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Chilling requirements of contrasting black currant (*Ribes nigrum* L.) cultivars and the induction of secondary bud dormancy

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ABSTRACT

Chilling requirements for bud dormancy release and subsequent flower development in contrasting black currant (*Ribes nigrum* L.) cultivars were assayed at temperatures ranging from +10 to −10 °C using single node cuttings, entire detached shoots, and intact plants. While single node cuttings underestimated the chilling requirements of intact plants, severed shoots and intact plants produced similar results. Chilling at −5 °C for 14 weeks or more were optimal for breaking of bud dormancy and promotion of flower development in most cultivars, flower development usually having greater chilling needs than bud break itself. Within certain limits, extension of the chilling period compensated for non-optimal chilling temperature. However, while exposure to −10 °C for 8 weeks caused bud dormancy release, continued chilling for another 8 weeks inhibited bud break completely. We propose that excessive chilling induces secondary bud dormancy, a principle that is well established in seeds, but has to our knowledge, not been recognized for bud dormancy before. Marked genotypic differences were found and discussed. The observed severe chilling requirements of black currants concur with the reported vulnerability of this crop to declining winter chill in the wake of the ongoing global warming. Furthermore, elevated autumn temperature was found to induce a particularly deep dormancy state that further increases the chilling need.

1. Introduction

Winter dormancy is an important adaptive strategy in perennial plants living in the temperate and cold regions of the world. Induction of and release from dormancy are governed by seasonal environmental signals such as photoperiod and temperature (Vegis, 1965). Short days (SD) and/or low temperature induce growth cessation and bud dormancy, while release from the dormant condition requires exposure to chilling at near-freezing temperatures and is usually promoted by long day (LD) conditions (Vegis, 1965; Heide, 1993; Howe et al., 1999; Basler and Körner, 2014). Dormancy is a condition that is gradually acquired and dissipated, resulting in a dynamic change of dormancy states (Lang et al., 1987). Because of seasonal changes in the dormancy-regulating environmental factors, dormancy is also strictly seasonal. In the northern hemisphere, dormancy is deepest in late autumn and is gradually lost during winter (Vegis, 1965). When studying chilling requirements of field-grown plants, it is therefore, important that chilling treatments are started when dormancy is maximal. In black currant, this dormancy state is attained in late October in the Nordic environment (Måge, 1976).

Dormancy regulation is of large ecological significance in nature and has important practical implications in horticulture when florist and fruit crops are manipulated to grow, flower and fruit outside their natural growing season. The predicted and ongoing climatic change (Solomon et al., 2007) may also strongly inflict on plant dormancy relations due to reduced chilling potential of milder winters. Declining levels of winter chill has thus been identified and symptoms of inadequate winter chill such as erratic bud break and reduced shoot growth, reduced and uneven flowering, and reduced fruit yields have been observed in a range of perennial fruit species in both Europe (Sunley et al., 2006; Atkinson et al., 2013) and North America (Baldocchi and Wong, 2008). The black currant (*Ribes nigrum* L.) is a soft fruit species that in recent years has been reported to be notably affected by inadequate winter chill (Jones et al., 2012; Atkinson et al., 2013). In the UK, there is particular concern regarding the ability of black currant crops to produce consistent crops under the present scenario of global warming (Jones et al., 2012). Examination of historical climate and cropping data showed that black currant fruit crops are at potential

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risk in mild winter areas of Europe due to the ongoing and forecasted projections of declining winter chill (Atkinson et al., 2004). Severe problems with inadequate winter chill in black currant crops have also been experienced in New Zealand, where development of low chill cultivars are given high priority (Snelling and Langford, 2002).

An early study of the chilling requirements of black currant was conducted by Hoyle (1960) using potted plants of cv. Boskoop Giant. She found that both near-freezing temperatures and LD were effective in breaking of bud dormancy in black currant. At a temperature of 3.3 °C, bud-break was progressively advanced with increasing duration of exposure for up to 15 weeks. However, in a recent study, Jones et al. (2012) concluded that "for blackcurrants, the effectiveness of chilling increases as temperature falls to 0 °C and even below for most cultivars". It has also been found that the chilling requirement and the optimal chilling temperature can vary considerably among commercial cultivars (Plancher and Dördrechter, 1983; Rose and Cameron, 2009; Jones et al., 2012; Atkinson et al., 2013). As an example, Rose and Cameron (2009) reported that temperature optima for chilling effectiveness varied from +2.2 °C in 'Ben Hope' to −3.4 °C in 'Ben Tirran'. However, as pointed out by Lantin (1973), cultivars with low chilling requirement also carry an increased risk of spring frost damage at anthesis which was identified by Atkinson et al. (2013) as one of the main limitations to commercial black currant production in the UK. Although the study by Sunley et al. (2006) revealed that also the frequency and severity of spring frost events have declined in UK as a result of the recent climatic warming, future black currant breeding programs will probably have to compromise between reduced chilling requirements and spring frost avoidance.

Assessment of chilling requirements for dormancy release is complicated by modifications of the dormant state by certain external factors. Thus, warm temperatures during SD dormancy induction in autumn result in a particularly deep and stable state of dormancy that is manifest as an increased chilling requirement and delayed bud-burst in spring. This has been demonstrated in black currant (Sønsteby and Heide, 2011; Sønsteby et al., 2012) and other soft fruit species (Måge, 1975; Palonen, 2006) as well as in a range of other temperate woody plants (Heide, 2003). Therefore, the dormancy state of field-grown plants can vary considerably between years, thus underlining the importance of repeating this type of experiments over several years. Furthermore, it has also been demonstrated that excessive chilling actually can inhibit subsequent development of black currant buds under permissive temperature conditions (Jones et al., 2012), a phenomenon termed "over-chill" by these authors. Such findings may, at least in part, explain why simulation models involving responses to accumulated chill often have failed to adequately describe observed chilling responses in black currant (Sunley et al., 2006; Rose and Cameron, 2009) and other temperate woody plants (cf. Linkosalo et al., 2006).

