

## **Effect of environmental factors on the propagation of deciduous azalea by cuttings. I. Influence of stock plant management on rooting and carbohydrate status**

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### **Abstract**

The aim of this study was to estimate the effect of stock-plant forcing in a greenhouse during spring as a management technique for stock plants on rooting and on changes of carbohydrate contents of cuttings from deciduous azalea cultivar 'Polārzvaigzne' during the rooting period. Forcing of the stock plants enabled to start propagation of deciduous azalea by cuttings one month earlier. Conditions in the greenhouse promoted accumulation of carbohydrates in the shoots of forced stock plants compared to control plants. However, the stock-plant forcing affected neither the number of rooted cuttings nor the duration of time necessary for maximum rooting. The patterns of carbohydrate changes were similar in forced and control cuttings. The amount of reducing sugars, total soluble sugars, and starch increased significantly in the cuttings after severing, while the level of sucrose generally fluctuated over time. After root emergence the content of reducing sugars, sucrose, and starch decreased initially in the stem base and later in the leaves of cuttings. The content of starch was better related to the rooting process than the content of total soluble sugars.

**Key words:** adventitious root formation, *Rhododendron*, soluble sugars, starch, stock plant forcing, vegetative propagation.

### **Introduction**

*Rhododendron* L. is one of the most popular ornamental woody plant genera planted in parks and gardens. Despite the opportunities offered by new micropropagation techniques, cuttings are still widely used to propagate deciduous azalea cultivars. However, there are several problems with cuttings related to both the rooting process and their survival.

Management of stock plants is often used to improve the rooting potential of difficult-to-root species. Some of the most widely-used methods include reducing of irradiance or etiolation, modifying mineral nutrition, winter pruning, as well as combination of different methods (Howard 1994; Hartmann et al. 2002). Reduced irradiance results in some anatomical changes such as decreased lignification and reduced cell differentiation; thereby increasing initiation of root primordia (Ernsten, Hansen 1986; Bassuk, Maynard 1987; Maynard, Bassuk 1991; Hansen, Potter 1997; Voltolini, Fachinello 1997). Fertilisation of stock plants promotes the supply of cuttings with mineral elements, improving adventitious rooting, but often resulting in decline of survival (Henry et al. 1992; Rowe et al. 1999; Spanos et al. 1999). Stock plant pruning can help to maintain high rooting

potential due to promotion of juvenile-like growth of plants (Cameron et al. 2001). Also, the growing of plants in a greenhouse conditions is included in pre-treatment of the stock plants. The forcing of stock plants at higher-than-normal temperatures and humidity not only promotes shoots development and allows to start propagation by softwood cuttings earlier, but also affects the morphology and physiology of cuttings, promoting rooting and further development of cuttings (Samostchenkov 1985; Polikarpova, Pilugina 1991). However, generally the benefit from each treatment is genotype dependent.

Some studies have focused on changes of carbohydrate concentrations during rooting of tree cuttings (Welander 1994; Murai et al. 1999; Pellicer et al. 2000), due to their importance as a source of energy and building blocks of macromolecules during the root development (Dey, Harborne 1997; Heldt 2005). Woody plant species differ in their requirements for propagation conditions, in relation to the rooting process and physiological processes including carbohydrate metabolism (Haissig 1990; Welander 1994; Hartmann et al. 2002; Rowe et al. 1999). The management of stock plants can influence the carbohydrate status of cuttings as well (Rowe et al. 1999; Hoad, Leakey 1996). Hartmann et al. (2002) described the general characteristics of changes in carbohydrate levels during the root formation, as follows. Starch hydrolysis during rooting releases soluble sugars, which can be used for root formation. The bases of cuttings acts as sinks attracting assimilates. Application of auxins for stem cuttings enhances both adventitious root formation and the movement of assimilates towards the auxin source. Although carbohydrates are essential for root formation of cuttings, they are not the cause of rooting, nor do they initiate it. However no extensive research on the carbohydrate status in deciduous azaleas during rhizogenesis has been conducted.

