Journal of Berry Research 1 (2010) 91–101
DOI:10.3233/BR-2010-010
IOS Press

Development and quality of cloudberry (*Rubus chamaemorus* L.) as affected by female parent, male parent and temperature

Inger Martinussen[a,*], Eivind Uleberg[a], Gordon J. McDougall[c], Derek Stewart[c] and Olavi Junttila[b]
[a]*Norwegian Institute for Agricultural and Environmental Research, Bioforsk Nord Holt, Tromsø, Norway*
[b]*Department of Arctic and Marine Biology, University of Tromsø, Tromsø, Norway*
[c]*Plant Products and Food Quality, Scottish Crop Research Institute, Dundee, Scotland*

Abstract. In this study we investigated the interaction between temperature and genotype on fruit development and levels of total phenols and anthocyanins in cloudberry. The experiment was done in a phytotron using one female ('Fjellgull') and one hermaphroditic ('Nyby') cultivar. Plants were grown at 9, 12, 15 and 18°C in 24-h photoperiod. The female cultivars were pollinated with pollen from a male ('Apollen') clone and from the hermaphrodite clone. Parthenocarpic fruit development was induced by gibberellic acid (GA_3). Ripe berries were frozen individually at −80°C and stored until analyses. There was a linear, double logarithmic relationship between temperature and number of days from pollination/GA_3-treatment to ripening. 'Fjellgull' had significantly larger berries than 'Nyby', and the largest berries were obtained at 12 and 9°C. Pollen clone did not have a significant effect on berry size. GA_3 induced parthenogenesis in 'Fjellgull' and partial parthenogenesis in 'Nyby'. In 'Fjellgull', the parthenocarpic berries were comparable to pollinated ones at low temperatures, but at 18°C their development was restricted. The level of total anthocyanins was significantly higher in 'Fjellgull' than in 'Nyby', and these levels were significantly enhanced at 9 and 12°C compared to higher temperatures. Levels of total phenolic compounds were not significantly affected. In conclusion, the present results indicate that low temperature is favourable both for size and quality of cloudberries.

Keywords: Cloudberry, temperature, gibberellin, berry development, anthocyanins, phenols, *Rubus chamaemorus*

1. Introduction

Cloudberry (*Rubus chamaemorus* L.) is a circumpolar plant species [13] growing on peat land, preferably *Sphagnum* bogs. Berries of cloudberry have traditionally been used by people living in these areas, and currently cloudberry is an economically important product for domestic and commercial use, particularly in Fennoscandia. The first cloudberry cultivars released in Norway, two female and two male cultivars, were selected for stability and high number of carpels ('Fjellgull' and 'Fjordgull') and for abundant pollen production ('Apollen' and 'Apolto') [24]. Unisexuality of cloudberry is one obstacle for cultivation, and clones with bisexual (hermaphrodite) flowers have been sought for some time. Such clones have been reported [9], and recently one hermaphrodite cultivar ('Nyby') was released in Finland (Uosukainen 2005, pers. comm.). In addition, cloudberry also has an ability to produce parthenocarpic fruits after a treatment with gibberellic acid (GA_3) [15].

*Corresponding author: Inger Martinussen, Norwegian Institute for Agricultural and Environmental Research, Bioforsk Nord Holt, Box 2284, N-9269 Tromsø, Norway. Tel.: +47 40604100; Fax: +47 77655143; E-mail: inger.martinussen@bioforsk.no.

Cloudberries contain several classes of phenolic compounds with ellagitannins/ellagic acid as the dominating ones [10, 16, 19, 27], and they are rich in vitamins C [28] and E [1, 2]. The antioxidative and/or potential human health beneficial effects of cloudberry are mainly due to the ellagitannins [16], which have been shown to exhibit antiproliferative effects on certain human cancer cell types [4, 6, 20]. In addition, phenolic extracts of cloudberry reportedly have antimicrobial activity against *Salmonella* and *Staphylococcus* [23]. Generally, the antioxidative activities and chemical composition of berries are affected both by the genotype and the environment, as shown for blueberries [12], strawberries [30] and raspberries [3]. Kähkönen et al. [16] found significant differences in contents of anthocyanins, flavonols, and ellagitannins in cloudberries collected at various locations in Finland. In order to develop a breeding program with the aim to produce new varieties with improved nutritional value combined with high production efficiency and berry quality, changes in the metabolism of bioactive compounds need to be understood [4].

To our knowledge there are no published studies on the effects of temperature on development and quality in cloudberries. Similarly, reports on the pollination stability of the hermaphroditic clone under contrasting temperature conditions are missing. The elucidation of such relationships is vital for further development of cloudberry as a cultivated crop. The main purpose of this study was to investigate the effects of temperature on development and targeted chemical composition of cloudberry cultivars 'Fjellgull' and 'Nyby'. Importantly, 'Fjellgull' was pollinated either with 'Apollen' or with 'Nyby', while 'Nyby' was self-pollinated. Further, since treatment with GA_3 can replace pollination, effects of GA_3 at various temperature treatments were also tested.

2. Materials and methods

2.1. Plant material

Clonally propagated plants of two cloudberry cultivars (*Rubus chamaemorus* L.) were used. The female cultivar 'Fjellgull' (32 plants in total) was propagated from rhizomes at the Norwegian Institute for Agricultural and Environmental Research (Bioforsk Nord Holt). The hermaphrodite cultivar 'Nyby' (64 plants in total) was obtained from Laukaa Research Station, Finnish Agricultural Research Organisation. 'Nyby' was originally propagated *in vitro*, but the plants were well established in pots when transported to Tromsø. All the plants were potted in fertilized peat in 12 cm plastic pots (0.7 l). The male cultivar 'Apollen' (16 plants in total), obtained from Bioforsk Nord Holt, and the hermaphrodite cultivar 'Nyby' were used for pollination. Plants were grown in the phytotron of the University of Tromsø (69°39′N lat.) for one season at 18°C in natural 24-h photoperiod. As a preparation for cold treatment, plants were exposed for 6 weeks to a 12 hr photoperiod at 6°C, after which they were stored at 4°C for about 6 weeks. The experiment was started October 10th, 2007. All the plants had abundant, normal flowering, and differences in propagation methods are not expected to influence pollination and berry development.

