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Effect of substrate moisture level on cloudberry seedling growth and development after propagation

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Abstract. Experimentation of cloudberry cultivation has recently started in Latvia. Propagation is an essential part of cloudberry cultivation strategy, and it is an important step to ensure cloudberry survival and high vitality in field conditions. Optimal moisture conditions have to be determined for seedling development in the greenhouse. Potted cloudberries, cultivar 'Nyby', were grown at four different relative moisture levels of the substrate (in percent of the full water-holding capacity of the peat) - 50%, 60%, 70%, and 80%. Physiological measurements were taken once every week, including the concentration of total *a* and *b* chlorophyll in SPAD units and stomatal conductance in mmol $m^{-2}s^{-1}$. Morphological parameters, such as the number of leaves per pot, leaf size (cm), number of winter buds and visual score (from 1 to 5) were measured at the end of the vegetation season. Results revealed significant differences between the substrate moisture treatments for chlorophyll content in leaves, winter bud development and visual scoring. The authors note that slightly higher results were found for all parameters for the 80% treatment, following the tendency of increased plant vitality in higher moisture levels. This study indicates that a relative moisture of at least 70% of the full water-holding capacity of the peat is necessary for successful cloudberry growth and development under greenhouse conditions.

Key words: cultivar Nyby, greenhouse, peat substrate, photosynthesis, *Rubus chamaemorus*, SPAD, transpiration, winter buds.

INTRODUCTION

Cloudberry *Rubus chamaemorus* L. is a perennial plant with circumpolar distribution (Thiem, 2003). Since the 20th century, cloudberries have mainly been studied and cultivated in the northern arctic and subarctic regions - Canada, Norway, and Finland (Rapp & Martinussen, 2002; Bellemare et al., 2009b). Recently, the first studies on the propagation and cultivation of cloudberries have been started in Latvia. To successfully grow cloudberries in the hemiboreal climate zone, optimal moisture and lighting conditions, and fertilization technologies have to be developed. According to Rapp (2004), the cultivation of cloudberry includes three basic steps: propagation by cutting of rhizomes, the rooting period during which plants develop new shoots, and planting in the field for berry harvest.

Studies regarding the growth conditions of cloudberry during the rooting period are scarce, and standards for substrate moisture and fertilization regimes have to be developed before commercial production can be established. Considering that the climate in Latvia is warmer with longer summers than in Northern countries, an optimal moisture regime is essential to successful growth and survival of cloudberry seedlings. As cloudberry naturally occurs in sphagnum peat (Rapp, 2004), it requires a stable water level. Berries like highbush blueberry *Vaccinium corymbosum* L. and American cranberry *Vaccinium macrocarpon* Aiton are already grown in cutover peat bogs in Latvia (Krīgere et al., 2019), and cloudberry is a potential berry plant for cultivation as well. For now, some of the requirements for other berries grown in peat soils could also be attributed to the cultivation of cloudberries. Few studies on cultivated *Vaccinium* species have been conducted regarding the influence of moisture conditions on plant development in the nursery. Low water availability decreases transpiration and photosynthetic rates in blueberries, indicating water stress (Glass et al., 2003). For cranberry, plant biomass growth was limited in insufficient moisture conditions (Lampinen, 2000).

Théroux-Rancourt et al. (2009) reported that stable water availability at the surface is beneficial for growth and rhizome extension during cloudberry establishment in plantations. However, if soil water content exceeds the optimal range, plant roots are subjected to reduced oxygen availability. Prolonged overwatering can inhibit branching and leaf growth (Pelletier et al., 2015; Guo et al., 2021) and cause root rot, resulting in plant death (Ward, 2013). Therefore, the prerequisites for cloudberry growing are sufficient water supply and well-aerated peat soil, which corresponds to fibric peat (H2–H3) (Rapp, 2004; Wendell & Alsanius, 2008; Bussières et al., 2015). Cloudberry rhizome vitality is essential for the development of roots and new ramets growth during propagation (Bellemare et al., 2009a). Rhizome segments (20–40 cm long) are commonly used as transplants (Rapp, 2004). However, mortality in field conditions can exceed 70%, which is mainly explained by insufficient reserves of carbon and nutrients in the rhizome segment, as well as by the formation of an underdeveloped root system (Bellemare et al., 2009a). To prevent this problem, it would be advantageous to carry out the period of rooting and shoot development in the greenhouse, with subsequent planting of seedlings in the field. Especially since it has been proven that cloudberry rhizomes can develop an extensive root system when grown in containers in a peat substrate under greenhouse conditions (Bussières et al., 2015; Boulanger-Pelletier & Lapointe, 2017). A critical issue for such propagation is the optimal water regime in the peat substrate to obtain healthy seedlings with a developed root system. Although in the northern countries, cloudberries are grown in nurseries, there is no available data on the growing temperature or moisture conditions under which cloudberries are propagated, or the optimal conditions. Considering the overall scarcity of available studies on cloudberry growing, starting cultivation in the climate of Latvia also requires obtaining data on the first step of cloudberry cultivation: propagation. Therefore, to determine the optimal conditions for propagation, we conducted a study testing different moisture treatments for potted cloudberries.

