**THE EFFECT OF THE FERTILISATION SCHEDULE DURING THE PROPAGATION PERIOD OF THE WITCH-HAZEL (*HAMAMELIS MOLLIS*** [Oliv.](http://www.ipni.org/ipni/idAuthorSearch.do?id=7128-1&back_page=%2Fipni%2FeditSimplePlantNameSearch.do%3Bjsessionid%3DD541F2CDBBB6D4BDFA0999DB5C774DFA%3Ffind_wholeName%3Dhamamelis%2Bmollis%26output_format%3Dnormal) ex Forb. & [Hemsl.](http://www.ipni.org/ipni/idAuthorSearch.do?id=3825-1&back_page=%2Fipni%2FeditSimplePlantNameSearch.do%3Bjsessionid%3DD541F2CDBBB6D4BDFA0999DB5C774DFA%3Ffind_wholeName%3Dhamamelis%2Bmollis%26output_format%3Dnormal)**) ON THE SUCROSE PROFILE: THE RELATION WITH HARDENING**

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**Abstract**

The problem of the first overwintering of just rooted cuttings is a serious one in many woody species, also in various witch-hazel species. The fertilisation strategy which affects the hardening of cuttings is very important. Cuttings of *Hamamelis* *mollis* [Oliv.](http://www.ipni.org/ipni/idAuthorSearch.do?id=7128-1&back_page=%2Fipni%2FeditSimplePlantNameSearch.do%3Bjsessionid%3DD541F2CDBBB6D4BDFA0999DB5C774DFA%3Ffind_wholeName%3Dhamamelis%2Bmollis%26output_format%3Dnormal) ex Forb. & [Hemsl.](http://www.ipni.org/ipni/idAuthorSearch.do?id=3825-1&back_page=%2Fipni%2FeditSimplePlantNameSearch.do%3Bjsessionid%3DD541F2CDBBB6D4BDFA0999DB5C774DFA%3Ffind_wholeName%3Dhamamelis%2Bmollis%26output_format%3Dnormal) were cut in the middle of June 2009 in the University Botanic Gardens 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 for which no fertiliser was added, also the variant of 0.2 g pure N l-1 substrate (using 3-4M Osmocote® Exact 16+11+11+3 Mg+Te), the variant of 0.2 g pure N l-1 substrate (using 3-4M Osmocote® Exact 11+11+18+3 Mg+Te) and the variant of 0.4 g pure N l-1 substrate (using 3-4M Osmocote® Exact 11+11+18+3 Mg+Te) were used. 19 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 cutting stems 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 stems and leaves in August and the highest, between 0.4 and 0.85 mg g-1 FW in stems 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, fertilising, winter surviving, *Hamamelis mollis*

**Introduction**

Witch-hazel(*Hamamelis*) is a well known genera of shrubs the ornamental value of which is especially fulfilled in spring when these plants begin to flower, from the end of January to the end of March. The 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, cuttings, layers, mound-layering and of course grafting. Leafy cuttings were often used to propagate different witch-hazel species. Plietzsch (1993) demonstrated that among different species also *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 different species, among them also in *Hamamelis virginiana*, with adding *Bacillus subtilis* to the substrate mixture.

The main problem corresponds to the overwintering period of the rooted plants, which is very problematic in witch-hazel regardless of the propagation method used. Twenty five years ago Spellerberg (1986) first made it official that in the propagation process, and in cutting propagation in particular, the problem of the first overwintering of the rooted plants as important as the rooting of cuttings. Ten years later overwintering was described as the most important factor in propagation, just like the physiological character of stockplants or the time of cutting severance (Spethmann 1997). Nevertheless, overwintering remains an open issue in many cases, such as in genera *Hamamelis*. There are frequent reports about witch-hazel species, which can be rooted very well but the rooted cuttings only survive the first winter with great looses (Spethmann 1997). The question how well a just rooted plant can overcome the winter period is the same as in open-field grown plants. For these plants winter survival rates closely depend on their growing conditions in the year before winter. 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 stage of its phenological cycle but the process of hardening has to be finished before winter starts. The hardening is characterised through the accumulation of sugars and other protective substances which causes 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 of 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 dependant 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 player in the root initiation phase as energy and C providers (Veierskov 1988).