2.2. Growth conditions

Plants were grown under controlled temperature treatments (9, 12, 15 and 18°C; variation ±0.5°C) in 24-h photoperiod. Daylight was supplemented with cool white fluorescent tubes giving the minimum of 150 $\mu mol\ cm^{-2}\ s^{-1}$ PAR. The humidity was regulated to ensure a 0.5 MPa water vapor deficit. Plants were watered daily, and fertilized with a complete nutrient solution once a week.

2.3. Treatments

Following treatments were used:

'Fjellgull': (1) Control, no treatment; (2) Pollination with pollen from 'Apollen'; (3) Pollination with pollen from 'Nyby'; (4) One application of 5 μg GA_3, dissolved in ethanol and given as a 10 μl microdrop to the carpels. For pollination, carpels were rubbed with stamens from 2 to 3 flowers of pollen cultivar.

'Nyby': (1) Control, no treatment; (2) Assisted self-pollination, flowers were rubbed gently with a brush, repeated over 2 days; (3) Application of 5 μg GA_3 as above.

These treatments were done at all four temperature conditions and number of treated flowers varied from 11 to 36. In addition, stamens from totally 57 flowers of 'Nyby' at 12 and 15°C were removed at flower bud stage and the emasculated flowers were either pollinated with pollen from 'Apollen', treated with GA_3 as above, or left untreated.

Both pollination and treatments with GA_3 were done daily during the flowering period, which lasted from one to two weeks. Flowers for the treatments were tagged randomly and, when possible, all the treatments were applied daily. Thus, one single plant could have two or three treatments, and some flowers in each plant were left untreated. Flowers were not isolated, but none of the unpollinated flowers of 'Fjellgull' or emasculated and non-pollinated flowers of 'Nyby' developed any fruit, indicating that no pollinating insects were active in the growth rooms and no unintended pollination took place. No indications of any carry-over effects between flowers on the same plant were observed. For example, in plants where several of the flowers were treated with GA_3, untreated and unpollinated flowers did not show any development.

2.4. *Sample collection and measurements*

Ripe berries (soft, easily detached from the sepals/pedicle) were harvested daily, fresh weight (yield) and number of developed drupelets (berry size), and number of days from the pollination/treatment to harvest were recorded for each berry. Seeds (stony endocarp and seed) were removed with forceps from six berries of each treatment, rinsed, air-dried overnight at room temperature and weighed. For each treatment and temperature combination, six intact berries (with seeds) and six seedless berries (berries were crushed and the seeds/stony endocarp picked out with forceps) were used. The samples were frozen individually at −80°C and stored at this temperature for chemical analyses. Berries for these samples, as well as for the measurements of the seeds, were selected randomly from normally developed berries during the whole harvesting period. Thus, berries in each sample originated from several plants.

Pollen germination was tested in 3–6 samples of 'Nyby' and 'Apollen' at all temperatures using a hanging-drop method [5]. A minimum of 400 pollen grains were counted from each sample. Both cultivars produced pollen with good germination capacity at all temperature treatments (data not shown). All pollinated flowers set fruit and data on number of developed drupelets provides information on the efficacy of fertilization.

2.5. *Chemical analyses*

For chemical analyses, three samples, each with two berries, were used. As the seeds were not crushed during extractions, differences between samples of intact and seedless berries reflect the effect of crushing the berries prior to freezing. Frozen berries were taken from the freezer and allowed to thaw. For each sample two berries were weighed and homogenized in a hand held glass tissue grinder for 2 min in acetonitrile containing 1% acetic acid in a 1 : 1 (fruit weight : solvent volume) ratio. The average sample size was 3.72 g for the seeded berries and 2.96 g for the seedless berries.

2.6. *Total phenolics and anthocyanins*

Total phenolic and anthocyanin contents were determined as described by McDougall et al. [20]. Samples were analyzed using two biological and three technical replicates.

2.7. *Statistical analyses*

Statistical analysis of growth data, berry weight, seed size and number of druplets were performed using Statview 4.0 (Abascus Concepts) to generate *t*-test, factorial ANOVA, and regression analyses. Statistical analysis of total anthocyanins and total phenols were performed using Minitab 15. The effects of female parent were analysed by comparing 'Fjellgull' pollinated with 'Nyby' with self-pollinated 'Nyby'; effects of the male parent by comparing 'Fjellgull' pollinated with 'Apollen' with 'Fjellgull' pollinated with 'Nyby'; and effects of GA_3-treatment were analysed separately for 'Fjellgull' and 'Nyby' by comparing pollinated with GA_3-treated plants. All four temperature treatments were included in these analyses (2×4 factorial). For berry data all harvested berries were included and, accordingly, number of berries per treatment varied from 12 to 36. Data on seed size was based on six berries per treatment; chemical analyses were based on 3 replications, each with two berries.

3. Results

3.1. Flowering, berry development

The time from beginning of forcing to beginning of flowering increased from 11 days at 18°C to 38–42 days at 9°C (143 and 160 degree days with 5°C as base temperature, respectively). At 9°C, 'Nyby' started to flower 4 days later than 'Fjellgull', whilst differences between the cultivars were minimal at higher temperatures. The main flowering period increased with decreasing temperature, from 4–5 days to 12–14 days at 18 and 9°C, respectively. At 9°C 'Nyby' continued to produce some isolated flowers for several weeks after the main flowering period had ended.