The objective of this study was to estimate the influence of stock-plant forcing on adventitious rooting and on the carbohydrate status of deciduous azaleas cuttings. Cuttings from the cultivar 'Polārzvaigne' were evaluated for rooting and analyzed for concentrations of soluble carbohydrates and starch in the leaves and the stem bases of cuttings during the rooting period.

## Materials and methods

As stock plants 7-year-old bushes of deciduous azalea cultivar 'Polārzvaigne' (♀ *Rhododendron* × *kosterianum* C.K. Schneid. × ♂ *Rh. roseum* Rehd.) propagated by cuttings were used.

At the end of February 1999 thirty stock plants were potted individually in 40 l plastic pots with a medium consisting of equal volumes of peat and semi-decomposed pine needles and were placed into a greenhouse for forcing, without additional heating and light. As a control stock plants (n = 30) maintained outdoors in the bed were used. Forced cuttings (FC) from the forced stock plants were collected on 28 May 1999, while control cuttings (CC) from the bed stock plants were collected on 29 June 1999.

Softwood cuttings were trimmed 6 to 8 cm in length. Five or six leaves were left on each cutting. The leaves were trimmed to approximately one-third of the original leaf area. The base of each cutting was wounded by removing a 1.0 to 1.5 cm strip (in length) of bark on one side of the stem and then treated with 0.5 % (w/w) indole-3-butyric acid powder in talc. The cuttings were inserted in a plastic flat (30 × 60 × 10 cm) filled with a medium consisting of equal volumes of peat and semi-decomposed pine needles. A total of 1700

cuttings for each variant were planted in 34 flats (50 cuttings per a flat). The cuttings were placed under a polyethylene tent in a non-mist semi-shadow greenhouse ( $40$  to  $90 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) under natural photoperiod with temperatures of  $23 \pm 4 \text{ }^\circ\text{C}$  (day) and  $15 \pm 4 \text{ }^\circ\text{C}$  (night).

Cuttings were sampled for measurement at 3 or 4-day intervals within 14 weeks. Samples were taken in three replications between 10:00 and 11:00 hours. At each sampling, five cuttings were randomly selected. The leaves and the basal 2.0 cm segment of the stem were separately removed and used for analysis. The cuttings were considered as rooted if there was at least one root  $\geq 5$  mm long. Rooting percentage was calculated, as a proportion of all cuttings in the sample. The samples were rinsed with water, fixed in water vapour and dried at  $60 \text{ }^\circ\text{C}$  for 48 h. Dry leaves were milled by laboratory-mill and the stems were scrupulously cut in approximately 0.4 mm thin slices and bruised in the pestle together with granulated glasses to powdery consistence. The concentrations of reducing sugars, sucrose and starch were determined.

