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Effect of environmental factors on the propagation of deciduous azalea by cuttings. II. Influence of an extended growth period on bud-break, overwinter survival and carbohydrate levels of rooted cuttings

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Abstract

The influence of stock-plant forcing in a greenhouse during spring, as well as an expanded photoperiod and ground heating during autumn and winter, on post-rooting growth and overwinter survival of cuttings was tested using cuttings from the deciduous azalea cultivar 'Polārzvaigzne'. The stock-plant forcing and extended lighting during the autumn supported the development of new shoots in the post-rooting period. During autumn, all starch stored in the leaves of cuttings was transported to perennial parts of plant. During winter, the use of starch depended on temperature. A high temperature (10 °C) promoted depletion of starch in the stem of cuttings if it could not be replenished. Additional light and heating promoted the retention of leaves and the photosynthesis ability of cuttings. Overwinter survival was affected by environmental conditions during winter mainly, while stock-plant forcing did not significantly influence survival. An extended light period together with additional heating in winter improved overwinter survival of cuttings, but delayed their further growth in spring..

Key words: carbohydrate, extended photoperiod, overwinter survival, *Rhododendron*, stock-plant forcing, vegetative propagation.

Introduction

One of the problems in practical vegetative propagation of woody plants is the loss of even successfully rooted cuttings during the first winter or spring. This is also true for many of the splendid cultivars of deciduous azaleas (Berg, Heft 1991). These problems also occur in other woody plant genera (*Acer* L., *Betula* L., *Cornus* L., *Corylopsis* L., *Hamamelis* L., *Magnolia* L., *Stewartia* L., *Viburnum* L.) propagated by cuttings (Smalley et al. 1987).

Poor overwinter survival may be caused by an insufficient level of stored carbohydrates in cuttings (Perkins, Bassuk 1995; Hartmann et al. 2002). Carbohydrate reserves depend on the initial level of carbohydrates at severing of cuttings, carbohydrate depletion or accumulation during rooting as well as on the ability to replenish them during the post-rooting period (Perkins, Bassuk 1995; Hoad, Leakey 1996). Hence, the post-rooting growth and development of new shoots during the current season is very important, because leaves of new shoots promote accumulation of photosynthates (Smalley et al. 1987). However, growth after rooting during the current season is not typical for rhododendrons.

To promote post-rooting growth, different methods including management of stock plants and various treatments of cuttings can be used. For example, light exclusion as pre-treatment of stock plants can affect bud break and subsequent growth. Stem banding (localized light exclusion) usually promotes growth effectively, but the influence of whole plant etiolation may be variable (Maynard et al. 1990; Sun, Bassuk 1991a; Sun, Bassuk 1991b; Blakesley et al. 1992). Also, stock-plant forcing as pre-treatment of stock plants can improve post-rooting growth. Stock-plant forcing in the greenhouse during spring promotes shoot development and allows to start propagation by cuttings earlier, leading to a head-start in cutting advancement and ensuring additional time for establishment of rooted cuttings (Samostchenkov 1985; Polikarpova, Pilugina 1991). Moser (1991) noted that this method can be used for deciduous azaleas. Treatments that may promote shoot growth of cuttings after rooting include foliar spray with plant growth regulators (gibberellins, cytokinins; McConnel, Herman 1980; Ernsten, Hansen 1986; Maynard et al. 1990), leaf defoliation (English 1981), provision with high nutrition levels (Johnson, Hamilton 1977, Rieckerman et al. 1999), warm temperatures (Dixon 1980), and long day or night interruption (Smalley et al. 1987; Rieckerman et al. 1999). In addition, auxins, which are used widely to favour adventitious root formation, prevent bud break and new shoot growth (Maynard et al. 1990; Sun, Bassuk 1991a). Berg and Heft (1991) observed that an extended growth season with an extended photoperiod and additional heating in a greenhouse during autumn can improve development of rooted cuttings as well as overwinter survival of azalea cuttings, probably due to improved carbohydrate supply.

The objective of this study was to examine the effects of stock plant forcing, together with extended light and additional heating in autumn and winter, on post-rooting growth and overwinter survival of rooted cuttings. The levels of sugars and starch in the cuttings were measured due to their importance as energy reserves.