Because of the continuing uncertainty of the chilling requirements of black currant cultivars and the recent problems with inadequate winter chill of black currant crops in many places of the world, we have carried out a series of experiments under well controlled environmental conditions with intact plants and detached shoots of a range of cultivars of widely different origin over a period of three years. The aim of the investigations was to more accurately determine the critical and optimal chilling temperatures and chilling periods for dormancy release in black currant genotypes, and to verify the modifying effect of the dormancy-inducing environment on the dormant state. A particular objective was to explore the evidence for secondary dormancy in buds, a principle that is well established in seed dormancy (Bewley and Black, 1994), but which to our knowledge, has not been recognized in buds of any species. The results of these experiments are presented and discussed below.

2. Material and methods

2.1. Plant material and handling

Field-grown annual shoots of a range of cultivars of widely different geographic origin (Sønsteby and Heide, 2013) were sampled in late autumn from a cultivar repository at the Bioforsk Experimental Centre Apelsvoll in the central part of South Norway (60°40′N). In a preliminary experiment in the 2010/2011 season, shoots of the cultivars 'Murmanschanka', 'Öjebyn', 'Kristin', 'Ben Tron', 'Ben Hope', 'Tiben', and 'Narve Viking' were sampled on 3 November and stored at 2 °C for 4–20 weeks before forcing as single node cuttings. Then, in the 2011/2012 season, shoots of the cultivars 'Imandra', 'Hedda', 'Ben Tron', and 'Narve Viking' were sampled on 19 October and stored at temperatures ranging from +10 °C to −10 °C for 4, 8, 12, and 16 weeks before forcing as single node cuttings. In the same season, shoots of the cultivars 'Ben Tron', 'Ben Alder' and 'Narve Viking' were sampled on 17 October and stored at temperatures ranging from +10 °C to −10 °C for 7, 14 and 21 weeks and forced as entire shoots. Finally, the latter experiment was repeated in the 2013/2014 season under identical conditions.

In all instances the temperature variation in the cold stores was maintained within ±1 °C. When sampled, the shoots were cut at the base, bunched and equipped with a plastic bag with a wad of moist tissue paper around the cut ends, and wrapped in a sheet of polyethylene. The bunches were stored in the dark at the various temperatures for periods as indicated for each experiment. Single node cuttings of 5–6 cm length and comprising one node with its subtending internode were cut from the middle part of stored shoots (five cuttings from each shoot). The cuttings were forced in a growth room maintained at 20 ± 2 °C and continuous light (approx. 40 μmol quanta m^{-2} s^{-1}) provided by warm-white fluorescent tubes (Philips TL 32). The cuttings were inserted in holes in pads of wet plastic foam contained in plastic flats with a 1 cm layer of tap water (see Fig. 3). The entire shoots were forced in a greenhouse with minimum temperature of 20 °C and 24 h photoperiod (daylight + continuous artificial light provided by 70 W incandescent lamps and Philips 400 W HPI T metal halide lamps at a flux of ~200 μmol quanta m^{-2} s^{-1}). A fresh cut was made at the base of the shoots immediately before they were placed in plastic buckets with tap water containing 1% Floralife 300 (Smithers-Oasis, UK Ltd.). During forcing, the water solution was changed weekly and a fresh cut made at the base of each shoot.

For experiments with intact plants, single-stemmed potted plants of the cultivars 'Imandra', 'Ben Tron', and 'Narve Viking' were propagated and raised in a greenhouse during the summer under LD conditions as described by Sønsteby and Heide (2011). On 10 September, when the plants had a height of about 100 cm and 25–30 leaves, the plants were moved into daylight compartments of the Ås phytotron for dormancy induction at temperatures of 9, 15, or 21 °C and natural day-lengths (59°40′N). After 6 weeks under these conditions, the plants were defoliated and placed in the dark in a cold store at 0 ± 1 °C for 5, 10, or 15 weeks for breaking of dormancy. During cold storage, the plants were covered by a plastic hood to prevent desiccation.

After completion of the cold storage, plants and shoots were allowed to thaw and acclimate to the forcing conditions by stepwise increasing temperatures of 0, 5, 10, and 20 °C over a period of 5 days. Potted plants were watered twice with temperate tap water during the acclimation period, while a hole was cut in the plastic wrappings of the shoot bunches to allow equilibration with the external air. The plants were then moved into a heated greenhouse and forced at min. 20 °C and 24 h photoperiod under the same light conditions as described above for entire shoots.

A separate experiment was carried out with intact plants of the cv. Murmanschanka in order to further expose the effect of autumn

Fig. 1. Three successive stages of bud break in a black currant cutting. Stage 2 was recorded as bud break.

temperature on bud break and flowering under natural spring conditions. Plants were raised as described above and induced to growth cessation and floral initiation under natural day-length conditions at Ås from 24 August at temperatures of 9, 15, and 21 °C. After 6 weeks of cultivation under these conditions, the plants were chilled for 27 weeks at 0 ± 1 °C (to simulate a full winter), and then allowed to reinitiate growth in a plastic greenhouse maintained at 8 ± 5 °C and 24 h photoperiod (natural day-length + extension with incandescent lamps at approx. 10 μmol quanta m^{-2} s^{-1}).