The unpollinated flowers of 'Fjellgull' did not develop any berries, indicating that no uncontrolled pollination took place in the growth rooms. However, at 9°C the carpels in some of the flowers did not dry out but remained green and increased to a fresh weight of 30–50 mg. All the emasculated, unpollinated flowers of 'Nyby' stopped in development and died, while most of the emasculated flowers produced ripe berries if pollinated or treated with GA_3 (Table 1). Lack of development in some of the flowers was probably due to damages to the carpels during emasculation.

Rate of berry development was mainly affected by temperature (Fig. 1A). At 18°C, ripening time was in average 35 days but at 9°C this lengthened considerably to around 62 days. There was a linear relationship between logarithm of days to ripening and logarithm of temperature ($p < 0.0001$). In general, berry development was slightly faster in 'Nyby' (44.2 days) than in 'Fjellgull' (46.3 days). This difference was significant when analyzed with *t*-test ($p = 0.03$). Neither pollinator ('Apollen' or 'Nyby' for 'Fjellgull') nor GA_3-treatment had any significant effect on the rate of berry development (Fig. 1A).

3.2. Berry size

Number of carpels is a good indicator for the potential size of cloudberry. 'Nyby' had in average significantly less carpels than 'Fjellgull', 15.3 ± 3.2 ($n = 38$) and 21.3 ± 3.6 ($n = 42$), respectively. Consequently, 'Fjellgull' had much larger berries than 'Nyby' and the maximum fresh weight values observed were 4.82 g (26 drupelets, 9°C, pollinated with 'Nyby') and 2.61 g (18 drupelets, 15°C, assisted pollination), respectively. Mean fresh weight of all berries was 2.33 g for 'Fjellgull' and 1.39 g ($p < 0.0001$) for 'Nyby' (both pollinated with 'Nyby'). However, interaction between clone and temperature was significant ($p = 0.0004$); 'Fjellgull' had the largest berries at 9°C and 'Nyby' at 12°C (Fig. 1B). In general, berry size decreased during the harvesting period.

In 'Fjellgull', berry size was not significantly affected by pollen parent, but the interaction between pollen parent and temperature was significant ($p = 0.001$); pollination with 'Nyby' produced larger berries than pollination with 'Apollen' at 9°C (2.83 and 2.16 g, respectively), while the opposite was the case at the other temperature treatments (Fig. 1B). In average, parthenocarpic berries of 'Fjellgull' were smaller than pollinated berries ($p = 0.0001$). This difference was mainly due to an inhibited development of GA_3-treated berries at 18°C (pollinated 2.38 ± 0.66 g,

Table 1

Development of emasculated flowers of 'Nyby' as affected by pollination and GA_3 treatment. Results are from 12 and 15°C. Mean values ±SD

Treatment	Ripe berries %	Fresh weight (g)	Number of developed drupelets
Control	0 ($n = 23$)	–	–
Pollinated with 'Apollen'	82 ($n = 11$)	1.02 ± 0.48 ($n = 9$)	8.0 ± 4.6 ($n = 9$)
Pollinated with 'Nyby'	67 ($n = 6$)	1.22 ± 0.56 ($n = 4$)	8.8 ± 3.3 ($n = 4$)
GA_3, 5 μg	53 ($n = 17$)	0.94 ± 0.34 ($n = 9$)	11.3 ± 3.8 ($n = 9$)