Carbohydrates are not only important as providers of the 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, the cutting 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 (Štefančič et al. 2008). Optimal photosynthesis resulted in higher concentration of primary metabolites (carbohydrates), which enhanced stronger cutting growth during the propagation season. As Spellerberg (1986) has already postulated with different plant species, stronger growth of cuttings reduced looses of cuttings during the subsequent winter. The fertilisation of cuttings during the propagation period is also crucial for the appropriate growth of cuttings and sugar synthesis. Numerous experiments cover the fertilisation topic in cuttings. Mac Carthaight and Eble (1989) described the importance of adding a slow release fertiliser to the propagation substrate to reduce the problems caused by the loss of nutrients as a result of the leaching effect. Carney and Whitcomb (1982) also emphasised the importance of adding a slow release fertiliser to the propagation media 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 media resulted in higher concentrations of added nutrients (N, P2O5, K2O) in the cutting tissues. In terms of winter survival rates, this is especially important in the case of potassium, which strengthens cellular membranes (Larcher 2001).

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. This paper presents the results of a study of 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 on 20 years old stockplants in the University Botanic Gardens in Ljubljana, Slovenia. The cuttings were harvested on 18th of June 2009 and adjusted to 12 cm long leafy cuttings with apical meristems. On average, the cuttings had three to four fully developed leaves.

The cuttings were treated with 0.5% IBA (indole-3-butyric acid + 10% Euparen on talcum basis) prior insertion into the peat/sand (1/1; v/v) substrate mixture. Before inserting the cuttings, the substrate mixture was fertilised using a slow-release fertiliser. Different N concentrations and different N/K ratios were tested in this experiment, therefore different variants were used:

1. variant: no added fertiliser (control variant)
2. variant: 0.2 g pure N l-1 substrate (1.25 g l-1 3-4M Osmocote® Exact 16+11+11+3 Mg+Te)
3. variant: 0.2 g pure N l-1 substrate (1.80 g l-1 3-4M Osmocote® Exact 11+11+18+3 Mg+Te)
4. variant: 0.4 g pure N l-1 substrate (3.60 g l-1 3-4M Osmocote® Exact 11+11+18+3 Mg+Te)

pH value of the substrate mixture was adjusted to 4.0 with lime. All fertiliser treatments were replicated 4 times with 19 cuttings (15 cuttings for rooting evaluation + 4 cuttings for sucrose analyses; the sucrose analyse followed on severance date and additionally on 4 different dates during propagation period) per plot.

The experiment was carried out in an unheated plastic house under a fogging system (Plantfog-Befeuchtungsanlagen Nebelsysteme, Fishamend, Austria). The daily air temperatures in the house fluctuated a lot (up to 50 °C during daytime and between 18 and 20 °C during the night). The substrate temperatures (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 in the same place in the plastic house without heating, where they had been rooted. Experiment evaluation followed in three different developmental states of the plants, in the pre-dormancy phase (26 November), in the true dormancy phase (12January) and in the post dormancy phase (25February) to evaluate the whole winter effect (Larcher 2001). On the first two dates three cuttings per replicate were measured, whereas on the spring evaluate date the rest of cuttings were measured. The rooting rate (%), the number of primary roots and the root bush length were measured.

***Extraction and analysis of sucrose***

Sucrose was analysed in cuttings five times during the propagation period (18 June – the severance date, 16 July, 13 August, 10September and 08 October) 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, leaves and stems, of each cutting were included into analyses. In the laboratory, 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, double distilled water was used, and an RI (refractive index) 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), using ANOVA. The experiment was analysed using two-factor design 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. The rooting of cuttings was evaluated on three different dates which were recognised as sampling dates in this case. Statistically significant differences among treatments were tested with the Duncan test at α = 0.05.