The aim of this study was to determine the effect of different moisture levels in peat substrate on the physiological and morphological parameters of cloudberry seedlings. Determining the optimal and lower limits of substrate relative moisture level for potted cloudberries in partly controlled greenhouse conditions is an important step in the development of cloudberry cultivation technology for the establishment of commercial production.

METHODS AND MATERIALS

Experimental condition and design

The experiment was conducted in a partly-controlled experimental greenhouse of the Institute of Biology, University of Latvia, located in Riga, Latvia, between March and September 2023. Non-conditioned greenhouse (size: 6×10×4 m, material: polycarbonate) conditions ensured no wind or precipitation influence on the plants. The lowering of the temperature in the summer months was carried out by ventilation during the day, which ensured that the greenhouse did not overheat. For these purposes, the greenhouse was equipped with 4 ventilation windows and doors at both ends of the greenhouse. As experiment was carried out in a greenhouse in Latvia, which is located in the hemiboreal climate zone, our findings could be applied in similar (temperate) environmental conditions in semi-controlled producing greenhouses.

Latvia is located in the hemiboreal climatic zone. The mean temperature for March, April, May, June, July, August and September were +1.6 °C, +7.4 °C, +11.3 °C, +16.6 °C, 16.8 °C, +18.5 °C, +15,8 °C, respectively, based on the records provided by SLLC 'Latvian Environment, Geology and Meteorology Centre' (2024).

Commercially produced peat substrate for forest seedlings and milled raw peat (Ltd. Laflora, Latvia) in a ratio of 1:2 was used as a substrate (level H2–H5 on von Post scale). Substrate agrochemical characteristics, determined from composite sample (2 L) taken in March before the experiment establishment, are given in Table 1. To determine plant-available nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) concentrations, peat samples were extracted using 1 M HCl in volume ratio 1:5; substrate electrical conductivity (EC) was measured in peat via distilled water extraction (1:5); peat reaction (pH) was measured in 1 M KCl soil-extractant mixture (1:2.5) as previously described (Karlsons et al., 2021). The experiment was carried out with the hermaphroditic cloudberry cultivar 'Nyby'. Cloudberries were propagated by sectioning rhizomes (15 cm each). Sections were then planted in 1 L plastic pots with the substrate. All pots with substrate were weighed to ensure identical weight. Seedlings were grown at four different relative moisture levels of the substrate (in per cent of the full water-holding capacity of the peat) - 50%, 60%, 70%, and 80%, labelled as 50M, 60M, 70M, 80M, respectively. Two months after setting up the experiment, a single dose (100 mg) of complex fertilizer Novatec Classic 12–8–16 with micronutrients (COMPO GmbH & Co, Germany) was added to each pot as a maintenance fertilizer.

Table 1. Nutrient concentration, pH and EC in the air-dry peat substrate before moisture experiment establishment

Plant available peat substrate nutrient concentration (g m^{-3}) in 1 M HCl extraction											pH_{KCl}	EC_{H2O}, mS cm^{-1}
N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B		
5	11	131	1,606	250	65	90	5.28	7.33	0.97	0.5	4.39	1.06

To determine the water-holding capacity (WHC) of the peat, a certain amount of peat substrate was placed in 1 L plastic pots (in three replications) and saturated with tap water (method adapted from Faran et al., 2019). After full saturation, the peat was removed from the pots and weighed. Samples were then dried in a drying oven at 100–105 °C to a constant mass, that is, the weighing was repeated until the mass of the sample did not change. The substrate WHC was the difference between the weights of the wet and dry substrate. The 50%, 60%, 70% and 80% WHC levels were determined as a respective fraction of the specified 100% WHC.

