



ELSEVIER

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: [www.elsevier.com/locate/scihorti](http://www.elsevier.com/locate/scihorti)

# Control of growth cessation and floral initiation in red raspberry (*Rubus idaeus* L.) cultivars of diverse origin in controlled and natural environments



R. Hodnefjell<sup>a</sup>, O.M. Heide<sup>b</sup>, R. Rivero<sup>c</sup>, S.F. Remberg<sup>a</sup>, A. Sønsteby<sup>c,\*</sup>

<sup>a</sup> Faculty of Biosciences, Norwegian University of Life Sciences, NO-1432, Ås, Norway

<sup>b</sup> Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, NO-1432 Ås, Norway

<sup>c</sup> NIBIO, Norwegian Institute of Bioeconomy Research, NO-1431 Ås, Norway

## ARTICLE INFO

### Keywords:

Biennial-fruiting raspberry  
Flower initiation  
Growth cessation  
Photoperiod  
Temperature  
Tip flowering

## ABSTRACT

The aim of the investigation was to assess and compare the environmental limits for growth cessation and floral initiation in a range of new and established biennial-fruiting red raspberry (*Rubus idaeus* L.) cultivars of diverse origin under phytotron and field conditions. The results confirmed that growth cessation and floral initiation in biennial-fruiting red raspberry are jointly controlled by the interaction of low temperature and short days (SD). When transferred from non-inductive high temperature and long day (LD) conditions to naturally decreasing autumn daylengths at varying phytotron temperatures on 18 August, growth immediately levelled off and ceased completely within 2 weeks in all cultivars at 9 °C. Serial dissections of lateral buds revealed that floral initiation simultaneously took place. At 15 °C on the other hand, the plants continued growing and remained vegetative until around 15 September when the daylength had decreased to approximately 13 h. The change to 9 °C resulted in an immediate but short-lasting floral induction response that did not bring about initiation in buds situated near the base of the canes, as was the case at 15 °C. At 18 °C, marginal floral induction took place only in the cultivars ‘Glen Ample’, ‘Balder’ and ‘Vene’, even at photoperiods down to 10 h, whereas at 21 °C, all cultivars grew vegetatively regardless of daylength conditions. However, exceptions were some plants of ‘Vene’ and ‘Anitra’ that initiated terminal flowers at 18 and 21 °C and flowered directly without chilling (so-called tip flowering). Although some cultivars of Northern origin ceased growing and initiated floral primordia somewhat earlier (at longer photoperiods) than those of more southerly origin, the differences were relatively minor and not consistent (no latitudinal cline). Results obtained in the field under decreasing autumn temperature and daylength conditions agreed closely with the results in the phytotron. We therefore conclude that results obtained with raspberry in properly controlled daylight phytotron experiments are generally applicable to field conditions.

## 1. Introduction

Environmental regulation of shoot growth and flower formation of biennial-fruiting red raspberry have mainly been studied under controlled environment conditions (Moore and Caldwell, 1985; Heide and Sønsteby, 2011). The cultivars ‘Malling Promise’ and ‘Glen Ample’, which have been thoroughly studied (Williams, 1960; Sønsteby and Heide, 2008), grew continuously and remained vegetative at 21 °C in both short day (SD) and long day (LD) conditions, while at 15 °C they ceased growing and initiated floral primordia in photoperiods < 15 h. At 9–10 °C on the other hand, they ceased growing and initiated floral primordia in both SD and LD. Growth cessation and initiation of floral primordia takes place simultaneously, and are thus jointly controlled by the interaction of photoperiod and temperature. However, extended SD

exposure under high radiation conditions resulted in floral initiation in ‘Glen Ample’ also at 18 °C (Sønsteby et al., 2013), while marginal SD induction at 21 °C, as observed by Woznicki et al. (2016), was due to marginally low temperatures during raising of the plants.

Although less studied under natural field conditions, growth cessation and floral initiation takes place simultaneously also in the field (Hudson, 1959), and the timing of the process under naturally decreasing daylength and temperature conditions in autumn, indicate control by the same photoperiod and temperature interactions as demonstrated under controlled environment conditions (Mathers, 1952; Robertson, 1957; Måge et al., 1989). Nevertheless, it is sometimes argued that results from controlled environment experiments are not directly applicable to the varying environment in the field, and that the results therefore should be verified under field conditions. Also, a wide

\* Corresponding author.

E-mail address: [anita.sonsteby@nibio.no](mailto:anita.sonsteby@nibio.no) (A. Sønsteby).