2.2. Experimental design, data collection and analysis

The experiments were factorial, with a split plot design, with chilling temperatures as main plots and with chilling durations and cultivars as subplots. The experiments were replicated in three blocks, each containing five single node cuttings, or three entire shoots or potted plants of each cultivar in each treatment.

During forcing, bud break was observed three times weekly and scored according to the stages shown in Fig. 1. Stage 2 was considered as bud break. In addition, when the observations were terminated, the number and distribution of sprouted buds were recorded along the entire shoot length of severed shoots and rooted plants, as well as the number of flowers at each node.

The data were subjected to analysis of variance (ANOVA) by standard procedures using a MiniTab® Statistical Software program package (Release 16; Minitab Inc., State College, PA, USA). Percentage values were always subjected an arc sin transformation before performance of the ANOVA.

3. Results

3.1. Results with single node cuttings

The results of the preliminary experiment with single node cuttings of seven cultivars are shown in Fig. 2. With 4 weeks of chilling at 2 °C, only cuttings of 'Ben Tron' and 'Murmanschanka' had a high proportion of buds that reached stage 2 in less than 16 days. All the other cultivars exhibited varying degrees of erratic bud burst, and needed on average of 21 to 25 days to reach stage 2. When the chilling period was extended to 6 or more weeks, all healthy buds were breaking and strongly advanced in all cultivars except Narve Viking. With more than 8 weeks of chilling, the advancement of budding levelled off in all cultivars except for Narve Viking which was notably slow and displayed an advancement of bud break with increasing chilling time all the way up to 20 weeks.

The results of a similar experiment with single node cuttings cut from shoots of four cultivars stored at varying temperatures for 4 to 16 weeks are shown in Fig. 3. The results show highly significant main effects of chilling temperature, chilling period, and cultivar

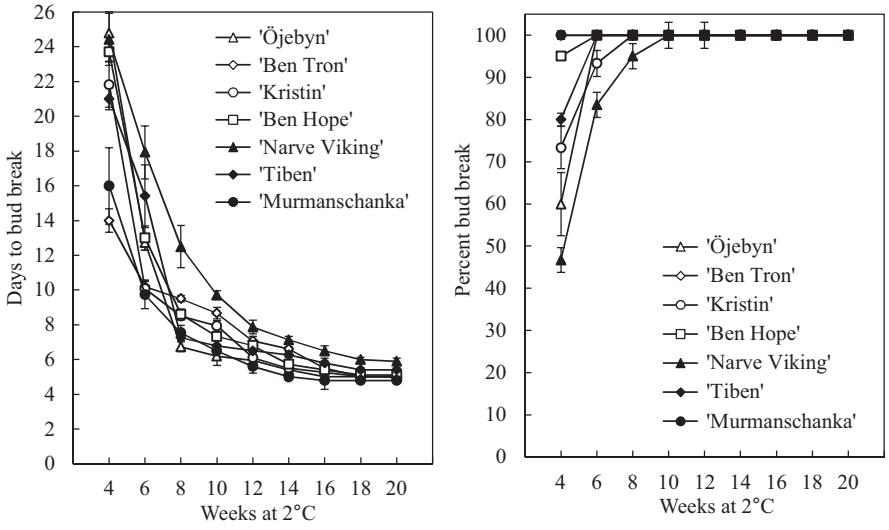

Fig. 2. Bud break in single node cuttings of seven black currant cultivars as influenced by increasing length of chilling at 2 °C. Values are the means ± SE of three replicates, each with five cuttings of each cultivar in each treatment.