**Results**

Regardless of the fertilisation strategy used, the sucrose concentrations in cutting stems reached values around 0.6 mg g-1 stem one month after inserting cuttings in the substrate (Fig. 1). This means that in the first month the values remained at the same level as they were on the date of severance, on 18 June. Later, sucrose values decreased, dropping to 0.2 and 0.3 mg g-1 stem in August and September. Sucrose concentrations in cutting stems increased again toward the end of the propagation period, with values between 0.4 and 0.85 mg g-1 stem. Different fertilisation variants did not show any significant differences in sucrose concentrations; 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 16 July, and reached between 0.6 and 0.8 mg g-1 leaves. At the end of the propagation season, in September and October, the values ranged between 0.4 and 0.6 mg sucrose g-1 leaves (Fig. 2). A significant fall in sucrose concentration was noticed in August with values below 0.25 mg g-1 leaves. Again, fertilisation variants did not show any difference.

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 great differences in the survival rates among different fertilisation variants (Table 2).

Average rooting success in all fertilisation variants was very good, reaching values around 80% (Fig. 3). Average rooting success did not differ considerably among the different sampling dates. In cuttings where no fertiliser was applied, quite a wide range of rooting success was measured, ranging between 50 and 95% at different sampling dates. Cuttings which were rooted and grew in fertilised media rooted more equally but these differences were not significant. Rooting heterogeneity of the control cutting was also monitored by measuring the number of the main roots and their length (Table 1). Fertilised variants showed 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 (using 16-9-12 fertiliser).

**Discussion**

AR is a process the duration of which is generally not very dependent on particular species. It lasts, in optimal conditions, between 3 and 5 weeks. The first four days after inserting cuttings in the substrate can be divided into the root induction an 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, the glucose and fructose, increased in the same experiment during root formation. Our results with *Hamamelis mollis* showed the same sucrose decrease in cutting stems 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 in those stem sections which were nearer to the cutting base. In our experiment, the sucrose decrease was also present in cutting leaves one month after the cuttings had been set. Not only older literature reports (Veierskov 1988) but also several new experiments reveal sucrose as the substance acting in sucrose-specific signalling pathways, which influence the plant hormone metabolism, transport and perception. Moreover, some of these reports even reveal 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) could show in *Prunus triloba* that an increase of carbohydrate concentration took place in the cuttings which were set to the propagation process earlier in the propagation season compared to the cuttings which were set later. In this experiment, earlier severance of the cuttings also correspond with better overwintering results. Larcher (2001) described the accumulation of sugars as the main step in the pre-hardening stage in woody plants, which is crucial for good survival of plants during winter.

The strongest sucrose accumulation in cutting stems before winter was evident in our variant where cuttings did not receive any fertilising before setting. These cuttings also showed the strongest oscillations 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 media 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 showed a significant effect on root bush length. These results correspond with some other reports on substrate fertilising during the propagation period in the past (Mac Carthaight and Eble 1989, Carney and Whitcomb 1982). 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. More targeted experiments will be necessary in the future to study the combined effect of the increased absorbed K and sucrose status in plants on the overwintering results.

**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. Additionally, we would like to thank the whole team in the University Botanic Gardens for giving us the opportunity to harvest the cuttings.