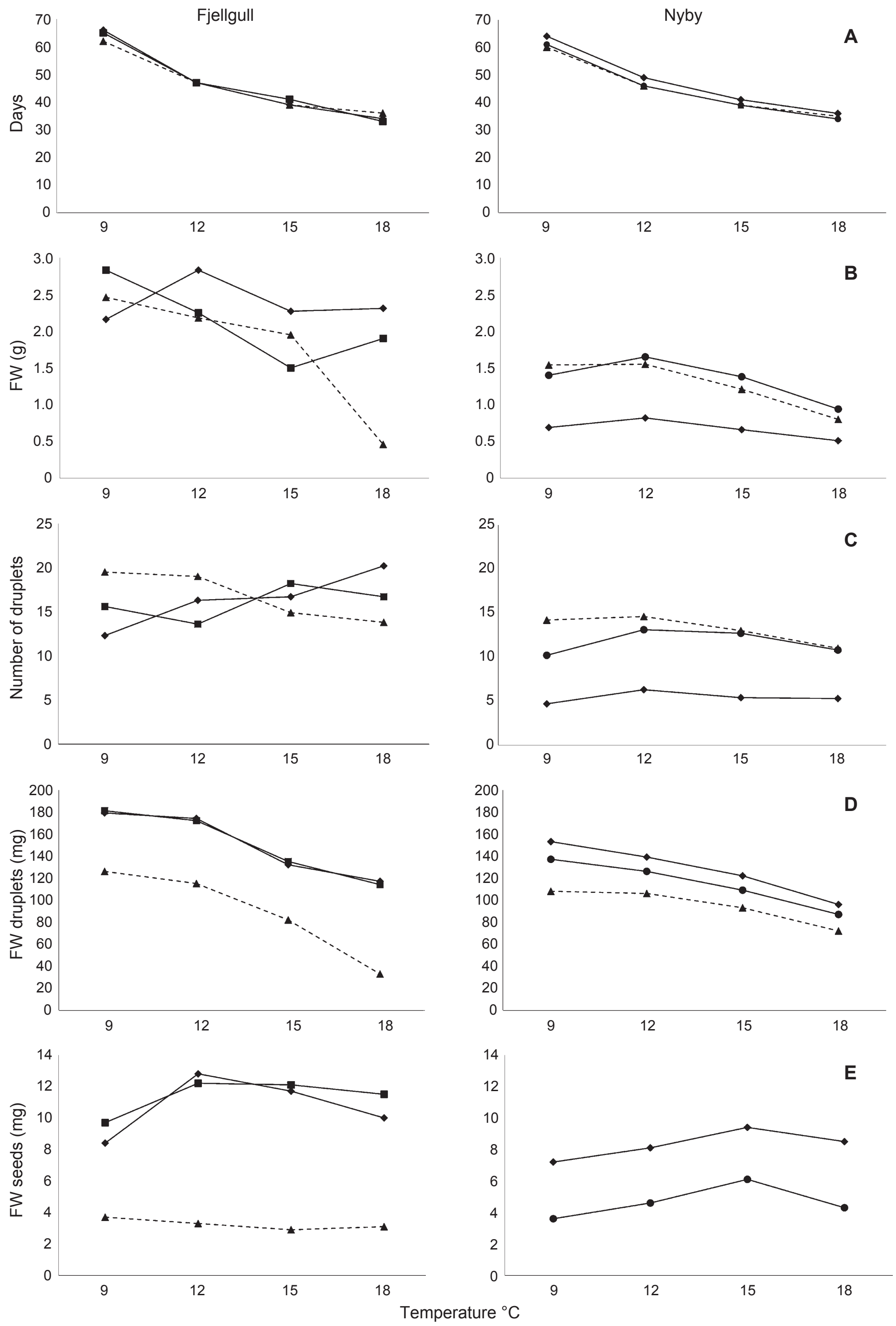

Fig. 1. Effects of temperature on development of berries in cloudberry cultivars ‘Fjellgull’ (left) and ‘Nyby’ (right). (A) Number of days from pollination/GA_3-treatment to ripening. (B) Fresh weight (FW) of berries in g. (C) Number of drupelets. (D) Fresh weight of drupelets in mg. (E) Fresh weight of seeds in mg. Symbols; (♦) for ‘Fjellgull’ pollinated med ‘Apollen’, ‘Nyby’ no treatment; (■) for ‘Fjellgull’ pollinated with ‘Nyby’; (•) for ‘Nyby’ self pollinated with brush, and (▲) treated with GA_3.

GA_3-treated 0.46 ± 0.38 g). However, at 9°C the GA_3-treated berries (2.46 ± 0.50) were comparable to pollinated berries (‘Apollen’ 2.16 ± 0.69, ‘Nyby’ 2.83 ± 0.64).

Unpollinated (treatment 1) flowers of ‘Nyby’ developed berries, but these were in average only half the size of berries obtained with assisted self-pollination or with GA_3, both with respect to fresh weight and number of developed drupelets (Fig. 1B and C). In unpollinated flowers generally only some of the peripheral carpels had been fertilized; these carpels are more or less covered by stamens. Following assisted self-pollination, most or all of the carpels developed into drupelets. As the stamens were not removed before treatment with GA_3, the treated berries had both parthenocarpic and pollinated drupelets. However, GA_3-treatment significantly increased the number of drupelets in ‘Nyby’ (pollinated 11.9 ± 3.9, GA_3-treated 13.8 ± 4.5, $p = 0.0009$), but fresh weight of berries was not significantly different in these treatments. In addition, the number of developed drupelets per berry was higher in ‘Fjellgull’ than in ‘Nyby’, and the fresh weight of single drupelets was approximately 30% higher in ‘Fjellgull’ than in ‘Nyby’ (Fig. 1D). The fresh weight of single drupelets increased significantly with decreasing temperature for both cultivars (Fig. 1D) whilst the fresh weight of single drupelets was significantly ($p = 0.0001$) smaller in GA_3-treated than in pollinated berries in both cultivars. In conclusion, total berry size was not significantly affected by GA_3-treatment although it increased the number of developing drupelets.

3.3. Seed size

Seeds from six berries from each treatment were carefully picked out with forceps, air dried and weighed. ‘Nyby’ had smaller seeds (seed plus endocarp) than ‘Fjellgull’ and for the pollinated berries the mean fresh weight of air-dry seeds was 11.4 mg and 8.3 mg ($p = 0.0001$) for ‘Fjellgull’ (pollinated with ‘Nyby’) and ‘Nyby’, respectively. In ‘Fjellgull’, pollen parent had significant effect on seed size, 11.4 mg and 10.8 mg ($p = 0.035$) for berries pollinated with ‘Nyby’ and ‘Apollen’, respectively (Fig. 1E). In GA_3-treated berries, all ‘Fjellgull’ seeds were without embryo, while in ‘Nyby’ only a proportion of the seed were without embryo and this was, of course, reflected in the average seed size in these treatments (Fig. 1E).

In cloudberry, seeds make a significant proportion of berry weight. Fresh weight of berries after removal of the seeds was determined and calculated as per cent of fresh weight of intact berries. This percentage was higher for ‘Nyby’ (76%) than for ‘Fjellgull’ (74%, $p = 0.009$), it was significantly ($p = 0.0001$) higher at 9°C than at other temperatures, and in ‘Fjellgull’ it was significantly ($p = 0.0001$) enhanced by GA_3-treatment.