**Table 1**  
Description of the origin of the raspberry cultivars used in the studies.

Cultivar	Pedigree	Country and year of release	Breeding institution/company
'Anitra'	N91631 x N92683	Norway, 2015	Graminor
'Balder'	'Norna' x 'Malling Jewel'	Norway, 1988	Norwegian Univ. Life Sciences
'Cascade Delight'	'Chilliwack' x WSU 994	USA, 2003	Western Washington Res. & Ext. Centre
'Glen Ample'	'Glen Prosen' x 'Meeker' (among others)	Scotland, 1994	James Hutton Inst.
'Malling Juno'	Cross between two early East Malling selections	UK, 1998	East Malling Research
'Ninni'	'Varnes' x RU00403067	Norway, 2015	Graminor
'Schönemann'	'Lloyd George' x 'Preussen'	Germany, 1950	W. Schönemann, Fellbach
'Tulameen'	'Nootka' x 'Glen Prosen'	Canada, 1989	Agric. Can. Res. Station
'Vene'	'Veten' x 'Newburgh'	Norway, 1987	NIBIO
'Veten'	'Preussen' x 'Lloyd George'	Norway, 1961	NIBIO
RU044003090	'Varnes' x RU00403067	Norway	Graminor

range of biennial-bearing red raspberry cultivars are commercially grown and new cultivars are steadily released, while only a couple of cultivars have been thoroughly studied for their environmental requirements. Of special interest is the question of whether raspberry cultivars of varying geographic origin constitute a latitudinal cline in their developmental temperature and daylength requirements, as is the case with other berry crops such as black currants (Sønsteby and Heide, 2013).

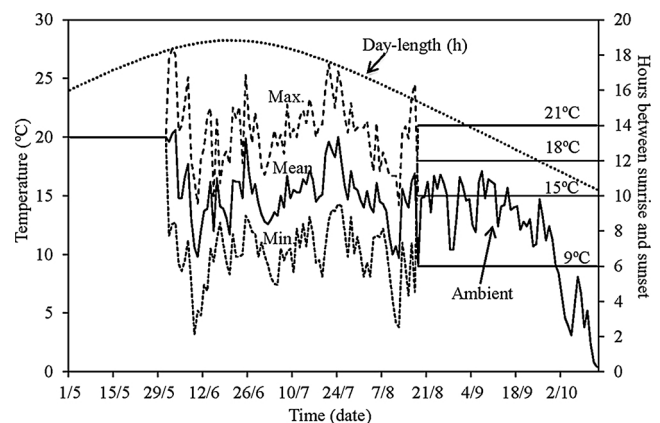
We have therefore, compared the environmental requirements for growth cessation and floral initiation in a range of new and established biennial-fruited red raspberry cultivars of diverse origin under phytotron and field conditions. The results of these investigations are presented and discussed below.

## 2. Materials and methods

### 2.1. Plant material and cultivation

A total of ten cultivars and one advanced breeding line were studied in two experiments. A short description of the origin of these cultivars is presented in Table 1. The cultivars 'Anitra', 'Balder', 'Glen Ample', 'Schönemann', 'Vene', 'Veten', and 'Tulameen' were used in a phytotron experiment conducted in 2016. Single cane plants were propagated and raised at the NIBIO Experimental Center Apelsvoll (60° 40' N, 10° 52' E) as described by Woznicki et al. (2016). Throughout the propagation period, the plants were maintained in natural long photoperiods (16–19 h) and a minimum temperature of 20 °C. Then, in early June the plants were moved outdoors and further cultivated until mid-August at ambient temperature and daylength as shown in Fig. 1. The plants were placed in rows with five plants per running meter and two meters between the rows. Only one cane per plant was allowed to grow, additional emerging shoots were removed by weekly pruning throughout the experiment. The plants were automatically fertigated during the summer with a fertilizer solution with electric conductivity (EC) = 1.5 mS cm<sup>-1</sup> and consisting of a 2:3 mixture of Superba Red™ (7-4-22% NPK + micronutrients) and Calcinite™ (15.5% N and 19% Ca), both from Yara International (Oslo, Norway).