Thirty pots per treatment were randomly placed on the greenhouse table. Moisture levels were continuously maintained by weighing the pots and adding tap water three times per week from March to September 2023. The moisture treatments started on March 23.

Plant measurements

The start of cloudberry plant growth was recorded on March 21, when buds were visible in 50% of the pots. Leaves were fully developed by the beginning of May and measurements were started on May 12. Non-destructive measurements of physiological parameters were carried out once a week on randomly selected leaves for each treatment ($n = 15$). The concentration of total *a* and *b* chlorophyll was measured using a chlorophyll meter SPAD–502Plus (Minolta, Warrington, UK). Stomatal conductance in mmol $m^{-2}s^{-1}$ was measured with METER SC–1 Leaf Porometer (Decagon Devices, Pullman, WA, USA).

Morphological parameters, including the number of leaves and winter buds per pot, leaf size (cm), and visual score were measured once, at the end of the vegetation season on September 1st. For each treatment, two diagonal measurements were taken for each randomly selected leaf to calculate the average size ($n = 30$). The number of leaves and winter buds were counted per pot ($n = 15$), pots from each treatment were selected randomly. Visual score scale (Fig. 1) was adapted from Anwar et al., 2010 for cloudberry leaves (5 – green and healthy, 4 – pale green, 3 – yellow, 2 – yellow and brown, 1 – brown (dead leaves)). Randomly selected pots ($n = 15$) were rated by the average condition of the leaves in the pot using the scale.

Figure 1. Representative example of visual scoring scale for evaluation of leaf condition in different substrate moisture conditions (scale adapted from Anwar et al., 2010).

Statistical analysis

Cloudberry growth results were analysed with descriptive statistics using the R programming language. A one-way ANOVA with post hoc Tukey HSD test were conducted to determine statistically significant differences ($P < 0.05$) between moisture treatments for all parameters, except for visual scoring of the plants, for which a Kruskal Wallis test with post hoc Dunn’s test was performed. ‘

RESULTS AND DISCUSSION

Physiological measurements

SPAD measurements in cloudberry leaves revealed significant differences ($P < 0.05$) between most of the substrate moisture treatments except between 70M and 80M. Both treatments had the highest mean results per growing season regarding chlorophyll content in leaves (Fig. 2). 70M and 80M were characterized by only small seasonal changes in leaf SPAD readings, and the values generally did not decrease during the summer. The only difference between these treatments, which can only be evaluated as a trend, was found in September when a slight decrease in chlorophyll concentration was more pronounced for 70M. The results on the dynamics of cloudberry leaf chlorophyll content revealed not only lower mean values for 50M and 60M but also a progressive decrease starting from the second half of July to the end of the growing season. For 50M, SPAD values decreased particularly rapidly until mid-August and were significantly different from those found in 60M during this period. SPAD indicates the overall vitality of the plant and its ability to photosynthesize (Uddling et al., 2007). Chlorophyll content is affected by environmental and genetic factors and positively correlates with nitrogen concentrations in leaves (Xiong et al., 2015). While drought conditions limit yield in crops even with adequate N fertilization, excessive water levels may contribute to N leaching, thus decreasing the chlorophyll contents (Széles et al., 2012). Studies show that drought conditions decrease chlorophyll content, thus decreasing photosynthetic capacity (Arunyanark et al., 2008). Considering our study was conducted to find the lower limit of moisture level for cloudberry seedlings, moisture and chlorophyll content appear to be related and indicate the effect of drought on plant vitality. Therefore, in greenhouse conditions, the relative moisture level of at least 70% and higher in the peat substrate is optimal for photosynthesis in cloudberry leaves.