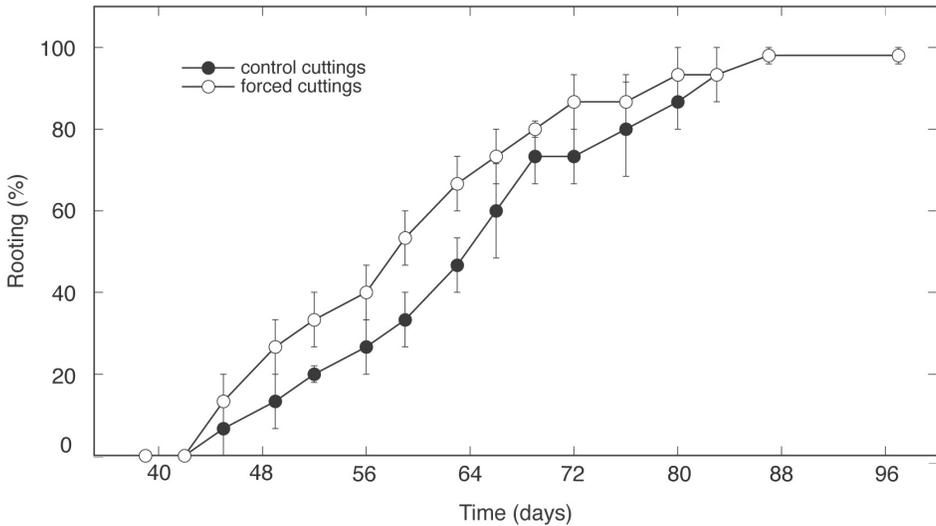
For carbohydrate analysis 0.25 g of leaf tissue and 0.10 g of stem tissue (dry mass) was used. To determine the concentrations of reducing sugars (glucose and fructose) and sucrose, dry plant material was ground with 10 ml 96 % (v/v) ethanol and 50 ml hot water was added. The extraction was performed in a water bath at  $75 \text{ }^\circ\text{C}$  for 45 min. After the extraction, the proteins, lipids and tannins were precipitated by alkaline 2 ml  $\text{Pb}(\text{CH}_3\text{COO})_2$  for 1 h in warm solution. To precipitate the remaining lead (Pb) ions, an equivalent amount of saturated  $\text{Na}_2\text{SO}_4$  was added. Then the solution was brought to 100 ml with distilled water and filtered. In the extracts the amount of reducing sugars was measured using the copper-iodometric titration method (Shaffer, Somogyi 1933; Strong, Koch 1974). For determination of sucrose, the total amount of sugars was measured first. Three ml of 8 % (w/v) oxalic acid was added to 25 ml filtrate. The solution was placed in a water bath at  $100 \text{ }^\circ\text{C}$  for 10 min to induce sucrose hydrolysis. After cooling, 1 N NaOH was used for neutralization and the solution was brought to 50 ml with distilled water. Then the total amount of sugars was determined as previously described for reducing sugars. The content of sucrose was calculated by subtracting the reducing sugar content from the total sugar content and multiplying the result by 0.95. To determine starch content, dry plant material was ground with 10 ml 80 % (w/v)  $\text{Ca}(\text{NO}_3)_2$  solution and boiled for 3 to 5 min to pass starch into the colloidal solution. The amount of starch was determined by the Berthram method of bichromate-sodium thiosulfate titration (Strong, Koch 1974). Carbohydrate concentration was expressed as a percentage of dry mass attributable to sucrose, reducing sugars and starch.

Statistical analyses were performed using SPSS 11.0 for Windows.

## **Results**

Forcing of the stock plants by planting them into a greenhouse enabled to start propagation of deciduous azalea by cuttings one month earlier – the control cuttings were collected for rooting on 29 June, and the cuttings from forced stock plants were already collected on 28 May.

There was no statistically significant effect of stock-plant forcing on rooting of cuttings (Fig.1). While the increase of rooting percentage tended to be slightly more pronounced in the forced cuttings, the first rooted cuttings in both variants were established only on



**Fig. 1.** Effect of stock plant forcing on rooting percentage of deciduous azalea cultivar 'Polārzaigzne'. Deciduous azalea cultivar cuttings from outdoor-raised stock plants (control cuttings) were collected on 29 June 1999, and cuttings from greenhouse-forced stock plants (forced cuttings) were collected on 28 May 1999. Mean values  $\pm$  SE are shown,  $n = 3$ . Root emergence was observed visually.

day 45. Also, maximum rooting (98 %) for forced and control cuttings was achieved nearly simultaneously (day 87). Thus, the stock-plant forcing affected neither the number of rooted cuttings nor the duration of time necessary for maximum rooting.