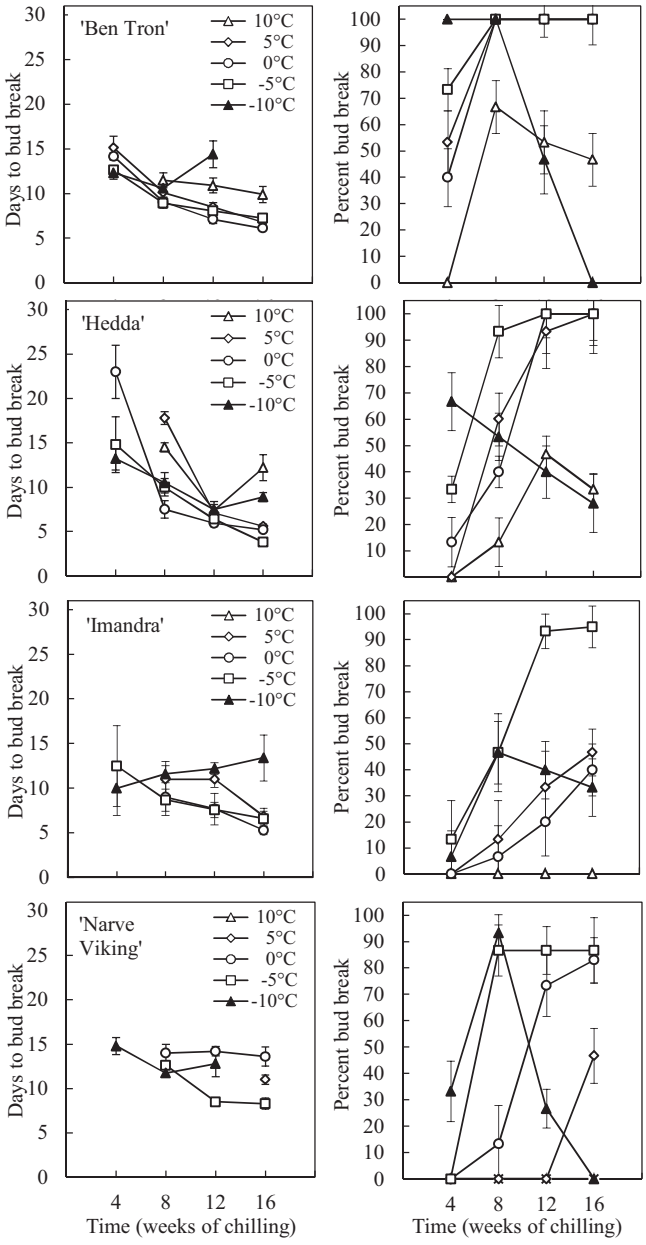

Fig. 3. Bud break in single node cuttings of four black currant cultivars as influenced by increasing length of chilling at five different temperatures as indicated. Values are the means ± SE of three replicates, each with five cuttings of each cultivar in each treatment.

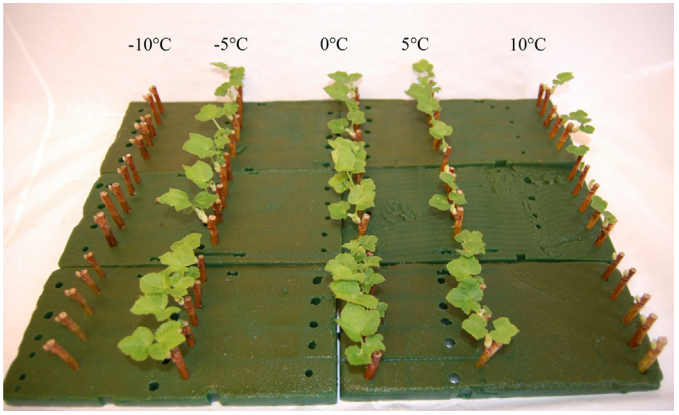

Fig. 4. The experimental system used for single node cuttings. The photo shows cuttings of the cultivar 'Ben Tron' chilled for 16 weeks at the temperatures indicated and forced at 20 °C and 24 h photoperiod for 25 days. Note the dormant state of the buds chilled at −10 °C for such extended time.

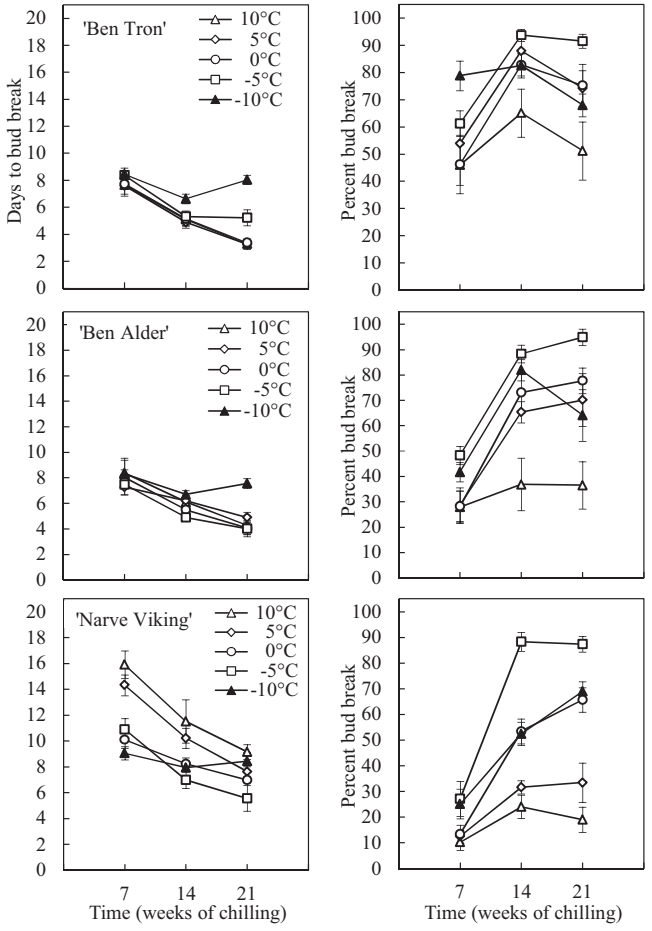

Fig. 5. Bud break in entire shoots of three black currant cultivars as influenced by increasing length of chilling at five different temperatures as indicated. Values are the means ± SE of two experiments in two different years, each with three replicates with five shoots of each cultivar in each treatment.

Table 1
Probability levels of significance for the main effects and interactions of chilling temperature, chilling period, and cultivar on the percentage and earliness of bud break in single node cuttings of four black currant cultivars (cf. Fig. 3).