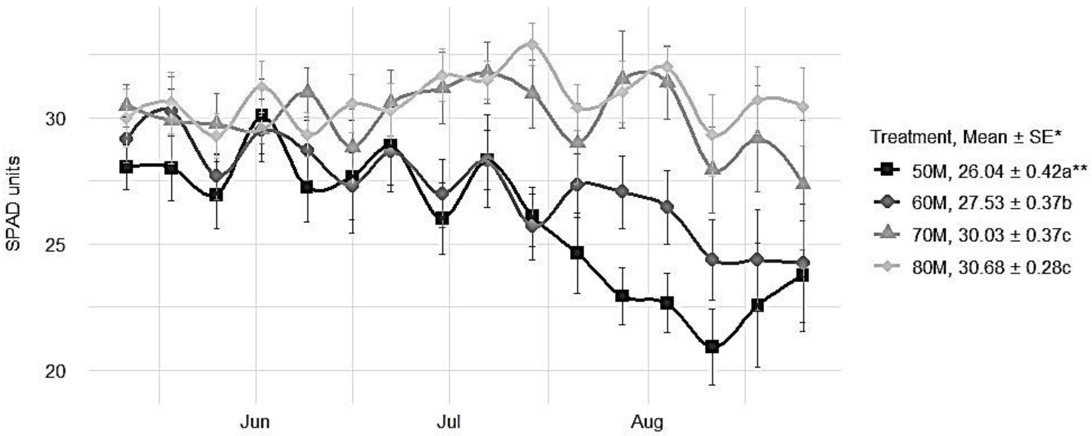

Figure 2. Effect of different substrate moisture levels on chlorophyll content of cloudberry leaves in SPAD units from May to September. 50M, 60M, 70M, and 80M – represent relative substrate moisture content at 50%, 60%, 70%, and 80% of full water-holding capacity.

*Given means and standard errors (SE) represent the means of all growing season chlorophyll data for each treatment. **Means with different letters indicate significant differences between treatments ($P < 0.05$).

Although the results revealed an increase in mean stomatal conductance (SC) in accordance with higher moisture levels, no significant differences ($P > 0.05$) were found between any of the moisture treatments (Fig. 3). This can be attributed to a type II error based on the limited number of replicates; however, the results do show a tendency for higher SC levels in 80M with more available water than for other treatments. The SC of cloudberry leaves slightly increased over the season for all moisture treatments, and only the last measurements in September showed a tendency to decrease or remain unchanged. The differences in SC under different substrate moisture conditions were more evident as the growing season progressed. From mid-July water stress (treatments 50M and 60M) reduced leaf stomatal conductance as compared to 70M and 80M. SC characterizes vital physiological processes as gas exchange and transpiration, which depend on various environmental stress factors at microclimatic scale, such as temperature, wind, relative humidity, and water availability (Lavoie-Lamoureux et al., 2016; Urban et al., 2017; Xiong et al., 2018). Low water availability reduces photosynthetic activity and inhibits growth due to reduced gas exchange capacity (Bryla, 2011; Osakabe et al., 2014). Once water loss exceeds water availability, stomata close thus decreasing transpiration rates (Asbjornsen et al., 2011). Therefore, SC is a good indicator for assessing how soil moisture levels impact the plant. Study of blackberry *Rubus* L. clones revealed that by adapting the stomatal apparatus in drought-stress conditions, plants maintained the photosynthetic rates (Zhang et al., 2017). Although our results did not confirm the impact of drought-stress on plant transpiration, a tendency was found of SC to slightly increase in accordance with higher moisture levels, similar to the SPAD results.