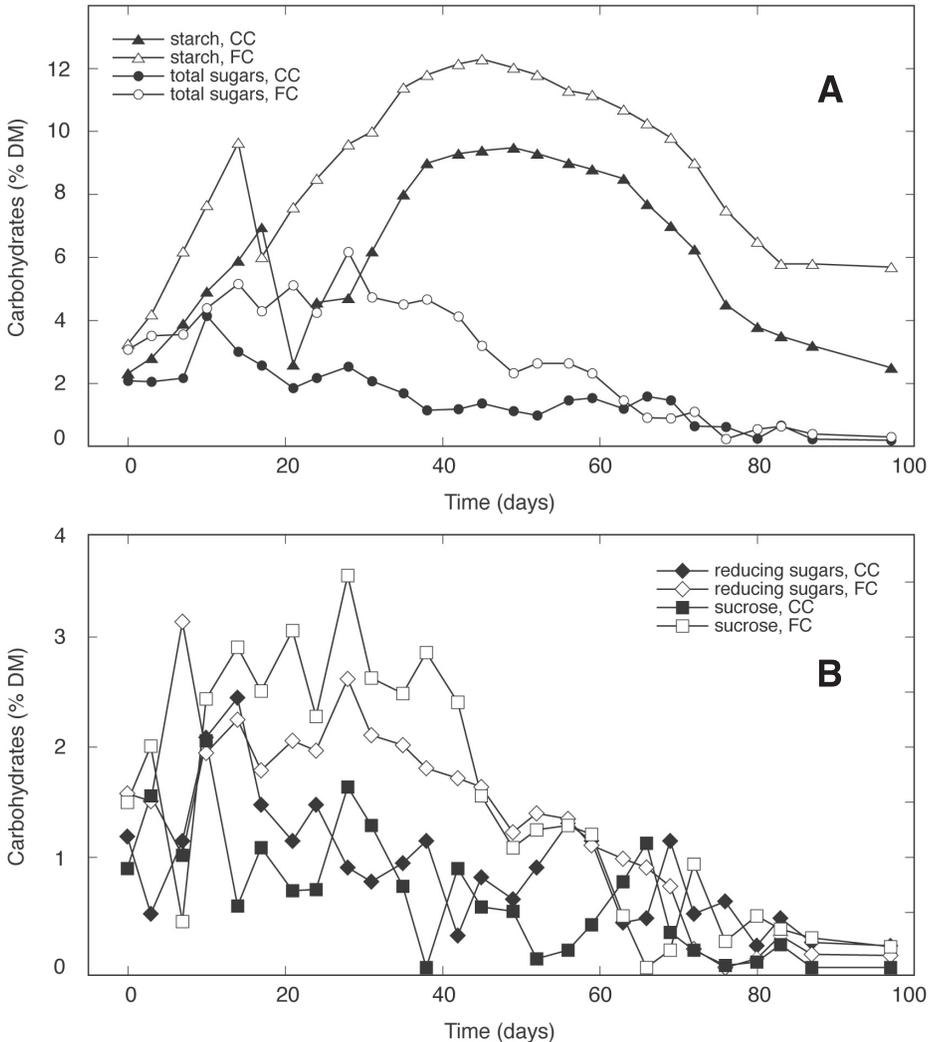
The different environmental conditions used for cultivation of stock plants influenced the amount of sugars and starch in shoots. Before day 0, the leaves of cuttings from forced stock plants contained more sugars than cuttings from control plants (Fig. 2 and 3). However, in the stem bases of forced cuttings all carbohydrates had higher amounts than in control cuttings.

Also, during the next three days the changes in contents of carbohydrates differed between the variants in the leaves of cuttings. The levels of all carbohydrates increased in the leaves of control cuttings, while in forced cuttings only the content of reducing sugars increased, but the level of sucrose and starch declined. Subsequently, until two weeks after propagation the starch level decreased in cuttings of both variants, while the amount of reducing sugars continued to grow.