Source of variation	Days to bud	Bud break
(ANOVA)	break	(%)
Length of chilling (*A*)	<0.001	<0.001
Temperature (*B*)	<0.001	<0.001
Cultivar (*C*)	<0.001	<0.001
A × *B*	<0.001	<0.001
A × *C*	0.004	0.001
B × *C*	<0.001	0.03
A × *B* × *C*	0.001	0.002

as well as significant two- and three-factor interactions of these factors (Table 1). As in the previous experiment, 'Ben Tron' had the lowest chilling requirement, while 'Narve Viking' and 'Imandra' had the largest requirements. Thus, in these latter cultivars, the 10 °C temperature was unable to break bud dormancy even when storage was extended to 16 weeks. Overall, −5 °C was the optimal temperature for bud dormancy release in all cultivars. The only exception occurred with 4-weeks of storage when −10 °C

Fig. 6. Appearance of shoots of 'Ben Tron' and 'Narve Viking' black currants chilled for 14 weeks at temperatures of 10, 5, 0, −5, and −10 °C (from left to right) and forced for 21 days at 20 °C in 24 h photoperiod. Note the effect of low chilling temperature on the advancement of bud break towards the base of the shoots of 'Narve Viking'.

usually was the most effective temperature. Generally, the proportion of breaking buds increased when storage was extended from 4 to 8 and 12 weeks and then, in most cases, remained even with further extension to 16 weeks. This was accompanied by a parallel reduction in the number of days to bud burst. However, at −10 °C, the proportion of breaking buds always declined significantly when the storage period was extended beyond 8 weeks. Thus in 'Ben Tron', the percentage of burst buds declined linearly from 100% with 4 and 8 weeks of chilling at −10 °C to zero when the chilling was extended to 16 weeks (Figs. 3 and 4). To a lesser extent, declining bud break was also observed with extended chilling periods in buds from shoots stored at +10 °C.

3.2. Results with entire shoots

Much the same results were obtained with forcing of entire cut shoots that had been stored at the same temperatures for 7, 14, and 21 weeks. Since the results were not significantly different in the two years of experimentation, they are presented as means of the two years (Fig. 5). As with single node cuttings, 'Ben Tron' had the lowest chilling requirement, and again, −5 °C was the most efficient temperature for breaking of bud dormancy, except with the shortest storage period, when −10 °C was equally effective. Chilling at 10 °C always had poor dormancy-breaking effect, and in 'Narve Viking' even 5 °C was only moderately effective (Fig. 5). Enhancement of bud break was greatest when storage was extended from 7 to 14 weeks, whereupon the effect usually levelled off, except at marginally effective storage temperatures in the slow-responding cultivars 'Narve Viking' and 'Ben Alder'. However, as in the previous experiment with single node cuttings, storage for extended periods of time at −10 °C resulted in declining and delayed bud burst, except in the case of 'Narve Viking'. The marked difference in chilling requirements of the cultivars 'Ben Tron' and 'Narve Viking' is illustrated in Fig. 6. The photos also illustrate the different chilling requirements of buds located at different positions along the shoot. When chilling was marginal, only the buds at or near the top were released from dormancy while, as the amount of chilling increased, dormancy release was spreading down the entire length of the shoot. In both cultivars, chilling at −5 °C was optimal for facilitating this process which is a prerequisite for high fruit yield. The late dormancy release of buds located at the middle part of the shoot explain why single node cuttings cut from the middle part of 'Narve Viking' shoots stored at 10 °C remained dormant even with extended periods of exposure (cf. Figs. 3 and 6).

Also flower development was strongly influenced by the chilling treatments in parallel with the effects on bud burst (Table 2). In all cultivars, and across the range of chilling temperatures, the percentage of flowering buds and the number of flowers per shoot and per flower truss increased with increasing amount of chilling. However, except in the case of 'Narve Viking', the increase usually levelled off at 14 weeks of chilling. The number of days to anthesis decreased in tandem. In general, the chilling requirement for flower development was somewhat larger than that for bud break, the −10 °C temperature usually being optimal. The results in Table 2 also show that flowering was less abundant in 'Ben Alder' than in the other two cultivars, an effect that can be related to differences in the timing of and temperature requirements for floral initiation in the autumn (cf. Sønsteby and Heide, 2013).

3.3. Results with intact plants

The growth responses of three black currant cultivars exposed to temperatures of 9, 15, or 21 °C and natural day-length at Ås from 10 September are shown in Fig. 7. Depending on cultivar and temperature conditions, elongation growth started to level off after 1 to 2 weeks followed by complete growth cessation within a few more weeks. The response was particularly fast in 'Imandra' which came to a complete growth cessation after 2 weeks at 21 °C. At lower

Table 2
Effects of chilling temperature and chilling period on subsequent flower development in shoots of three black currant cultivars when forced at 20 °C under LD conditions.