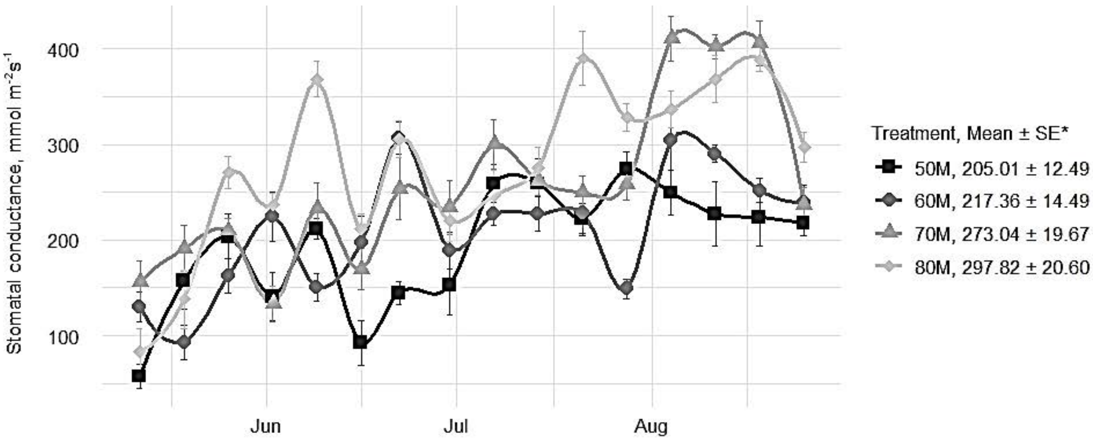

Figure 3. Effect of different substrate moisture levels on stomatal conductance (mmol $m^{-2}s^{-1}$) of cloudberry leaves from May to September. 50M, 60M, 70M, and 80M – represent relative substrate moisture content at 50%, 60%, 70%, and 80% of full water-holding capacity.

*Given means and standard errors (SE) represent the means of all growing season stomatal conductance data for each treatment. No significant differences were found between treatments ($P > 0.05$).

Morphological measurements

Morphological parameters such as the number of leaves and winter buds per pot, and leaf size were measured at the end of the vegetative period of cloudberry seedlings to compare cloudberry morphological development under different substrate moisture treatments. These measurements thus revealed the effect of moisture on the development of cloudberry seedlings after one season of rooting period.

The number of leaves per pot did not differ significantly between any of the treatments (Table 2), however, by the end of the vegetative period 80M had on average the most leaves per pot that were developed and had survived. Although the leaf number for 70M were on average lower than for the rest of the treatments, treatments did not differ significantly within this parameter, thus this is not a contradictory finding. An increase in soil bulk density and a decrease in pore size increases water-holding capacity, which depends on soil physical properties. In other studies, cloudberries in peat with low bulk density and high porosity developed more leaves than those in more compacted peat soil (Théroux-Rancourt et al., 2009). The most suitable peat for cloudberry growing in field conditions is fibric peat, classified H2–H3 on the von Post scale (Bussières et al., 2015). In this study, the peat substrate used was within the range of H2–H5 on the von Post scale, which is generally suitable for cloudberry propagation and growing in the greenhouse. In addition, the physical properties and water-holding capacity of the substrates in pots of all treatments were identical. Therefore, the differences in the number of leaves produced in different moisture treatments could be related to the amount of water held in the peat substrate. Although no significant differences were found between the treatments, which could be related to the limited number of experimental replicates, a tendency was found for the number of leaves in the pot to be the highest at 80M. This is generally consistent with other parameters, for which the highest results were observed at 80M.

Table 2. Number of leaves and winter buds per pot, and mean leaf size (mean ± standard error (SE)) at the end of the growing season 2023 for cloudberry seedlings in different substrate relative moisture treatments. 50M, 60M, 70M, and 80M – represent relative substrate moisture content at 50%, 60%, 70% and 80% of full water-holding capacity

	Mean ± SE		
Treatment	Leaves per pot	Winter buds per pot	Leaf size (diagonal), cm
50M	12.73 ± 0.64	11.67 ± 1.10a*	4.19 ± 0.13
60M	12.80 ± 1.00	14.40 ± 1.36a	4.03 ± 0.25
70M	11.60 ± 0.70	16.13 ± 1.81ab	4.65 ± 0.28
80M	14.80 ± 0.90	21.47 ± 1.70b	4.80 ± 0.31

*Means with different letters in a column were significantly different ($P < 0.05$). Number of leaves per pot and leaf size did not differ significantly.

The number of winter buds per pot revealed significant differences between 50M–80M and 60M–80M treatments. 80M had the highest number of winter buds per pot, however, there were no significant differences found between 70M and 80M. This suggests that relative substrate moisture level can vary within 70–80% without a significant effect on cloudberry winter bud development. Rhizomes are essential for root and new shoot growth and for cloudberry growing this is especially important, considering that high-density cloudberry patch produces more berries (Brändle & Crawford, 1987; Rapp, 2004). Thus, it is important to maintain the vitality of rhizomes and enhance their development, as winter buds are developed in the vegetative period to overwinter. Currently, no studies report results on cloudberry development in soil with excessively high water content, and it is not clear what is the upper limit for water content in peat for cloudberries. However, as literature suggests, waterlogging can reduce oxygen availability for roots leading to rotting, and eventually nutrient leaching, which is especially high in peat soils (Bryla, 2011). For cloudberries in a greenhouse, this is often indicated by rhizomes that have died and appear black as a result of overwatering (pers. obs.).