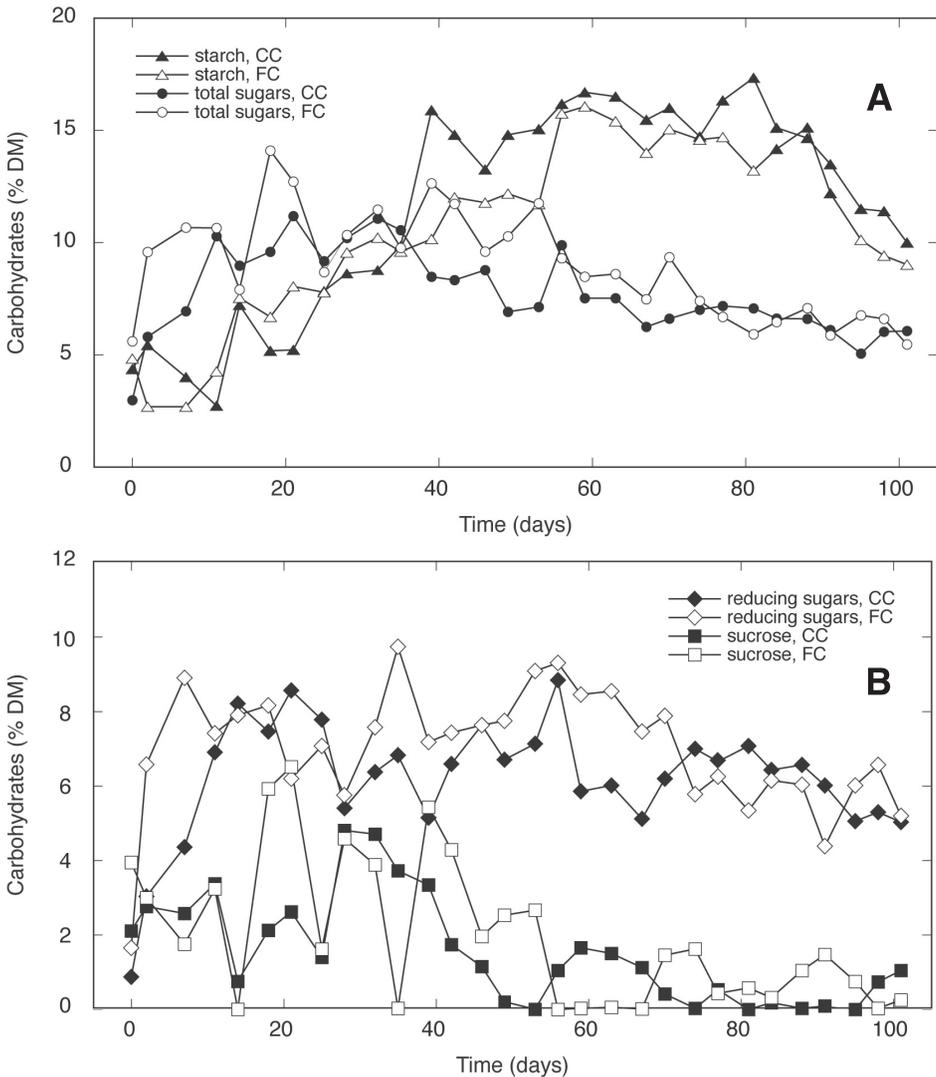
In total, during the following course of rooting the trends of changes in carbohydrate amounts were similar in the leaves and the stem base of both cutting variants. During the first half of the rooting period the amount of starch increased in both stems and leaves of azalea cuttings, followed by a decrease during the second half (Fig. 2A and 3A). The level of sugars continuously decreased after the initial stage of rooting in the stems of cuttings, reaching extremely low levels at the end of the experiment (Fig. 2A).

In general, both the amount of starch and total soluble sugars in the stems during rooting were higher in azalea cuttings taken from forced stock plants in comparison with control cuttings (Fig. 2A). The differences in soluble sugar content were due to both reducing sugars and sucrose (Fig. 2B).