Materials and methods

As stock plants, 7-year-old bushes of deciduous azalea cultivar 'Polārzvaigzne' (♀ *Rhododendron* × *kosterianum* C.K. Schneid. × ♂ *Rh. roseum* Rehd.) propagated by cuttings were used. The stock plants were grown as described previously (Apine, Kondratovičs 2005). Forced cuttings from forced stock plants were collected on 28 May 1999, while control cuttings from bed stock plants were collected on 29 June 1999. The cuttings were prepared and propagated as described previously (Apine, Kondratovičs 2005).

The cuttings were placed under a polyethylene tent in a non-mist semi-shadow greenhouse (40 to 90 $\mu mol\ m^{-2}\ s^{-1}$) under a natural photoperiod with temperatures of 23 ± 4 °C (day) and 15 ± 4 °C (night). After 6 August 1999, when the day length became shorter than 16 h, an extended light period was provided to maintain a 16-h photoperiod for half of the cuttings from both variants (variants with additional light). The supplementary light (25 - 35 $\mu mol\ m^{-2}\ s^{-1}$) treatment was white fluorescent tubes, suspended 80 cm above the middle of the plant block (1.2 m width). The tubes were set above the polyethylene tent. During autumn and winter, flats with cuttings in the greenhouse were covered by a twofold polyethylene tent. After 20 October 1999, to maintain a stable temperature during winter, the soil was heated: for the variants with additional light to 13 ± 4 °C, for the variants without additional light to 10 ± 2 °C. As a control, four flats from each variant were overwintered in the greenhouse without extended light and heating (temperature fluctuated from -9 °C to +4 °C). The variants without additional light and heating were assessed only during the spring because the cuttings of these variants soon lost their leaves.

Bud-break and new shoot growth were estimated during first season on 1 October 1999 and during the second season on 4 April 2000. The survival of overwintered cuttings was determined after the beginning of growth during the spring of the second season in 2000. Four replications (four flats, approx. 45 cuttings per flat) from each variant were used to determine the percentage bud break, new shoot lengths and survival of cuttings. The mean of new shoot length and percentage of cuttings with new shoots were calculated. Cuttings that did not start to grow in the period before 15 May 2000 were defined as dead. The percentage of overwintered cuttings was calculated as the proportion of cuttings that continued to develop over all cuttings in the sample.

For determination of carbohydrates in the leaves, the cuttings were sampled at 7- to 10-day intervals from 1 October until 27 December 1999 and twice per month until 30 March 2000. On 15 March 2000, before bud break, the concentration of starch in stems of cuttings was determined. In both cases the samples were taken in three replications between 10:00 and 11:00 hours. At each sampling, five cuttings were randomly selected. The samples were rinsed with water, fixed in water vapour and dried at 60 °C for 48 h. Dry leaves were milled by laboratory-mill, and 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 as described previously (Apine, Kondratovičs 2005).

Statistical analyses were performed using SPSS 11.0 for Windows. The data were analyzed using ANOVA. Pair-wise comparisons were made by testing for the least significant difference (LSD) at a 95 % confidence level. Pearson correlation coefficients were generated to describe the relationships among several carbohydrates.

Results

Post-rooting growth

Both the forcing of stock plants and the extended photoperiod during the end of the growth season significantly ($P \leq 0.0001$) stimulated the formation of new shoots (Fig. 1). However, the forced cuttings showed the best results in both variants (with and without additional light) compared to the control cuttings. Development of new shoots on rooted cuttings occurred from mid-August till October, but during the subsequent period till spring the development had terminated. At the end of October the lengths of new shoots were 7.7 ± 0.1 cm for the forced cuttings with additional light, 4.0 ± 0.2 cm for the forced cuttings without additional light and 5.0 ± 0.6 cm for the control cuttings with additional light (data not shown).

From mid-October the leaves of cuttings from variants without additional light and heating started to senescence, and at mid-November the cuttings lost leaves. The leaves of cuttings from variants with additional heating but without additional light gradually began to turn yellow only from the end of November and the leaves abscised at the end of December. However, all new shoots of cuttings from these variants retained their leaves.