Leaf size (average diagonal, cm) did not differ significantly between any of the treatments. Prolonged drought stress impacts plant vegetative growth (Osakabe et al., 2014). Leaf health further has effect on other development stages of the plant (Bellemare et al., 2009a). Although this is not significantly evident in the study, we note the relatively smaller leaves in 50M and 60M as compared to the higher moisture level treatments.

Visual scoring revealed significant differences between the highest and the lowest moisture treatments (Fig. 4). 80M had the highest scores - all of the samples were given the highest scores of 5 (green and healthy) or 4 (pale green). Thus, 80M had visually the most green cloudberry leaves. For 70M, 73.33% of the samples were given the highest scores of 4 and 5, which was also indicative of good cloudberry vitality. 60M and 50M had no scores of 5 and overall had similar rating score percentages. Brown leaves indicate that plant tissue has dried out and photosynthesis has stopped. Considering that at the end of the vegetation season most of the 60M and 50M cloudberry leaves were visually yellow to brown (80.00% for both treatments), we conclude that relative peat moisture levels below 70% are insufficient in maintaining vital cloudberry seedlings.

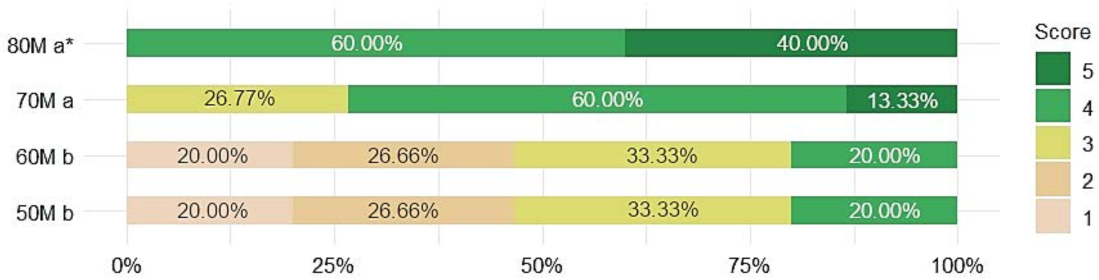

Figure 4. Visual scoring of cloudberry seedling leaf condition in different substrate moisture conditions in September 2023 (shown in per cent). 50M, 60M, 70M, and 80M – represent relative substrate moisture content at 50%, 60%, 70%, and 80% of full water-holding capacity. 5 – green and healthy, 4 – pale green, 3 – yellow, 2 – yellow and brown, 1 – brown (dead leaves).
*Different letters indicate significant differences between treatments.

As 70M and 80M were not significantly different, we can assume moisture levels of 70–80% are suitable for ensuring sufficient vitality of cloudberry seedlings, based on leaf status. Visual score related most to SPAD results, revealing 80M and 70M as the most successful in terms of leaf development.

The above study confirmed that 80M treatment had the highest vitality indicating results in all the parameters measured, however, the differences were significant for chlorophyll content in leaves, winter bud development and visual scoring. In conditions of insufficient water supply, stomatal conductance and CO_2 uptake decrease, resulting in a general decrease in photosynthesis of the plant (Osakabe et al., 2014). The development of optimal leaf size is also vital for photosynthesis. Indeed, our results indicate that all the determined indicators are interrelated and well describe the success of the seedlings at different levels of substrate moisture.

The importance of moisture conditions is highlighted in several studies regarding berry plant growth in plantations and experimental sites. Soil moisture was positively correlated with node number per shoot, leaf number per shoot and leaf chlorophyll content for arctic bramble (*Rubus arcticus* L. ssp. *arcticus*), a cloudberry related species (Vool et al., 2011). Although this study demonstrated the beneficial effect of higher moisture on the vegetative growth of arctic bramble, it did not provide precise information on the moisture content of the growing medium. Our study showed a similar trend for leaf numbers to increase with higher peat moisture content.