In contrast, in the leaves of cuttings, the effect of stock-plant forcing was less pronounced (Fig. 3). During the first half of the rooting period not only the amount of starch, but also the level of reducing sugars and total soluble sugars, significantly increased in the leaves of azalea cuttings. During the period of intensive root formation (since the day when the first root emerged) the levels of all soluble sugars decreased. However, in leaves the decrease of sugars was considerably delayed and less dramatic than in the stem base of cuttings. In the leaves the decline of starch content started only at the end of experiment when maximal rooting was achieved.



**Fig. 2.** Changes of sugar and starch amounts in the 2-cm stem base of azalea cuttings during rooting. A, starch and total soluble sugars; B, reducing sugars and sucrose. Deciduous azalea cultivar 'Polärzvaigzne' cuttings from outdoor-raised stock plants (CC) were collected on 29 June 1999, and cuttings from greenhouse-forced stock plants (FC) were collected on 28 May 1999. Mean values are shown,  $n = 3$ .



**Fig. 3.** Changes of sugar and starch amounts in the leaves of azalea cuttings during the rooting period. A, starch and total soluble sugars; B, reducing sugars and sucrose. Deciduous azalea cultivar 'Polarzvaigzne' cuttings from outdoor-raised stock plants (CC) were collected on 29 June 1999, and cuttings from the greenhouse-forced stock plants (FC) collected on 28 May 1999. Mean values are shown,  $n = 3$ .

## Discussion

In the present study, stock-plant forcing enabled to start propagation by cuttings one month earlier. Similar results have been obtained with pear cultivars, apple rootstocks and others cultures (Tarasenko, Omeltchuk 1985; Polikarpova, Pilugina 1991). However, in a study with apple rootstocks, root formation in cuttings from forced stock plants

occurred within 14 days, while the cuttings of control variants rooted in 26 to 36 days. Furthermore, the forced cuttings often showed a higher percent of rooting than control cuttings (Polikarpova 1990). The results of the present study did not consistent with these results (Fig. 1). These differences can be explained by the fact that in the conditions of the present experiments, maximum rooting was achieved even with control plants. In previous studies, stock-plant forcing was observed to have a greater positive effect on the rooting of difficult-to-root plants (Polikarpova, Pilugina 1991).

Generally, the patterns of carbohydrate changes widely vary among the cuttings of different species. Similar carbohydrate levels as in the present study were found for leafy cuttings of *Rhododendron catawbiense* 'Roseum Elegans' (Davis, Potter 1987): in the base of cuttings both the starch and the sucrose amount increased much after excision and later declined during root development, but the glucose level fluctuated variably. Also, in experiments with red maple (*Acer rubrum* 'Red Sunset'; Smalley et al. 1991) the total soluble carbohydrate level rose, after an initial decrease, till the start of rooting and decreased afterwards, but, in this case, changes in the starch concentration did not demonstrate an evident pattern. In contrast, the level of reducing sugars in *Betula pubescens* cuttings was observed to remain nearly constant during rooting, although the sucrose amount increased by several times (Welander 1994).

In the present study, the differences in environmental conditions used for stock plant management significantly affected the initial levels of sugars and starch in azalea cuttings (Fig. 2, 3). Higher initial levels of carbohydrates in forced stock plants might reflect better conditions for photosynthesis in the greenhouse. It has been shown that leaves of forced shoots have several anatomical changes that positively affect photosynthesis (Polikarpova 1990). In plant leaves starch is deposited in the form of transitory starch, which is degraded during the following night (Heldt 2005). Since the samples in the present experiment were collected in the morning the observed level of starch in the leaves most probably reflects changes in the basal content of starch. It is possible that leaves of the forced stock plants accumulated more starch during a day than leaves of the control plants.