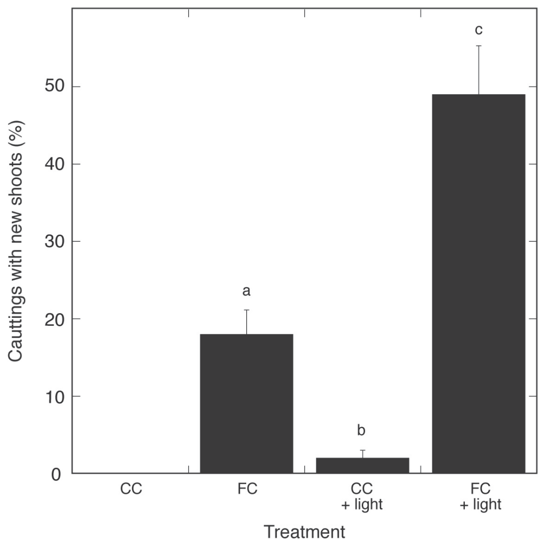


Fig. 1. Effect of stock plant forcing and extended photoperiod on the formation of new shoots on deciduous azalea cultivar 'Polārzvaigzne' stem cuttings. Measurments were made on 1 October 1999, 126 days after the start of the experiment. Mean values are shown, n = 4. Columns with different letters are significantly different at the $P \leq 0.05$ level..

The cuttings from variants with additional heating and light retained almost all their leaves till the end of experiment (data not shown).

Carbohydrate status in the leaves

The carbohydrate status in the leaves of rooted cuttings was assessed in all of the experimental variants. The additional light regime only insignificantly affected the time course of sugar and starch content in the leaves, therefore, for reason of clarity, only data from the experimental variants with additional light and heating are shown in Fig. 2.

In October there were significant differences in the amounts of starch, reducing sugars and total soluble sugars between the variants (Fig. 2A). The control cuttings contained 11.5 % starch, while forced cuttings contained only 6.9 % starch. In the following period, the starch level gradually decreased in both variants. However, a significant difference in respect to starch content was observed between the forced and control cuttings. While starch was depleted almost completely and fluctuated from 0.3 % to 0.5 % in the leaves of control cuttings, the level of starch remained relatively high (1.6 % to 3.8 %) in the leaves of forced cuttings. After February, the starch level gradually increased in the cuttings of variants subjected to extended light (which kept leaves). Differences in the amount of total soluble sugars between experimental variants during October and November were due to higher content of reducing sugars in the leaves of control cuttings in comparison with forced cuttings.

Similar to starch, during autumn and winter the amount of reducing sugars continually decreased in the leaves of cuttings from both variants, while the amount of sucrose increased slightly (Fig. 2B). During winter the levels of sucrose and reducing sugars were quite stable for all of the experimental variants. But at the end of the study period, during

