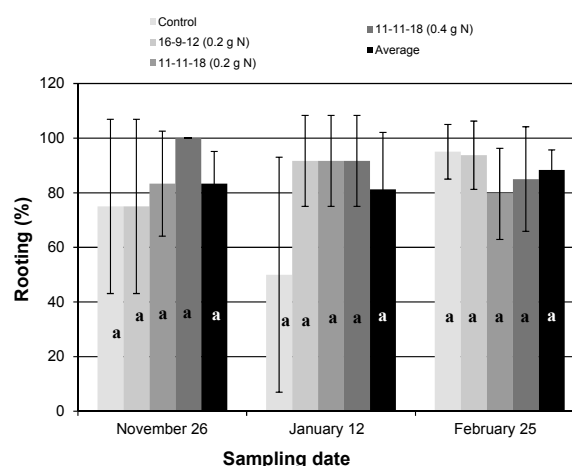


Fig. 1. Effect of rooting substrate fertilisation on rooting of *Hamamelis mollis* cuttings. The means (\pm SD) indexed with the same letters are not significantly different ($p \leq 0.05$, Duncan-test).

(using 16-9-12 fertiliser). Rooting heterogeneity of the control cutting was evidently again (Table 1).

The survival of cuttings was excellent in the first phase of winter (100% survival), later the survival decreased to values between 47.22 and 58.33%. There were no significant differences in the survival rates among different fertilisation variants (Table 2).

Regardless of the fertilisation strategy used, the sucrose concentrations in cuttings reached values around 0.6 mg g⁻¹ cutting one month after inserting cuttings in the substrate (Fig. 2). This means that in the first month the values remained at the same level as they were on the date of severance, on June 18. Later, sucrose values decreased, dropping to 0.2 and 0.3 mg g⁻¹ cutting in August and September. Sucrose concentrations in cuttings increased again toward the end of the propagation period, with values between 0.4 and 0.85 mg g⁻¹ cutting. Different fertilisation variants did not show any significant differences in sucrose concentrations;

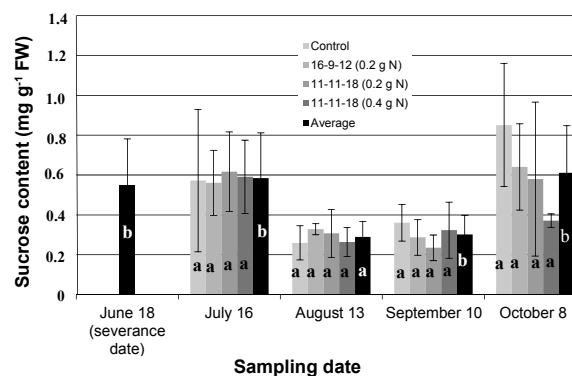


Fig. 2. Effect of rooting substrate fertilisation on sucrose content in cuttings of *Hamamelis mollis*. The means (\pm SD) indexed with the same letters are not significantly different ($p \leq 0.05$, Duncan-test).

Table 1. The number of main roots and the length of the root bush of *Hamamelis mollis* cuttings in different fertilisation variants at different sampling dates.

	November 26		January 12		February 25		Average	
	Number (n)	Length (cm)	Number (n)	Length (cm)	Number (n)	Length (cm)	Number (n)	Length (cm)
Control	10.5 ± 8.1 a	11.5 ± 7.7 a	5.3 ± 8.1 a	6.7 ± 8.2 a	20.3 ± 25.6 a	10.3 ± 8.0 a	12.0 ± 13.9 a	9.5 ± 8.0 a
NPK 16-9-12 (0.2 g N l ⁻¹)	12.1 ± 10.6 a	7.6 ± 7.3 a	16.2 ± 10.5 a	12.9 ± 7.8 ab	15.2 ± 14.1 a	14.2 ± 9.5 a	14.5 ± 11.8 a	11.6 ± 8.2 a
NPK 11-11-18 (0.2 g N l ⁻¹)	15.1 ± 11.5 a	13.8 ± 7.2 a	14.4 ± 10.3 a	19.0 ± 10.0 b	19.6 ± 16.5 a	14.1 ± 9.7 a	16.4 ± 12.8 a	15.7 ± 9.0 b
NPK 11-11-18 (0.4 g N l ⁻¹)	20.8 ± 20.6 a	13.3 ± 4.3 a	21.9 ± 23.6 a	12.6 ± 8.1 ab	9.9 ± 8.2 a	11.9 ± 7.8 a	17.5 ± 17.5 a	12.6 ± 6.7 ab

Means (± SD) indexed with the same letters are not significantly different ($p \leq 0.05$, Duncan-test).

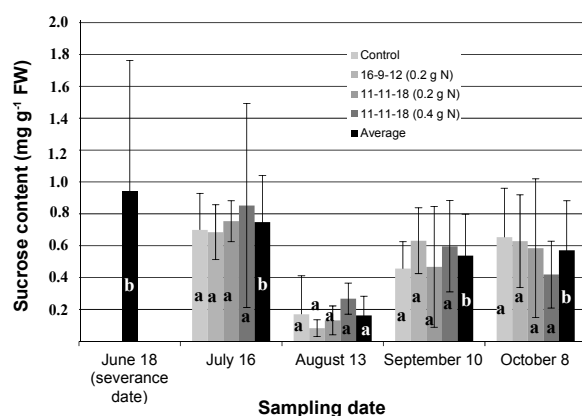


Fig. 3. Effect of rooting substrate fertilisation on sucrose content in cutting leaves of *Hamamelis mollis*. The means (± SD) indexed with the same letters are not significantly different ($p \leq 0.05$, Duncan-test).

however, the fall in the concentration in the middle of the period was significant.

