EFFECT OF FERTILISATION SCHEDULE DURING PROPAGATION PERIOD OF WITCH-HAZEL (*HAMAMELIS*) ON SUCROSE PROFILE: RELATION WITH HARDENING

Gregor Osterc and Franci Štampar

University of Ljubljana, Biotechnical Faculty, Chair for Fruit Growing, Viticulture and Vegetable Growing, Jamnikarjeva 101, 1000 Ljubljana, Slovenia, Fax: +386-1-4201088, E-mail: gregor.osterc@bf.uni-lj.si

Abstract

The problem of the first overwintering of just rooted cuttings is a serious problem in many woody species, also in different witch-hazel species. The fertilisation strategy which affects hardening of cuttings is very important. Cuttings of *Hamamelis mollis* Oliv. ex Forb. & Hemsl. were cut in the middle of June 2009 in a Botanical garden 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 control variant where 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 fog system. The sucrose accumulation in cutting stems and leaves was different during 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 winter and after the winter period differed considerably, between 50 and 95% especially in the control variant, in all fertilised variants was more uniform, reaching average values around 80%.

Key words: Leafy cuttings, substrate, fertilising, winter surviving, fog system, *Hamamelis mollis*

INTRODUCTION

Witch-hazel (*Hamamelis*) is well known genera of shrubs which ornamental value is especially fulfilled in spring when these plants begin to flower, from end of January till end of March. The production of these plants follows the strategy of other groups of shrubs. This holds true for all production phases, included propagation phase. We can propagate these plants using different methods known for woody species, cuttings, layers, mound-layering and of course grafting. Very often leafy cuttings were used to propagate different witch-hazel species. Plietzsch (1993) demonstrated that among different species also *Hamammelis*

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 overwintering period of rooted plants which is very problematic in witch-hazel regardless propagation method used. Twenty five years ago Spellerberg (1986) firstly made official that in the propagation process, exactly in cutting propagation the problem of the first overwintering of rooted plants is not less important than rooting of cuttings. Ten years later overwintering was described as the most important factor in propagation, like physiological character of stockplants or the time of cutting severance (Spethmann 1997). Never less overwintering stays in many cases a great problem, like in genera *Hamamelis*. It was often reported about witch-hazel species which can be rooted very well but thereafter, rooted cuttings survived the first winter with great looses (Spethmann 1997). The question how good a just rooted plant can overcome the winter period is the same as it is in the case of open field growing plants. For these plants holds true that winter surviving closely depends on growing conditions in the year before winter. A woody plant is not able to acquire freezing tolerance 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 effect cells to become less turgid (Larcher 2003).

Kommentar [P1]: Is this a factor in your experiment?

Sugar synthesis and their accumulation during propagation process are important for carbohydrate synthesis. Carbohydrates have important role in the process of adventitious rooting (AR). Numerous reports covered relationships between carbohydrate status in cuttings and different sub-phases during AR in the past. Emphasised, the formation of roots and their growing is tightly dependant on carbohydrate status. Carbohydrates are carriers of assimilated C which is used for the root formation, they are used for forming the structural compounds which are necessary in the process of building up the cell wall during the formation of roots (Druege 2009). Moreover, some reports recognise carbohydrates also as potential necessary player in the root initiation phase as energy and C providers (Veierskov 1988).

Kommentar [P2]: You mentioned the hardening in the title only and so the information on adventitious rooting should be linked to hardening.

Kommentar [P3]: Which ones?

Carbohydrates are not only important as provider the energy and C-skeletons for other essential organic compounds formation needed for AR but also has a crucial role to prepare plants properly for winter period (Veierskov 1988, Larcher 2003, Druege 2009). Therefore, propagation strategy may not be directed only in root formation but has to make the rooted plant possible to form enough essential sugars. In the case of cuttings the cutting surrounding after their severance has to minimise the appearance of stress at cuttings to make able the process of photosynthesis also during propagation process. This can be achieved only through including appropriate irrigation system (Štefančič et al. 2008). On the other hand the fertilisation of cuttings during propagation period is crucial for appropriate growth of cuttings

Kommentar [P4]: Please show the relation between rooting and over wintering

and sugar synthesis. Numerous experiments cover the fertilisation topic in cuttings. Mac Carthaight and Eble (1989) described the importance of adding slow release fertiliser to the propagation substrate to lower the problems by cuttings caused by loosing of nutrients as a result of leaching effect. Carney and Whitcomb (1982) also emphasised the importance of adding slow release fertiliser to the propagation media to enhance growth of cuttings except potassium fertilising which did not cause stronger growth. They also showed that stronger fertilising the rooting media resulted in higher concentrations of adding nutrients (N, P_2O_5, K_2O) in cutting tissues. Regarding winter survive this is especially important in the case of potassium which strengthens cellular membranes (Larcher 2003).