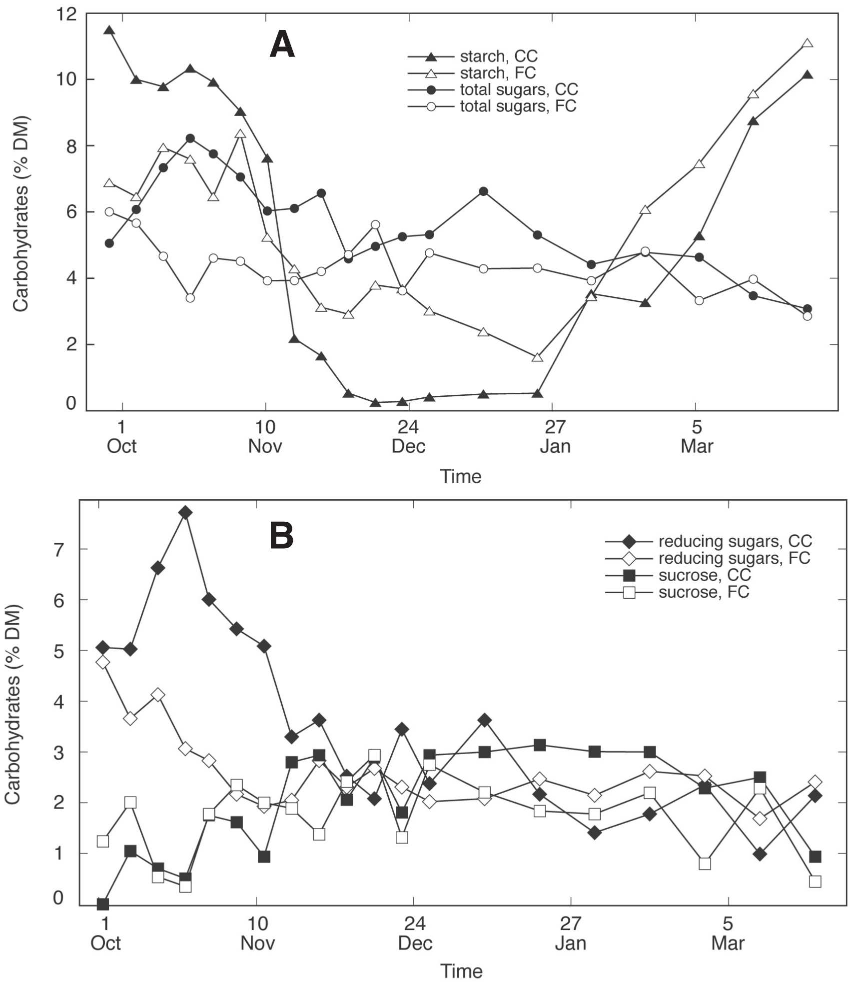


Fig. 2. Changes of carbohydrate concentrations in the leaves of rooted cuttings of deciduous azalea cultivar 'Polārzvaigzne' during the overwintering period. A, starch and total soluble sugars (TSS); B, reducing sugars and sucrose. Cuttings were taken from outdoor-raised stock plants on 29 June 1999 (control cuttings, CC) and from greenhouse-forced stock plants on 28 May 1999 (forced cuttings, FC). Mean values of variants subjected to an extended photoperiod and additional heating are shown, n = 3.

March the amount of soluble sugars tended to decrease in both control and forced cuttings mainly due to a decline of the level of sucrose.

Assessment of overwintering

The environmental conditions during the overwintering period influenced survival more significantly than stock-plant forcing in spring. The overwinter survival of rooted cuttings was assessed after bud-break. The cuttings from variants with additional light and heating had the best overwinter survival compared to the cuttings from the other variants (Fig. 3).

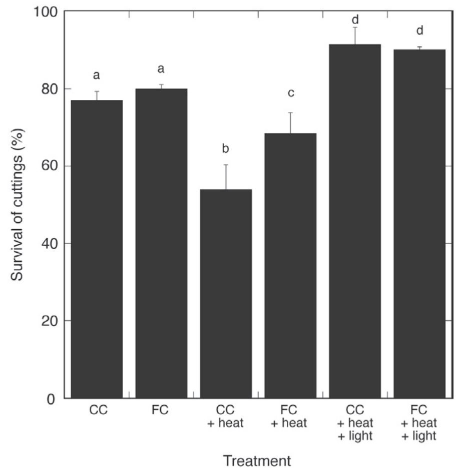
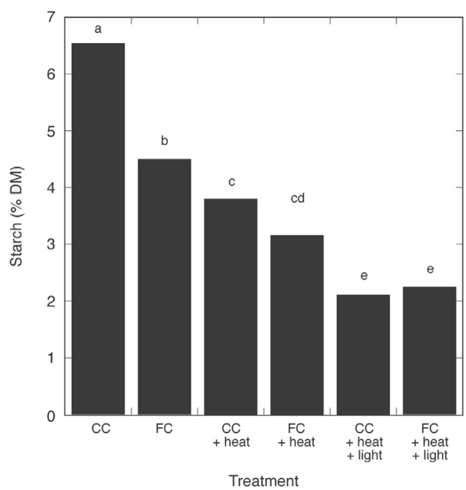


Fig. 3. Effect of experimental conditions on the overwinter survival of deciduous azalea cultivar ‘Polārzvaigzne’ stem cuttings. Cuttings were evaluated on 1 April 2000. Mean values are shown, n = 4. Columns with different letters are significantly different at the P ≤ 0.05 level..