The highest sucrose concentrations in leaves were measured on the severance date and one month later, on July 16, and reached between 0.6 and 0.8 mg g⁻¹. At the end of the propagation season, in September and October, the values ranged between 0.4 and 0.6 mg sucrose g⁻¹ (Fig. 3). A significant fall in sucrose concentration

was noticed in August with values below 0.25 mg g⁻¹. Again, fertilisation variants did not show any difference.

DISCUSSION

AR is a process whose duration is generally not highly dependent on particular species. It lasts, in optimal conditions, between 3 and 5 weeks. The first four days after inserting cuttings to the substrate can be divided into the root induction and root initiation phase, which are followed by the root development phase after day four. It has already been documented that during the root initiation phase (a few days after severance), a decrease in carbohydrate content in cuttings often occurred (Veierskov 1988). In addition, Spellerberg (1985) showed in his experiment with *Acer palmatum* 'Atropurpureum' and *Prunus triloba* that sucrose concentrations in cuttings decreased after severance during the propagation season. Concentrations of both monosaccharides, glucose and fructose, increased in the same experiment during root formation. Our results with *Hamamelis mollis* showed the same sucrose decrease in cuttings during the first months after the severance of cuttings. Cheffins and Howard (1982) also found a decrease of carbohydrates in apple rootstock cuttings during the first month after the severance, especially in cuttings which rooted successfully. The decrease was especially strong at the base of the

Table 2. The survival of cuttings at different times during the winter period for different fertilisation variants.

Fertilisation	November 26	January 12	February 25
Control	100.0 ± 0.0 a	100.0 ± 0.0 a	47.2 ± 10.6 a
NPK 16-9-12 (0.2 g N l ⁻¹)	100.0 ± 0.0 a	100.0 ± 0.0 a	52.8 ± 10.6 a
NPK 11-11-18 (0.2 g N l ⁻¹)	100.0 ± 0.0 a	100.0 ± 0.0 a	58.3 ± 10.6 a
NPK 11-11-18 (0.4 g N l ⁻¹)	100.0 ± 0.0 a	100.0 ± 0.0 a	50.0 ± 19.2 a
Average	100.0 ± 0.0 a	100.0 ± 0.0 a	52.1 ± 12.8 a

Means (± SD) indexed with the same letters are not significantly different ($p \leq 0.05$, Duncan-test)

cuttings. In our experiment, the sucrose decrease was also present in cutting leaves one month after the cuttings had been set. Not only previous reports (Veierskov 1988) but also several new experiments reveal sucrose as the substance acting in sucrose-specific signalling pathways, which influence the plant growth regulator metabolism, transport and perception. Moreover, some of these reports reveal even that sugars act as signals in regulating adventitious rooting (Druege 2009). Cheffins and Howard (1982) also analysed the non-rooted apple rootstock cuttings, where an increase of carbohydrates was observed during the same time period.

The increase of sucrose toward the end of the propagation season is a reaction of the plant to the process of hardening, because sucrose is the main storing form for carbohydrates. From the overwintering point of view the strong sucrose accumulation at that time is positive. Spellerberg (1986) showed in *Prunus triloba* that an increase of carbohydrate concentration took place in the cuttings set to the propagation process earlier in the propagation season, compared to the cuttings set later. In his experiment, earlier severance of the cuttings also correspond with better overwintering results. Larcher (2003) described the accumulation of sugars as the main step in the pre-hardening phase in woody plants, which is crucial for good survival of plants during winter.

The strongest sucrose accumulation in cuttings before winter was evident in our variant where cuttings did not receive any fertilising before setting. These cuttings also showed the highest variation in overwintering results. For a more constant overwintering success, optimal growing conditions during rooting period are necessary. Nair et al. (2008) stressed the importance of optimal growing substrate during the rooting process. It is evident that beside carbohydrate accumulation, sufficient mineral nutrition is also necessary for a successful overwintering process. Our fertiliser variants affected root growth of cuttings and the most evident was the effect of the 11-11-18 fertiliser. Fertilising with a 0.2 g N l⁻¹ substrate had a significant effect on root system length. These results correspond with some other reports on substrate fertilising during the propagation period in the past, dealing with different species and genus *Rhododendron*, *Ilex*, and *Pyracantha* (Carney and Whitcomb 1983, MacCarthaigh and Eble 1989). On the other hand, the survival of cuttings decreased with the strongest rate in the last third of the winter period and there were no great differences among fertilisation variants. This clearly shows that the plant species itself also has a great effect on survival success (Spethmann 1997). Different N/K fertiliser ratios did not show any effect on sucrose status or overwintering results in our experiment.

Acknowledgements: The research reported in this paper has been supported by the Programme Horticulture No. P4-0013-0481 granted by the Slovenian Ministry of Higher Education, Science and Technology.

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