After detachment the shoots experience water stress, which may cause stomatal closure and subsequent reduction of photosynthesis (Smalley et al. 1991). This may explain the decline of starch concentration in the leaves during the first two weeks of rooting (Fig. 3A). The effect of stock-plant forcing on changes in carbohydrate levels during the first days of the experiment could be related to differences in the anatomical structure of shoots of forced stock plants. Growth under greenhouse conditions leads to a raised density of palisade parenchyma, volume of intracellular space as well as the number of stomata (Polikarpova 1990). These changes may result in greater sensitivity of forced cuttings to water stress and a subsequent decline of the starch amount, as well as an increase of the reducing sugar amount in leaves of these cuttings. In orange trees, it was observed that during water stress, the content of starch and sucrose decreased in leaves, while the content of reducing sugars slightly increased (Vu, Yelenosky 1989). The changes of carbohydrate levels in the leaves of forced cuttings found in the present study conform to these results.

After severing, cuttings produce wound periderm and callus, as well as form root initials and root primordia (Hartmann et al. 2002). These processes may help to explain the intensive utilization of starch in the stem base of cuttings from both variants during the third week of propagation. However, as the rooting of rhododendrons is a long process (Kondratovics, Megre 1999; Hartmann et al. 2002) an increase in the starch concentration

continues and a high level of reducing sugars remains in both leaves and the stem base of the cuttings (Fig. 2 and 3). The high accumulation of starch, particularly in the base of cuttings, suggests that the early stages of adventitious root development do not request large energy resource. The results indicated that, during the subsequent stage of root development, root formation was supported by starch accumulated in the basal part of the stem at first, and the starch pool in the leaves is used for further root development. Growing roots act as a sink for assimilates that are utilised very rapidly there. This can explain why all of the determined carbohydrate levels declined during intensive root development.

Newly synthesized carbohydrates are generally very important for root growth and development of leafy cuttings (Hoad, Leakey 1996; Pellicer et al. 2000). An optimal photosynthesis process is essential for woody plant cuttings with a long rooting period, to compensate the used carbohydrate store during the rhizogenesis period (Aminah et al. 1997; Pellicer et al. 2000). The results of our study also confirm these conclusions and suggest that the concentration of starch in cuttings could be used as an indicator of the rooting process.

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## **Vides faktoru ietekme uz vasarzaļo rododendru pavairošanu ar spraudņiem. I. Mātesaugu apstrādes ietekme uz apsakņošanas un ogļhidrātu saturu**

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### **Kopsavilkums**

Pētījuma mērķis bija novērtēt vasarzaļo rododendru mātes augu pavasara steidzināšanas siltumnīcā ietekmi uz spraudņu apsakņošanas un ogļhidrātu satura izmaiņām tajos apsakņošanās laikā. Mātes augu steidzināšana deva iespēju uzsākt pavairošanu mēnesi agrāk, nekā izmantojot kontroles augus. Attīstībai labvēlīgie apstākļi siltumnīcā veicināja ogļhidrātu uzkrāšanos steidzināto mātes augu dzinumos. Tomēr mātes augu steidzināšana būtiski neietekmēja ne apsakņoto spraudņu daudzumu, ne arī apsakņošanās procesam nepieciešamo laiku. Gan steidzinātajiem, gan kontroles spraudņiem ogļhidrātu satura izmaiņas apsakņošanās gaitā bija visumā līdzīgas. Spraudņos būtiski pieauga reducējošo cukuru, kopējais šķīstošo cukuru un cietes daudzums, bet saharozes saturs bija salīdzinoši svārstīgs. Reducējošo cukuru, saharozes un cietes daudzuma samazināšanos spraudņiem novēroja tikai pēc adventīvo sakņu parādīšanās – vispirms spraudņu pamatnē, bet vēlāk arī to lapās. Cietes satura izmaiņas spraudņos raksturoja spraudņu apsakņošanās procesu labāk nekā kopējā šķīstošo cukuru daudzuma izmaiņas tajos.