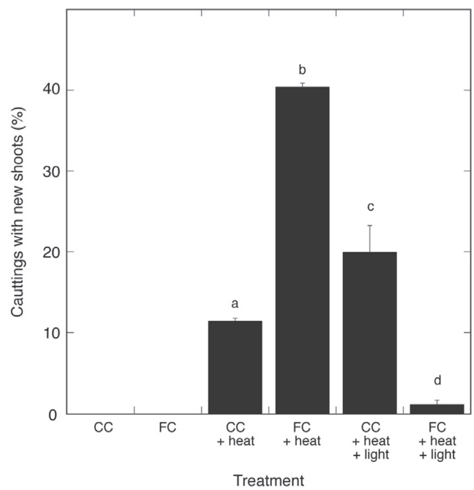


Fig. 4. Effect of experimental conditions on formation of new shoots after overwintering of deciduous azalea cultivar ‘Polārzvaigzne’ stem cuttings in the spring of the second season spring. Cuttings were evaluated on 1 April 2000. Mean values are shown, n = 4. Columns with different letters are significantly different at the P ≤ 0.05 level..

Fig. 5. Effect of experimental conditions on the starch concentration in the stems of rooted cuttings of deciduous azalea cultivar ‘Polārzvaigzne’ after overwintering. Samples were taken on 15 March 2000, 291 days after the start of the experiment. Mean values are shown, n = 3. Columns with different letters are significantly different at the P ≤ 0.05 level..

All cuttings that formed new shoots before winter and the cuttings that had retained their leaves throughout the winter survived (data not shown). In general, the conditions during the overwintering period affected survival of cuttings significantly (P ≤ 0.001), while the effect of stock plant forcing was not significant.

During spring, bud-break was first observed in the variants with additional heating and without extended light, but the development of new shoots in cuttings from the variants with additional light and heating was significantly weaker (Fig. 4). The lengths of the new shoots were 3.6 ± 0.8 cm in control cuttings from the variant with additional light and heating; 2.4 ± 0.6 cm in control cuttings from the variant with additional heating but without light; 2.5 ± 1.2 cm in forced cuttings with additional light and heating and 2.3 ± 0.7 cm in forced cuttings with additional heating but without light. Bud-break for control and forced cuttings from the variants without additional light and heating was initiated later, but during their development they reached the growth achieved by the cuttings that overwintered with additional heating but without additional light (data not shown).

Both stock-plant forcing ($P \leq 0.05$) and environmental conditions during winter ($P \leq 0.001$) significantly affected the starch level in the stem of cuttings. The highest amount of starch was found in the cuttings that overwintered without additional light and heating, and the lowest – in the cuttings that were provided with additional light and heating (Fig. 5). The control cuttings from the experimental variants that entered dormancy contained considerable more starch than forced cuttings from these variants.

Discussion

The present study clearly showed that pre-treatment of stock plants and the treatments of rooted cuttings significantly affected their further development. In general, the stock-plant forcing promoted formation of new shoots during the post-rooting period, but did not significantly affect the condition of leaves and survival of cuttings. Additional light positively influenced all parameters: new shoot growth, condition of leaves and survival of cuttings. Additional heating promoted retention of leaves, especially together with additional light, while the effect on the survival was conflicting. Heating (10 °C) alone without additional light diminished the survival of cuttings, but together with additional light increased survival. All of these observed effects on growth and survival were also reflected in changes of carbohydrates status of cuttings.

The extended photoperiod and especially the stock-plant forcing, which ensured extra time for post-rooting growth, promoted bud-break and new shoot growth of cuttings (Fig. 1). Poor bud-break was noticed for control cuttings, because of unfavourable conditions for these cuttings. The control cuttings were used for propagation one month later than forced cuttings, but progressing in the growth season reduces the ability for bud break (Smalley at al. 1987). The treatment of cuttings with additional light and heating significantly improved the condition of leaves of rooted cuttings, possibly due to a more beneficial ratio of growth promoters to growth inhibitors (Smalley at al. 1987; Hartmann et al. 2002). The retention of leaves maintains plant capacity for photosynthesis, which allows to replenish the carbohydrate reserves. Also the development of new shoots is very important for the development of the cuttings (Turetskaya, Polikarpova 1968; Polikarpova 1990; Hartmann et al. 2002), promoting supplement of carbohydrate stores due to an increase of the leaf area (Smalley at al. 1987). The importance of new shoots for photosynthesis and provision with photosynthates can explain the higher starch level in forced cuttings compared to the starch level in control cuttings during the December.