Length of storage (weeks)	Storage temp. (°C)	'Ben Tron'				'Ben Alder'				'Narve Viking'			
		Flowering buds (%)	Days To anthesis	Total no. of flowers	Flowers per truss	Flowering buds (%)	Days to anthesis	Total no. of flowers	Flowers per truss	Flowering buds (%)	Days to anthesis	Total no. of flowers	Flowers per truss
7	10	19.5	23.3	16.7	4.4	0.0	>50	–	–	0.0	>50	–	–
	5	29.7	21.0	26.0	4.5	1.5	35.0	2.0	2.0	4.5	45.0	5.0	1.7
	0	26.0	21.0	36.0	4.3	1.7	20.7	9.0	3.5	6.0	21.3	6.3	4.2
	−5	23.2	20.7	17.3	4.5	1.9	34.5	2.0	4.0	14.7	22.0	15.0	4.1
	−10	46.7	20.7	46.7	5.0	1.2	20.0	5.0	5.0	7.9	22.0	9.5	3.2
	Mean	29.0	21.3	28.5	4.5	1.1	26.6	5.4	3.6	6.6	25.0	7.2	2.6
14	10	14.4	14.8	16.4	5.9	0.9	>50	3.0	3.0	6.3	21.3	13.0	5.0
	5	20.6	14.3	23.0	3.6	9.8	16.0	5.0	3.9	5.3	20.0	10.5	5.3
	0	35.7	15.2	55.0	6.3	5.3	12.8	7.5	4.8	31.5	17.5	31.3	5.3
	−5	33.9	14.2	47.2	6.5	10.5	17.8	11.2	5.2	34.0	14.0	54.4	5.7
	−10	69.5	17.7	77.7	6.6	18.2	15.6	19.2	4.8	38.2	19.2	47.8	6.8
	Mean	34.8	15.4	46.0	5.9	7.5	15.5	7.7	4.7	23.1	18.1	34.5	5.7
21	10	11.0	12.5	16.8	5.3	0.0	>50	–	–	6.0	19.5	26.5	6.1
	5	10.8	13.0	22.7	6.3	6.8	17.8	9.0	4.3	13.2	16.7	38.0	6.8
	0	25.5	14.8	31.6	6.2	11.1	13.7	21.0	3.8	38.2	16.3	50.7	7.5
	−5	35.3	14.3	37.8	5.4	22.4	14.2	24.8	5.4	40.0	15.4	50.1	7.3
	−10	48.6	15.2	52.3	7.8	15.9	17.0	36.0	5.7	43.0	15.8	48.7	6.8
	Mean	26.2	14.2	34.8	6.3	11.2	15.6	18.2	4.9	23.4	14.0	35.7	6.9
Probability levels of significance (ANOVA)													
Source of variation													
Temperature (*A*)		<0.001	n.s.	0.003	0.006	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
Storage time (*B*)		0.04	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$A \times B$		n.s.	0.02	0.003	0.004	n.s.	<0.001	0.02	0.04	n.s.	<0.001	n.s.	<0.001

Data are the means of three replicates, each with three entire shoots of each cultivar in each treatment. n.s—not significant.

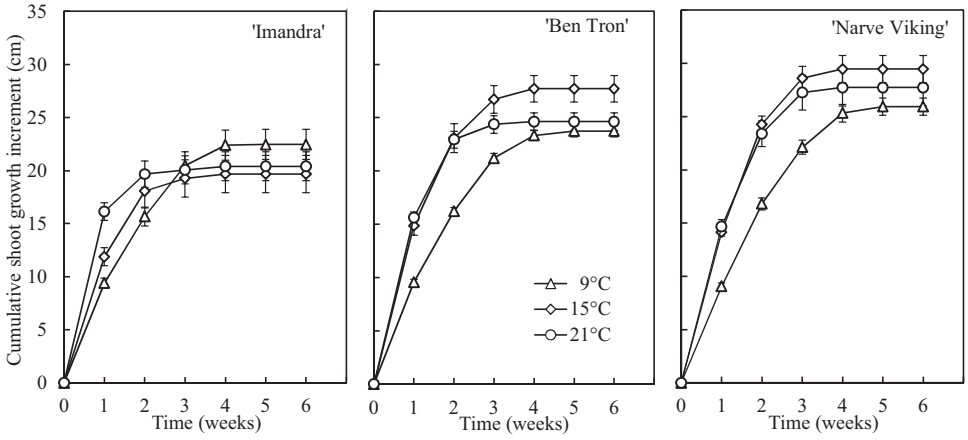

Fig. 7. Time-courses of shoot growth and cessation in three black currant cultivars as affected by the temperatures indicated under natural autumn day-length at Ås (from 10 September onwards). Values are the means ± SE of three replicates, each with five plants of each cultivar in each treatment.

temperatures, the response was gradually slower so that at 9 °C, growth continued for two more weeks. The same temperature responses were found in 'Ben Tron' and 'Narve Viking' which followed behind in due course. Thus, growth cessation in 'Narve Viking' took five weeks at 9 °C.

After 6 weeks of exposure to these conditions, the plants were chilled at 0 °C for 5, 10, or 15 weeks, whereupon they were forced at 20 °C and LD conditions as described above. The results in Table 3 and Fig. 8 show highly significant effects of autumn temperature and length of chilling on both the percentage of breaking buds and the earliness of bud burst and anthesis. However, due to a highly significant interaction of autumn temperature and length of chilling on the percentage of breaking buds, the main effect of autumn temperature was not significant for that parameter. Autumn temperature also had a highly significant effect on the number of flowers per plant and per flower truss, the numbers increasing with increasing temperature, while length of the chilling period had no significant effect. There was also a significant cultivar effect on all the flowering parameters, flowering being more abundant in 'Ben Tron' than in the other cultivars. Due to differences in the optimal autumn temperature among the cultivars, there was also a significant interaction of cultivar and autumn temperature on both abundance and earliness of flowering (Table 3).

In the high-latitude cultivar Murmanschanka, there was an immediate reduction in growth rate when the plants were exposed to natural autumn day-length, and after 2 weeks, growth had come to a complete stop at all temperatures (data not shown). After 27 weeks of cold storage, buds of the plants from 9 °C had reached stage 2 on the day when they were removed from chilling conditions, whereas in plants from 15 and 21 °C, bud burst was delayed by 7 and 14 days, respectively (Table 4). The percentage of breaking and flowering buds decreased with increasing autumn temperature with a concurrent significant delay in the timing of bud burst and anthesis. On the other hand, the number of flowers per plant and flower per truss were not significantly affected by autumn temperature in this high-latitude cultivar which has a low temperature optimum for flower initiation (Table 4).