3.4. Chemical analyses

The total contents of phenolic compounds were broadly similar in both cultivars. Significant effects of treatment (pollination) and seed removal were found. There was also significant effect of temperature, but this effect was not consistent. Phenolic levels were quite similar at 9°C and 15°C while at 12°C and 18°C the levels were markedly lower. The lack of consistency indicates that the temperature effect was likely due to random variation. Comparison of ‘Nyby’ with assisted self-pollinated against ‘Fjellgull’ pollinated by ‘Nyby’ showed a significant effect of clone ($p = 0.025$) and, in general, levels of total phenolic compounds tended to be higher in ‘Nyby’ than in ‘Fjellgull’ (Fig. 2). In ‘Fjellgull’ the highest levels were found in berries from 9°C, but in ‘Nyby’ the highest values were recorded for berries from 15°C (Fig. 3). Comparison of ‘Fjellgull’ pollinated by ‘Apollen’ against ‘Fjellgull’ pollinated by ‘Nyby’ showed a significant effect of male genotype ($p = 0.046$), where pollination by ‘Nyby’ gave enhanced levels of phenolic contents. This effect was clear in intact berries (Fig. 2A) ($p = 0.001$) but not in the berries from which the seeds were removed before freezing (Fig. 2B). In ‘Fjellgull’ there was also significant effect of GA_3-treatment (Fig. 2A). Again, this effect was observed only on intact berries. In ‘Nyby’, there was no effect of GA_3-treatment.

Conversely, female genotype, temperature and GA_3-treatment had significant effect on levels of anthocyanins (Fig. 3). Anthocyanin levels were about four times higher in ‘Fjellgull’ than in ‘Nyby’. In ‘Fjellgull’, the highest levels were found in berries from 9 and 12°C. This was corroborated by the outward appearance of the fruit with the berries of ‘Fjellgull’ exhibiting a strong reddish color the intensity of which increased with decreasing temperature (Fig. 4). However the berries of ‘Nyby’ were pale yellow, except at 9 and 12°C, where some berries had drupelets that displayed an orange-reddish tinge. GA_3-treatment reduced the anthocyanin levels in ‘Nyby’ ($p = 0.006$) when

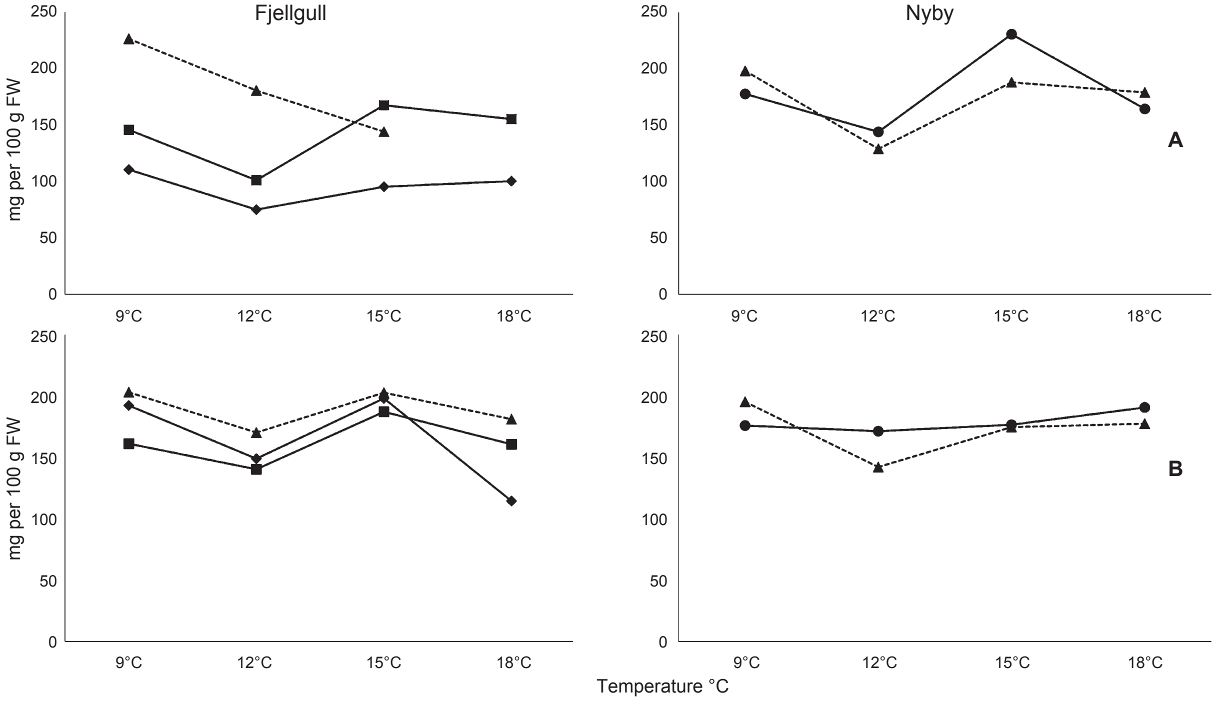

Fig. 2. Levels of total phenolic compounds (mg per 100 g fresh weight of berries) in berries of cloudberry cultivars ‘Fjellgull’ (left) and ‘Nyby’ (right), for intact berries (A) and seedless berries (B). Symbols; (♦) for ‘Fjellgull’ pollinated with ‘Apollen’; (•) for ‘Nyby’ self pollinated with brush; (■) for ‘Fjellgull’ pollinated with ‘Nyby’ and (▲) treated with GA_3.

only intact berries were included in the analysis (Fig. 3A). GA_3-treatment had no effect on the cultivar ‘Fjellgull’. Pollen parent had no significant effect on anthocyanin levels in ‘Fjellgull’.