A decline of the starch amount in leaves and an interruption of new shoot growth suggest that the provided supplementary light could not compensate the decrease of natural irradiance and day lengths (Fig. 2). During late autumn and December, the reason for a reduced starch level could be both a decrease of photosynthesis and transport of assimilates to perennial parts of the plant. In softwood cuttings the leaves are an important store of assimilates. Our results suggest that the rooted cuttings completely utilized the carbohydrates accumulated in leaves either for development or stored them in the perennial parts.

Although the overwinter survival was affected mainly by the conditions during winter, the stock-plant forcing-related effect of new shoot growth during the post-rooting period on overwinter survival was evident (Fig. 3). In several studies, it was established that cuttings developing new shoots overwinter better (Jermakov, 1975; Loach, Whalley, 1975; Goodman, Stimart, 1987). In Acer rubrum, all cuttings breaking bud were observed to survive the overwintering period (Smalley et al. 1987). Also, in our study, all cuttings with new shoots survived and in spring they grew more rapidly than the cuttings without new shoots. As all new shoots maintained leaves and photosynthetic capacity obviously they were able to replenish carbohydrate stores, which helped to initiate growth.

The results of the present experiment showed a correlation between the overwintering conditions and the starch amount in the stem of cuttings (Fig. 5). The cuttings subjected to low temperature during the overwintering period could reduce the amount of carbohydrate used in respiration. The condition of additional heating without additional light promoted the depletion of carbohydrates in cuttings without the ability to replenish the stores, which resulted in the lowest survival rate of these cuttings. The treatment of cuttings with additional light and heating during winter, despite the lowest level of starch in the stems, showed the best overwintering, because these cuttings maintained photosynthetic capacity. However the poor growth of new shoots of these cuttings in spring suggests that the natural seasonal dynamics of plants was disturbed due to prevention of the dormancy period.

While sufficient carbohydrate storage is important for winter survival of cuttings (Smalley at al. 1987; Perkins, Bassuk 1995), in our study the starch concentration in stems of the cuttings was not related to survival ability. Further studies should consider also the accumulation of carbohydrates in roots. The importance of roots for storages has been shown in several studies using both intact plants (Loescher et al. 1990; Nguyen et al. 1990; von Fricks, Sennerby-Forsse 1998) and cuttings (Smalley et al. 1987; Hartmann et al. 2002).

Generally, stock-plant forcing of deciduous azalea enhanced post-rooting growth of cuttings, and an extended photoperiod and additional heating in winter improved the survival of the cuttings. However, the natural seasonal dynamics were disturbed and the development of plants was delayed in spring. To improve the conditions for rooting and survival of cuttings, stock-plant forcing during spring and an extended photoperiod with heating during the autumn are recommended, although, during the winter a dormancy period with the respective temperature should be provided so that the natural seasonal dynamics are not disturbed.

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Vides faktoru ietekme uz vasarzaļo rododendru pavairošanu ar spraudeņiem. II. Paildzināta augšanas perioda ietekme uz apsakņoto spraudeņu attīstību, pārziemošanu un ogļhidrātu satura izmaiņām

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Kopsavilkums

Pētījuma mērķis bija novērtēt, kā vasarzaļo rododendru mātes augu pavasara steidzināšana siltumnīcā un papildus apgaismojums un augsnes apsilde rudenī ietekmēja apsakņoto spraudeņu tālāko attīstību un pārziemošanu. Mātes augu steidzināšana pavasarī un papildus apgaismojums rudens periodā veicināja jauno dzinumu attīstību jau tajā pašā rudenī. Spraudeņu lapās uzkrātā ciete rudenī tika transportēta uz pārziemojošajām auga daļāmm. Rezerves ogļhidrāta cietes izmantošana ziemas laikā bija atkarīga no temperatūras. Salīdzinoši augsta temperatūra (10 °C) veicināja uzkrātās cietes patēriņu. Papildus apgaismojums un apsilde veicināja lapu saglabāšanos spraudeņiem un, līdz ar to, arī spraudeņu fotosintēzes iespējas. Vides apstākļi ziemošanas laikā ietekmēja spraudeņu izdzīvošanu, bet mātes augu steidzināšana pārziemošanu būtiski neietekmēja. Papildus apgaismojums kopā ar augsnes apsildīšanu ziemošanas laikā uzlaboja spraudeņu izdzīvošanas iespējas, bet aizkavēja to attīstību pavasarī.