4. Discussion

In concurrence with the findings of White et al. (1999) for raspberry, the present results show that the chilling requirement for bud dormancy release is underestimated when assayed as single node cuttings (Fig. 2). The underlying physiological mechanism is that, when severed, the buds are released from both apical dominance and the mutual correlative inhibition between adjacent buds. Because of this, buds of single node cuttings are able to burst with less chilling. It should be noted however, that in the present experiments in which single node cuttings were cut only from the middle part of the shoot where dormancy release is particularly late (Fig. 6), single node cuttings often had greater chilling requirements than entire shoots (cf. Figs. 3 and 5). When using multi-bud hard-wood cuttings as used and recommended by Jones et al. (2012), it should be kept in mind that cuttings from different parts of the shoot have divergent chilling requirements. On the other hand, the concurrent results with chilling and forcing of intact plants and severed entire shoots, demonstrate that the results obtained with the latter technique are quite representative for intact plants and is therefore recommended.

All the studied black currant cultivars had generally low optimum temperatures for breaking of bud dormancy, temperatures of −5 and −10 °C being optimal. This concurs with the results of Jones et al. (2012), who found that temperatures of 0 °C and below were optimal in the wide range of cultivars used by them. This is below the +5 to −0 °C range which is considered the general temperature optimum for bud dormancy release in temperate woody plants (Vegis, 1965). This low temperature optimum may explain why black currant has proved particularly vulnerable to the recent declines in winter chilling (Jones et al., 2012; Atkinson et al., 2013). Within certain limits, extended chilling periods could compensate for non-optimal chilling temperatures. However, as previously reported by Jones et al. (2012), excessive chilling at sub-zero temperatures were often inhibitory to bud break. In the present experiments this occurred in all tested cultivars, but mainly at −10 °C (Figs. 3 and 5), while in the study by Jones et al. (2012) it was limited to certain (older) cultivars and with chilling at −5 °C, which was the lowest temperature used by these workers. In the present experiments with single node cuttings, the effect of excessive chilling was quite dramatic, reducing bud break from 100% to zero when chilling at −10 °C was extended from 8 to 16 weeks in 'Ben Tron' and 'Narve Viking', and with significant inhibition also in 'Hedda' and 'Imandra' (Fig. 3). Such results illustrate the inherent problems involved with modelling of the accumulation of chilling units at different temperatures (cf. Rose and Cameron, 2009; Jones et al., 2012).

The physiological explanation for such an over-chill response is not understood. However, the fact that dormancy was fully broken at −10 °C after 8 weeks of exposure show that a state of secondary dormancy must have been induced by additional chilling. The principle of secondary dormancy is well established and documented in seed dormancy where it is induced in non-dormant seeds in response to temperature and light conditions that are unfavourable

Table 3
Effects of autumn temperature and length of chilling period at 0 °C on bud break and flowering in intact plants of three black currant cultivars.

Cultivar	Autumn temp.(°C)	Length of storage (weeks)	Bud break (%)	Days to bud break	Flowering buds (%)	Days To anthesis	Total no. of flowers	Flowers per truss
'Imandra'	9	5	81.6	6.7	28.3	18.8	57.8	4.6
		10	83.0	5.8	17.1	17.6	29.8	4.8
		15	98.5	5.0	19.0	16.8	39.6	5.4
		Mean	87.7	5.8	21.5	17.7	42.4	4.9
	15	5	88.8	9.5	61.9	14.8	150.0	4.9
		10	84.3	7.7	54.1	14.6	164.8	6.2
		15	92.5	5.7	66.1	11.4	194.0	6.7
		Mean	88.5	7.6	60.7	13.6	169.6	5.9
	21	5	88.7	10.8	64.1	19.0	226.2	7.7
		10	80.6	10.2	55.5	17.6	218.6	7.9
		15	94.0	8.4	63.1	15.4	214.0	8.0
		Mean	87.8	9.8	60.9	17.3	219.6	7.9
'Ben Tron'	9	5	100	6.1	38.0	20.6	85.2	7.0
		10	100	5.0	35.4	21.2	93.4	7.9
		15	100	5.1	45.6	19.2	99.8	6.6
		Mean	100	5.4	39.6	20.3	92.8	7.2
	15	5	100	6.8	57.8	19.6	163.8	8.8
		10	100	6.3	63.2	17.6	201.0	9.1
		15	100	5.3	65.4	16.5	188.8	9.1
		Mean	100	6.2	61.9	18.0	184.2	9.0
	21	5	100	7.3	59.8	23.2	266.0	13.3
		10	98.8	7.1	61.8	21.0	308.0	15.6
		15	100	6.4	65.5	19.3	302.7	14.8
		Mean	99.6	6.9	62.6	21.1	292.9	14.6
'Narve Viking'	9	5	95.5	6.6	36.1	22.2	74.6	5.7
		10	100	6.2	24.4	23.2	58.2	6.6
		15	100	6.1	20.9	22.6	45.2	6.5
		Mean	98.5	6.3	27.1	22.7	59.3	6.3
	15	5	95.6	8.2	25.0	21.0	50.4	5.3
		10	97.4	7.9	24.5	20.6	35.8	3.7
		15	98.9	7.3	40.0	19.2	97.4	6.6
		Mean	97.3	7.8	29.8	20.3	61.2	5.2
	21	5	97.3	9.1	30.9	22.0	115.4	9.2
		10	92.6	8.5	46.3	22.0	171.8	9.4
		15	100	8.0	41.8	21.2	120.4	7.7
		Mean	96.6	8.5	39.7	21.7	135.9	8.8
Probability levels of significance (ANOVA)								
Source of variation								
Autumn temperature (*A*)			n.s.	<0.001	<0.001	<0.001	<0.001	<0.001
Length of storage (*B*)			0.002	<0.001	n.s.	0.001	n.s.	n.s.
$A \times B$			0.009	0.03	0.006	n.s.	0.008	0.04
Cultivar (*C*)			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$C \times A$			<0.001	<0.001	n.s.	0.01	n.s.	n.s.
$C \times B$			n.s.	<0.001	<0.001	0.002	<0.001	<0.001
$A \times B \times C$			n.s.	n.s.	n.s.	n.s.	n.s.	0.002