4. Discussion

Both the female cultivar ‘Fjellgull’ and the hermaphrodite cloudberry cultivar ‘Nyby’ were able to produce berries over the temperature range from 18 to 9°C. Although ‘Nyby’ is self-pollinated, mechanical pollination, or insect pollination, is needed for good fruit set. Treatment with GA_3-solution also increased the number of fertilized drupelets. Hermaphroditic cultivars are beneficial for cultivation and allow the whole field to be planted with fruit bearing plants. However, compared to ‘Fjellgull’, ‘Nyby’ has rather small berries with pale color and low levels of anthocyanins making them distinctly different from what is perceived by the consumers and processors as ideal or “good” fruit [11, 21]. Berry size is an important yield and quality character, but the yield capacity is also dependent on the number of flowers/berries per unit area. This was not studied in the present investigation, but ‘Nyby’ grew vigorously and flowering was very abundant. Further analysis of the production capacity of these cultivars will be addressed in separate experiments.

The two cultivars ‘Fjellgull’ and ‘Nyby’ originates from Northern Norway (Ifjord, 70°N and 27°E, and West-Finland at about 63°N and 22°E). The mean temperature of June–September is about 7–12°C and 13–14°C in Ifjord and Nyby, respectively. In spite of the climatic differences at their original locations, the present results do not show any significant, consistent differences in temperature responses of these genotypes, which could indicate climatic adaptations between these two clones. However, at 9°C ‘Nyby’ flowered slightly later than the more northern ‘Fjellgull’, while there was no difference between the two genotypes at higher temperatures. Furthermore, there

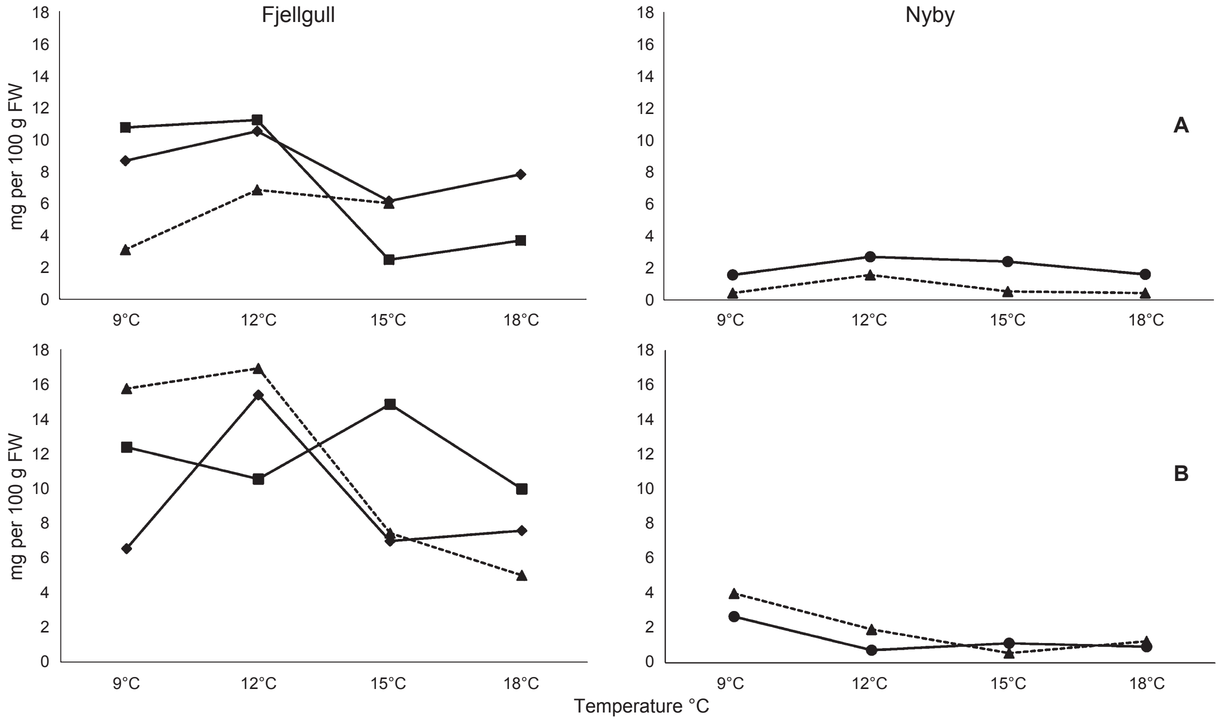

Fig. 3. Total anthocyanin levels (mg per 100 g fresh weight of berries) in cloudberry cultivars ‘Fjellgull’ (left) and ‘Nyby’ (right), for intact berries (A) and seedless berries (B). Symbols; (⧫) for ‘Fjellgull’ pollinated with ‘Apollen’; (•) for ‘Nyby’ self pollinated with brush; (■) for ‘Fjellgull’ pollinated med ‘Nyby’ and (▲) treated with GA_3.

Fig. 4. Ripe berries of ‘Nyby’ (left) and ‘Fjellgull’ grown at 12°C.

were no differences in ripening time at the different temperatures between ‘Fjellgull’ and ‘Nyby’. Berry size was highest in ‘Fjellgull’ at 9°C, while ‘Nyby’ produced the biggest berry at 12°C. The present results indicate that high temperatures, 18°C or more, have a negative impact on berry development in both cultivars. In particular, the fresh weight of individual drupelets was negatively correlated with temperature. In general, berry development in cloudberry is favoured by low and moderate temperatures. As cloudberry is distributed in northern boreal, subarctic and arctic regions, such responses could indicate adaptation of the species to those conditions.

Genetic differentiation in Finnish populations of cloudberry has been reported to be low [18]. Various clones derived from England (lat. 54°N) to Spitzbergen (lat. 78°N) showed only minor differences in growth behavior under different temperatures, although the northern clones initiated growth earlier than the southern ones [J. Nilsen, unpublished] [14]. Thus, so far we do not have experimental evidence for strong climatic adaptation between various cloudberry populations. On the other hand, the berries in the two cultivars were highly different both in respect to size, number of drupelets, color and chemical composition. In the present study, pollen parent had no significant effect on these characters (except for phenolic contents of 'Fjellgull' which was enhanced by pollination with 'Nyby' compared to 'Apollen'). These findings are significant and highlight the necessity to determine the variation in physiological and biochemical characters of cloudberry before further significant effort is put into the construction of an economically viable breeding program for cloudberry. Combination of beneficial berry characteristics found in 'Fjellgull' with hermaphroditism is, however, an obvious goal for breeding.