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**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. The means (± SD) indexed with different letters indicate significant differences (*P* = 0.05, Duncan-test)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 26 November | | 12 January | | 25 February | | Average | |
| Number (n) | Length (cm) | Number (n) | Length (cm) | Number (n) | Length (cm) | Number (n) | Length (cm) |
| Control | 10.46±8.09 | 11.50±7.72 | 5.29±8.10 | 6.67±8.15 | 20.27±25.58 | 10.31±8.04 | 12.01±  13.92 **a** | 9.49±  7.97 **a** |
| NPK 16-9-12  (0.2 g N l-1) | 12.08±10.63 | 7.58±7.32 | 16.17±10.54 | 12.88±7.81 | 15.15±14.09 | 14.24±9.53 | 14.47±  11.75 **a** | 11.57±  8.22 **a** |
| NPK 11-11-18  (0.2 g N l-1) | 15.13±11.52 | 13.83±7.15 | 14.42±10.33 | 19.04±10.01 | 19.59±16.50 | 14.11±9.68 | 16.38±  12.78 **a** | 15.66±  8.95 **b** |
| NPK 11-11-18  (0.4 g N l-1) | 20.75±20.61 | 13.33±4.29 | 21.88±23.58 | 12.63±8.13 | 9.94±8.24 | 11.90±7.76 | 17.52±  17.48 **a** | 12.62±  6.73 **ab** |

**Table 2:** The survival of cuttings at different times during the winter period for different fertilisation variants. The means (± SD) indexed with different letters indicate significant differences (*P* = 0.05, Duncan-test)

|  |  |  |  |
| --- | --- | --- | --- |
|  | 26 November | 12 January | 25 February |
| Control | 100 ± 0 **a** | 100 ± 0 **a** | 47.22 ± 10.64 **a** |
| NPK 16-9-12  (0.2 g N l-1) | 100 ± 0 **a** | 100 ± 0 **a** | 52.78 ± 10.64 **a** |
| NPK 11-11-18  (0.2 g N l-1) | 100 ± 0 **a** | 100 ± 0 **a** | 58.33 ± 10.64 **a** |
| NPK 11-11-18  (0.4 g N l-1) | 100 ± 0 **a** | 100 ± 0 **a** | 50.00 ± 19.25 **a** |
| Average | 100 ± 0 **a** | 100 ± 0 **a** | 52.08 ± 12.79 **a** |

**Fig. 1:** The sucrose content in stems of *Hamamelis mollis* cuttings dependant on different fertilisation variants during propagation period 2009. The means (± SD) indexed with letters show statistic differences, different letters indicated significant differences (*P* = 0.05, Duncan-test)

**Fig. 2:** The sucrose content in leaves of *Hamamelis mollis* cuttings dependant on different fertilisation variant during propagation period 2009. The means (± SD) indexed with letters show statistic differences, different letters indicated significant differences (*P* = 0.05, Duncan-test)

**Fig. 3:** The rooting success of *Hamamelis mollis* cuttings dependent on different fertilisation variants at different sampling dates. The means (± SD) indexed with letters show statistic differences, different letters indicated significant differences (*P* = 0.05, Duncan-test).

Answers to the reviewers' comments

Add Reviewer 1.

* **There are problems with English language:** The new manuscript was corrected by the English professor.

Add Reviewer 2.

* **It should be distinguish between effects of fertilisation on time course and rooting quality and hardening:** The aim of this study (we added the aim in this new version of the manuscript) was to look the effects of fertilisation of cuttings during propagation period and their ability to overcome winter. There are some reports (Mbabu and Spethmann 2007) which stress the importance of potassium regarding this problem. Therefore we toook in our experiment two fertilisers with different potassium ratio.
* **Sucrose analyses were not carried out at the same time as rooting evaluation:** Meassuring of sucrose accumulation was used for study the accumulation of primary metabolites, therefore these analyses were carried out during propagation period. Rooting evaluation was used to evaluate the winter effects on rooting, therefore this evaluation was carried out during the period from autumn to spring.
* **We do not know at what time roots are formed:** This was not the aim of this study, therefore the speed of root formation was not put in the results. But we know well from other reports, that the formation of roots lasted between 3 to 5 weeks, also in Hamamelis (Spethmann 1997; Hartmann et al. 1997).
* **For overwintering there are no results shown:** the results were added to the new manuscript version.

Add Reviewer 3.

* **Some papers are missing:** we added Nair et al. (2008) which covered this topic.