The data are means of 3 replicates, each with 3 plants of each cultivar in each treatment. n.s., not significant.

to germination (Bewley and Black, 1994). However, the principle of secondary dormancy has, to our knowledge, not been recognized in buds of any species before, although an over-chill response was reported by Jones et al. (2012). Analogous to the situation in seeds, we propose a mechanism whereby dormancy-released buds are induced to become dormant again (secondary dormancy) if they remain in low temperature conditions that are unfavourable and even fatal for growing buds. This would be an important survival mechanism that might have evolved by natural selection in plants living in seasonally cold and variable environments. In seeds with secondary dormancy, the dormant state is particularly hard to break, and it would therefore be interesting to study the chilling requirements for breaking of secondary dormancy in black currant buds. Clearly, the phenomenon of secondary bud dormancy needs to be further studied in this and other temperate woody plants.

Reduced and delayed bud break was also observed in single node cuttings of some cultivars when stored at +10 °C for extended periods (Fig. 3). This has probably nothing to do with secondary dormancy, but rather seems to be caused by depletion of stored energy sources due to increased respiratory losses at such an elevated temperature.

The results also confirm the considerable genotypic differences in chilling requirements that exist among black currant cultivars

Fig. 8. Effect of temperatures as indicated during autumn dormancy induction on the advancement and distribution of spring bud break in plants of three black currant cultivars. Photo taken after 15 weeks of chilling at 0 °C and subsequent forcing in LD at 20 °C for 14 days.

Table 4
Effects of autumn temperature on spring bud break and flowering in intact plants of 'Murmanschanka' black currant. The plants were chilled at 0 °C for 27 weeks before transfer to a plastic greenhouse with 8 ± 5 °C and 20 h day-length.

Tempera-ture (°C)	Breaking buds (%)	Days to bud burst	Flowering buds (%)	Days to anthesis	Flowers per plant	Flowers per truss
9	82.2 a*	0.0 a	50.6 a	14.9 a	243.6 a	10.5 a
15	83.6 a	7.2 b	45.5 ab	16.6 b	215.0 a	10.4 a
21	76.3 b	14.8 c	41.1 b	19.3 c	211.3 a	11.2 a
Mean	80.7	7.0	45.7	16.9	223.3	10.7
P-value	0.05	<0.001	0.05	<0.001	n.s.	n.s.

Data are the means of nine replicates with three plants in each treatment.
* Values within the same column followed by different lower-case letters are significantly different at $P \leq 0.05$ by Tukey's test. n.s.—not significant.

(Plancher and Dördrechter, 1983; Rose and Cameron, 2009; Jones et al., 2012; Atkinson et al., 2013). Of the cultivars tested here, the Scottish-bred 'Ben Tron' and the high-latitude Russian cultivar 'Murmanschanka' had the least chilling requirement, whereas 'Narve Viking' and 'Imandra' had the largest chilling requirement for breaking of bud dormancy. Late spring budburst in 'Narve Viking' is well known among black currant growers in Norway, as a characteristic that is valued as an avoidance mechanism against premature growth and spring frost damage. In Denmark, however, there are reports of irregular bud burst and flowering of this cultivar during the last few years, which has been related to insufficient winter chill (Personal communication, Dr. L. Andersen, Aarhus University). This is not surprising in light of the present results where temperatures at or above 0 °C had weak dormancy-release effect in this cultivar, whereas sub-zero temperatures of −5 and −10 °C were highly effective (Figs. 3 and 5). It can, therefore, be concluded that 'Narve Viking' is well adapted to regions with cold winters and incidences of frost during flowering, but poorly adapted to mild winter areas under the present scenario of global warming. On the other hand, the Scottish-bred 'Ben Tron', which had a relatively low chilling requirement and a wide range of effective chilling temperatures, appears well suited for such mild winter areas. The large chilling requirement and late budburst of 'Imandra' single node cuttings (Fig. 3) was unexpected and surprising, and at variance with the results with intact plants (Table 3). This cultivar is the mother of 'Murmanschanka' which was found to have a particularly low chilling demand (Fig. 3, Table 4). Also, in an earlier investigation (Sønsteby and Heide, 2013), both cultivars were very early in all developmental aspects and exhibited identical seasonal response patterns. We have no good explanation for the deviating and contrasting responses observed here, but suspect that they somehow may be related to induction of secondary dormancy.

Modification of the bud dormancy state by temperature during dormancy induction in autumn is well established, not only in black currant (Sønsteby and Heide, 2011; Sønsteby et al., 2012), but in a wide range of temperate trees and shrubs (Måge, 1975; Heide, 2003; Palonen, 2006). As shown in Table 3 and Fig. 8, elevated autumn temperature renders the buds in a deep state of dormancy that is manifest as increased chilling requirements and delayed bud break in spring, even after 27 weeks of chilling at 0 °C (cf. Table 4). Accordingly, because of this response mechanism, elevated autumn temperature as a result of global warming will further increase the chilling demand and further accentuate the reported problem of inadequate winter chill in black currant in regions with mild winters.

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