There was a significant effect of female genotype on total anthocyanins, the levels were higher in 'Fjellgull' than in 'Nyby'. Similar genetic differences have been reported for raspberry (*Rubus idaeus*) [3] and blueberry [7]. Differences in anthocyanin levels (Fig. 3) were reflected in color of the berries (Fig. 4). Total phenols were slightly higher in 'Nyby' than in 'Fjellgull'. No consistent effects of male genotype on the levels of total anthocyanins and phenols were detected. Connor et al. [7] reported heritability estimates for antioxidant capacity (0.43), total phenolics (0.46) and total anthocyanins (0.56) in blueberry (highbush blueberry, *Vaccinium corymbosum*) progeny, but also found that antioxidant capacity, total phenolics and total anthocyanins varied considerably over two growing seasons. Similar results have been reported for blueberry by Howard et al. [12]. Furthermore this effect has also been reported in raspberry, a sister species to cloudberry, by Kassim et al. [17], who found significant effects of harvest year and genotype × harvest year interaction for anthocyanin content. The consensus is that certain genotypes vary in their capacity to synthesize polyphenolics under different growing conditions. More extensive analyses are needed to map the genetic variation of chemical composition in cloudberry.

Temperature affected the level of total anthocyanins whilst there was no consistent effect of temperature on the level of total phenols. The results show that the content of total anthocyanins in cloudberry is positively related to low temperatures as described earlier in apple and pear peel [8, 26]. Contrary to this, Wang and Zheng [29] reported an increase in flavonol and anthocyanin content in strawberries with increasing day/night temperatures. In addition, Wang et al. [30] found that two cultivars of strawberry ('Earliglow' and 'Kent') grown at high day/night temperature (30/22°C) produced fruit with higher phenolic content, as well as antioxidant capacity, than fruits from plants grown at a lower day/night temperature (18/12°C). Rieger et al. [25] showed a decrease of anthocyanins in berries of *Vaccinium myrtillus* with rising altitude, and they explained this as a low temperature effect. There are no previous, systematic studies on effects of environmental conditions on chemical composition of cloudberry. Kähkönen et al. [16] found small but significant differences in levels of anthocyanins, flavonols, ellagitannins and total phenols in samples collected from different, distant localities in Finland, but these results confound both genetic and environmental effects. Määttä-Riihinen et al. [19] have reported significant differences in ellagitannins between two harvest years, but also these differences can be caused by several factors, not only climatic conditions. The environment had a significant effect on the levels of quercetin in raspberry, however, the variation was lower between growing seasons than between different locations [3].

The present study confirmed that parthenocarpy in cloudberry can be induced by GA_3 [15]. At low temperatures development of parthenocarpic berries of 'Fjellgull' was comparable to that of pollinated berries, but at 18°C only some of the treated flowers produced ripe berries. Nilsen [22] has reported similar results, but the interaction between GA_3 and temperature requires further focused research. No such interaction was observed with 'Nyby', but in this case only some of the drupelets were parthenocarpic, the others pollinated. In female clones, GA_3-treatment significantly reduced the size and weight proportion of seeds of intact berries and this can be considered as an improvement of quality. GA_3-treatment tended to increase the levels of total anthocyanins and phenols. GA_3-treatment has the potential, therefore, to rescue berry development in the case of incomplete pollination and will form part of the cloudberry breeder's tool box.

In conclusion, low temperature significantly enhances size and quality of cloudberries. The hermaphroditic 'Nyby' is stable at the temperature treatments used in this study, but successful fertilization is dependent on mechanical or insect pollination. From a consumer's point of view, berries of 'Fjellgull' are superior to those of 'Nyby'. No significant effect of male parent on berry size or on levels of total anthocyanins and phenols were observed. Breeding should therefore concentrate on the screening of female genotypes from nature and from crosses. An approach would be to cross female varieties with big berries and high concentrations of bioactive compounds with male varieties with vigorous rhizome growth and numerous shoots. There is, however, need for further studies on genetic variation of generative and vegetative characters in cloudberry.

Acknowledgements

The authors acknowledge funding from Graminor AS and the Norwegian Research Council MATPROGRAMMET. DS and GMcD thank the Scottish Government Rural and Environment Research and Analysis Directorate for their continued support. Leidulf Lund and the staff at the phytotron are acknowledged for assistance during the experiment.

References

[1] S.C. Andersson, K. Rumpunen, E. Johansson and M.E. Olsson, Tocopherols and tocotrienols in sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening, *J Agr Food Chem* **56** (2008), 6701–6706.

[2] Anon, Fineli® – Finnish Food Composition Database, Cloudberry entry, 2009. http://www.fineli.fi/food.php?foodid=449&lang=en (accessed on 11/18/2009).

[3] M.J. Anttonen and R.O. Karjalainen, Environmental and genetic variation of phenolic compounds in red raspberry, *J Food Compos Anal* **18** (2005), 759–769.

[4] M. Battino, J. Beekwilder, B. Denoyes-Rothan, M. Laimer, G.J. McDougall and B. Mezzetti, Bioactive compounds in berries relevant to human health, *Nutr Rev* **67** (2009), S145–S150.

[5] J.L. Brewbaker and B.H. Kwack, The essential role of calcium ion in pollen germination and pollen tube growth, *Am J Bot* **50** (1963), 859–865.