Studies on berry responses in various moisture levels showed that water stress effects may differ depending on the plant development stage and fluctuate in the vegetative period. Experiments with rooted cuttings of cranberry in a greenhouse revealed the greatest plant growth rates under the wettest conditions and no root mortality was found even at a high water table (Bauman et al., 2005). A study conducted on potted blackberry tissue clones revealed high drought-stress tolerance and adaptive stomatal mechanisms to maintain photosynthesis and prevent tissue damage (Zhang et al., 2017). This might also be true for cloudberry seedlings, as SPAD and SC results fluctuated for each treatment throughout the vegetative season. However, measurements for different moisture treatments during specific plant development stages need to be obtained to better explain how the fluctuation of SPAD indices and SC relate to cloudberry development. Our study showed that cloudberry seedling total chlorophyll content in leaves indicating the rate of photosynthetic activity is related to SC and to some extent could be regarded as cloudberry response reaction in drought-stress conditions.

The purpose of propagation is to obtain healthy rhizomes for field plantations. Successful rhizome development is essential, as rhizomes are source of carbohydrates during vegetative plant growth (Kaur et al., 2012). Rhizomes shorter than 15 cm are associated with lower survival when planted, which is related to lower carbohydrate reserves (Bellemare et al., 2009a). Thus, the development of rhizomes of adequate length and vitality in the nursery is essential for a successful plantation. In addition, leaves indicate the vitality of the plant during the vegetative period. Optimal cloudberry leaf health is also essential for fruit development (Bellemare et al., 2009b). The propagation of cloudberries requires relatively stable moisture conditions for vegetative development, and based on our results, the relative moisture in the peat substrate should not be less than 70% of water saturation.

These results are significant for ventilated greenhouse conditions. It should be noted that currently the cultivation of forest seedlings, ornamental seedlings and berry seedlings takes place in most cases under partially controlled conditions (without high-cost maintenance of a certain air temperature or humidity). Therefore, it is expected that the results of the study will have wide applicability within the temperate climatic conditions.

Planting berries in extracted peat bogs requires drainage management such as ditches near the planting site. Cloudberry requires a stable groundwater level of 30–40 cm; however, the water table should be assessed along with soil physical properties (Rapp, 2004; Théroux-Rancourt et al., 2009). This is true for greenhouse-grown cloudberries as well, as substrate with higher bulk density and lower porosity than H2–H3 level peat would hold water for longer thus reducing the water stress effect on seedlings and require less maintenance in the nursery. Research on this aspect is still to be continued, as the maximum moisture level for successful cloudberry growing in greenhouse conditions needs to be determined. Research is also needed to determine the optimal moisture level for cloudberries grown in field conditions in Latvia.

CONCLUSIONS

In this study, we compared how four different relative moisture levels (50%, 60%, 70%, 80%) of peat substrate influence cloudberry growth and development in greenhouse conditions during propagation. Significant differences were found between the lowest (50% and 60%) and highest (70% and 80%) moisture level treatments for three parameters: chlorophyll content in leaves, winter bud development and visual scoring. The highest visual scores indicating green leaves of cloudberry plants were given for substrate relative moisture treatments of 80% and 70%: 100% and 73% of the samples, respectively. Notably, the 80% treatment had visually the greenest cloudberry leaves, which suggests higher photosynthetic rates, potentially resulting in more successful seedling development as compared to the other treatments.

We also note that slightly higher results were found for all parameters for the 80% treatment, including stomatal conductance intensity, number of leaves, and leaf size. Although these three parameters did not differ significantly between treatments, these results do follow the tendency of increased plant vitality in higher moisture levels.

Therefore, the results suggest that a moisture level of at least 70% of the full water-holding capacity of the peat is required for optimal cloudberry survival and development rates during the rooting period in the greenhouse in temperate climate. The results suggested close interrelations between parameters like chlorophyll content, stomatal conductance, and leaf and winter bud development, which are inhibited in low water availability for potted cloudberries. We conclude that the relative moisture in the peat substrate should not be less than 70% of water saturation. Meanwhile, the question is open regarding the maximum moisture content in the peat, which would not cause cloudberry growth disturbances. Research in this direction should be continued.

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