MATERIALS AND METHODS

Plant material

The cuttings of *Hamamelis mollis* were harvested on 20 years old stockplants in a Botanical garden 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 slow-release type of 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) per plot.

The experiment was carried out in an unheated plastic house under a fogging system (Plantfog-Befeuchtungsanlagen Nebelsysteme, Fishamend, Austria). The air temperatures in

house differed strongly during the whole day (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 qualitative fogging system. 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.

Kommentar [P5]: What is that?

Cuttings stayed over the winter on the same place in the same plastic house without heating where they had been rooted. Experiment evaluation followed on three different developmental states of plants, on pre-dormancy phase (26th of November), on true dormancy phase (12th of January) and on post dormancy phase (25th of February) to evaluate also the whole winter effect (Larcher 2001). On first two dates always three cuttings per replicate were measured, whereas on spring evaluate date the rest of cuttings were measured. The rooting rate (%), the number of primary roots and the root bush length were measured.

Kommentar [P6]: But the sugar content was analysed at other dates? Why? Not mentioned in Material and Methods so far.

Extraction and analysis of sugars

Samples, one cutting per fertiliser variant, were analysed for their content levels of sucrose. Two different tissues, leaves and sprouts, of each cutting were included into analyses. In the laboratory, between 0.3 g and 2.0 g 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. The extracted samples were afterwards 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 sampling date was the date of sampling the cuttings from the propagation bed, 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 $\alpha = 0.05$.

Kommentar [P7]: In Table 1 also?

RESULTS

The sucrose concentrations in cutting stems reached independent on fertilisation strategy values around 0.6 mg g^{-1} stem one month after inserting cuttings in the substrate (Fig. 1). This means that the values stayed this first month on the same level as they were on the date of severance, on 18th of June. Sucrose values decreased later, in August and September to 0.2 and 0.3 mg g^{-1} stem. 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, the fall in concentration in the middle of the period was significant.

The highest sucrose concentrations in leaves were measured on severance date and one month later, on 16th of July, between 0.6 and 0.8 mg g^{-1} leaves. At the end of the propagation season, in September and October ranged values 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. Fertilisation variants did not show any difference again.

Average rooting success in all fertilisation variants was very well, reaching values around 80% (Fig. 3). Average rooting success did not differ considerable among different sampling dates. In cuttings, where no fertiliser was applied a quite width range of rooting success, between 50 and 95% at different sampling dates was measured. Cuttings which were rooted and grew in fertilised media rooted more equally. Anyway, these differences were not significant. Heterogeneously rooting of control cuuting was also observed by measurements of number of 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 control variant and fertilised with 0.2 g N l^{-1} substrate (using 16-9-12 fertiliser).

DISCUSSION

It has been already documented that during root initiation phase (few days after severance) often a decrease in carbohydrate content in cuttings occurred (Veierskov 1988). Spellerberg (1985) additionally 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 cutting stems during first months after severance of cuttings. Cheffins and Howard (1982) also found a decrease of carbohydrates in apple rootstock cuttings during first month after cutting 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 present also in cutting leaves one

Kommentar [P8]: That means only: the cuttings are photosynthetic active.

Kommentar [P9]: In accordance with light and clima conditions?

Kommentar [P10]: Is this difference important?

Kommentar [P11]: Is this also true for Hamamelis and when root formation in Hamamelis starts?

month after cutting setting. Not only older literature reports (Veierskov 1988) but also several new experiments reveal sucrose as substance acting in sucrose-specific signalling pathways, which influence 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) analysed also non-rooted apple rootstock cuttings, where an increase of carbohydrates were observed during the same time period.

Kommentar [P12]: We need information when root formation in Hamamelis starts

The increase of sucrose toward the end of the propagation season is a reaction of a 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 cuttings which were set to the propagation process earlier in the propagation season with regard to cuttings which were set later. Earlier severance of cuttings correspond in this experiment also with better overwintering results. Larcher (2001) described the accumulation of sugars as a main step in pre-hardening stage in woody plants which is crucial for good surviving of plants during winter.

Kommentar [P13]: You have protected conditions for your plants, what about the temperatures during autumn and winter?

The strongest sucrose accumulation in cutting stems before winter was evident in our variant where cuttings did not receive any fertilising before setting and these cuttings showed the strongest oscillating in overwintering results. It is evident that beside carbohydrate accumulation also sufficient mineral nutrition is necessary for positive overwintering process. Our fertiliser variants affected root growth of cuttings and the most evident was the effect of 11-11-18 fertiliser. Fertilising with 0.2 g N l^{-1} substrate showed a significant effect on root bush length. These results correspond with some other reports dealt with the substrate fertilising during propagation period in the past (Mac Carthaight and Eble 1989, Carney and Whitcomb 1982). Different N/K fertiliser ratio did not show any effect on sucrose status or overwintering results in our experiment. More target experiments will be necessary in the future to follow the combined effect of increased absorbed K and sucrose status in the plants on overwintering results.

Kommentar [P14]: No results shown

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