[6] E.M. Coates, C.I.R. Gill, G. Popa, M. McCann, G.J. McDougall, D. Stewart and I. Rowland, Colon-available raspberry polyphenols exhibit anti-cancer effects on *in vitro* models of colon cancer, *Carcinogenesis* **6** (2007), 1–13.

[7] A.M. Connor, J.J. Luby, B.S. Tong, C.E. Finn and J.F. Hancock, Genotypic and environmental variation in antioxidant capacity, total phenolic content, and anthocyanin content among blueberry cultivars, *J Am Soc Hortic Sci* **127** (2002), 89–97.

[8] J.D. Faragher, Temperature regulation of anthocyanin accumulation in apple skin, *J Exp Bot* **34** (1983), 1291–1298.

[9] M. Gustafsson and J. Kortesharju, Occurrence of hermaphroditism in a dioecious plant, *Rubus chamaemorus*, in northern Finland, *Aquilo Series Botanica* **36** (1996), 53–60.

[10] S. Häkkinen, M. Heinonen, S. Kärenlampi, H. Mykkänen, J. Ruuskanen and R. Törrönen, Screening of selected flavonoids and phenolic acids in 19 berries, *Food Res Int* **32** (1999), 345–353.

[11] P. Holloway, Managing wild bog blueberry, lingonberry, cloudberry and crowberry stands in Alaska, *Plant Management Notes*, U.S. Department of Agriculture Natural Resources Conservation Service, 2006.

[12] L.R. Howard, J.R. Clark and C. Brownmiller, Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season, *J Sci Food Agr* **83** (2003), 1238–1247.

[13] E. Hulten, The Flora of Alaska and the Yukon, *Lunds Univ Arsskr N F Avd 2 Bd* **45**(1) (1950), 416 pp.

[14] O. Junttila, J. Nilsen and K. Rapp, Research on cloudberry in Norway, in: Luonnonmarja- ja sienitutkimuksen seminaari, osa I, *Metsäntutkimuslaitoksen Tiedonantoja* **90** (1983), 23–33.

[15] O. Junttila, I. Martinussen, G. Nilsen, A. Ernstsen and T.V. Bhuwaneswari, Parthenocarpic fruit development in cloudberry (*Rubus chamaemorus* L.) is induced by 3β-hydroxylated-gibberellins, *J Hortic Sci Biotech* **77** (2002), 9–12.

[16] M.P. Kähkonen, A.I. Hopia and M. Heinonen, Berry phenolics and their antioxidant activity, *J Agr Food Chem* **49** (2001), 4076–4082.

[17] A. Kassim, J. Poette, A. Paterson, D. Zait, S. McCallum, M. Woodhead, K. Smith, C. Hackett and J. Graham, Environmental and seasonal influences on red raspberry anthocyanin antioxidant contents and identification of quantitative traits loci (QTL), *Mol Nutr Food Res* **53** (2009), 625–634.

[18] H. Korpelainen, K. Antonius-Kleemola and G. Werlemark, Clonal structure of *Rubus chamaemorus* populations: Comparisons of different molecular methods, *Plant Ecol* **143** (1999), 123–128.

[19] K.R. Määttä-Riihinen, A. Kamal-Eldin and A.R. Törrönen, Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (Family *Rosaceae*), *J Agr Food Chem* **52** (2004), 6178–6187.

[20] G.J. McDougall, H.A. Ross, M. Ikeji and D. Stewart, Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown *in vitro*, *J Agr Food Chem* **56** (2008), 3016–3023.

[21] G.S. Nilsen, Cloudberry: The northern gold, *International Journal of Fruit Science* **5** (2005), 45–60.

[22] G.S. Nilsen, Role of plant hormones in parthenocarpic development of Rubus chamaemorus fruit and in *in vitro* propagation of the plant, Ph.D. Dissertation, University of Tromsø, Norway, 2008.

[23] R. Puupponen-Pimiä, L. Nohynek, S. Hartmann-Schmidlin, M. Kähkönen, M. Heinonen, K. Määttä-Riihinen and K.-M. Oksman-Caldentey, Berry phenolics selectively inhibit the growth of intestinal pathogens, *J Appl Microbiol* **98** (2005), 991–1000.

[24] K. Rapp and I. Martinussen, Breeding cloudberry (*Rubus chamaemorus* L.) for commercial use, *Acta Hortic* **585** (2002), 159–160.

[25] G. Rieger, M. Muller, H. Guttenberger and F. Bucar, Influence of altitudinal variation on the content of phenolic compounds in wild populations of *Calluna vulgaris*, *Sambucus nigra*, and *Vaccinium myrtillus*, *J Agr Food Chem* **56** (2008), 9080–9086.

[26] W.J. Steyn, S.J.E. Wand, G. Jacobs, R.C. Rosecrance and S.C. Roberts, Evidence for a photoprotective function of low-temperature-induced anthocyanin accumulation in apple, *Plant Physiol* **136** (2009), 461–472.

[27] B. Thiem, Flow cytometric analysis of nuclear DNA content in cloudberry (*Rubus chamaemorus* L.) *in vitro* cultures, *Plant Sci* **164** (2003), 129–134.

[28] P.G. Walker, S.L. Gordon, R.M. Brennan and R.D. Hancock, High-throughput monolithic HPLC method for rapid vitamin C phenotyping of berry fruit, *Phytochem Analysis* **17** (2006), 284–290.

[29] S.Y. Wang and W. Zeng, Effect of plant growth temperature on antioxidant capacity in strawberry, *J Agr Food Chem* **49** (2001), 4977–4982.

[30] S.Y. Wang, W. Zheng and J.L. Maas, High plant growth temperatures increase anioxidant capacities in strawberry fruit, *Acta Hortic* **626** (2003), 57–63.