On 18 August, when the plants had about 20 nodes and were approximately 1 m tall, they were moved into the phytotron at the Norwegian University of Life Sciences at Ås, Norway (59° 40' N, 10° 45' E) and exposed to naturally decreasing photoperiod and temperatures of 9, 15, and 21 °C for 8 weeks and 18 °C for 10 weeks (to test whether prolonged exposure would induce flowering). Temperatures in the phytotron were controlled to ± 1.0 °C, and a water vapour pressure deficit of 530 Pa was maintained at all temperatures. Whenever the photosynthetic photon flux density (PPFD) fell below approximately 150 μmol quanta m<sup>-2</sup> s<sup>-1</sup> as on cloudy days, an additional 125 μmol



**Fig. 1.** Temperature and daylength conditions during plant raising and experimentation in the controlled environment experiment. Ambient temperature conditions at Apelsvoll during the 8-week experimental period are also included.

quanta m<sup>-2</sup> s<sup>-1</sup> were automatically added using Philips HPT-1 400W lamps. In addition, one set of plants were left outdoors at NIBIO Apelsvoll and grown under ambient temperature and daylength conditions for comparison with the phytotron treatments. All treatments had three replications of five plants each. During the treatment period, the plants were fertigated one or two times per day with the same fertilizer solutions as described above (EC = 1.5). Biological pest control measures were established when the treatments were started. Temperature and daylength conditions during plant raising and experimentation are shown in Fig. 1.

For the field experiment, the cultivars 'Anitra', 'Cascade delight', 'Glen Ample', 'Malling Juno', 'Ninni', 'Veten', and the breeding line RU044003090 were used. The plants were purchased from an authorized plant nursery and planted in the experimental orchard of the Norwegian University of Life Sciences at Ås in early summer 2015. The plants were planted in open soil without mulching in three single rows with a spacing of 0.5 m in the rows and 4 m between the rows. The experiment comprised three randomized blocks, each with six plants of each cultivar. No fertilizers were applied at planting in 2015, but in early spring 2016, 20 g of Fullgjødsel™ (12-4-18% NPK + micronutrients) and 10 g Nitrabor™ (15.4% N, 18.5% Ca, 0.3% B), both from Yara International (Oslo, Norway) were applied per m running plant row. In addition, 10 g Fullgjødsel™ was applied per m running row at the time of flowering. Three representative canes of each cultivar per plot were selected and marked with a ribbon for monitoring of shoot growth and leaf (node) production, giving a total of nine measured canes per cultivar. Photoperiod and temperature conditions during the entire 2016 growing season are presented in Fig. 2.

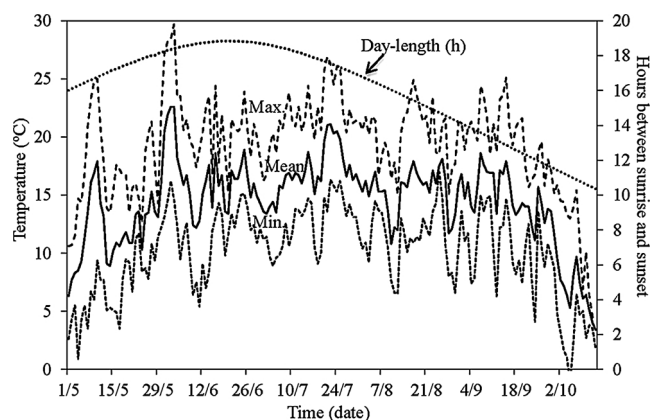


Fig. 2. Temperature and daylength conditions in the field at Ås during the 2016 growing season.

## 2.2. Scanning electron microscopy

Lateral buds with fully differentiated flower primordia of most cultivars in the field experiment were sampled at random on 1 November and examined by scanning electron microscopy for detection of specific cultivar differences in shape and structure of floral primordia. For this purpose, the buds were fixed and processed as described by Woznicki et al. (2016).

## 2.3. Experimental design and data collection

The experiments had a factorial randomized block design with three randomized blocks with eight or six plants per treatment in the phytotron and field experiments, respectively. In the phytotron experiment, five plants were used for monitoring of shoot growth and floral initiation, while three plants were used for dissection and examination of the profile of floral differentiation along the entire length of the cane. In the field experiment, only three of the six plants in each treatment and block combination were used for monitoring of shoot growth, and the other three for monitoring of the progress of floral initiation (different plants being used for the two purposes). Plant growth was monitored by weekly measurements of plant height, and counting of leaf numbers during the experimental period in the phytotron, or for ten weeks in the field, starting on 3 August. Buds were also sampled every week during the same periods for dissection and determination of the successive stages of floral bud initiation and differentiation. One of the buds in positions 5–7 below the apex of each plant was removed each week by a shallow longitudinal slit and stored in 70% ethanol until dissected and examined under a stereo microscope as described by Sønsteby and Heide (2008). This sampling technique did not affect the continued growth of the canes, and since new nodes were initiated weekly, a new bud was available every week. Floral development stages were scored by the six-stage scale developed by Woznicki et al. (2016), where stage 1 is a vegetative bud with no sign of change at the apex, while stage 2 indicates the first visible sign of floral initiation, and stage 6 a fully differentiated floral bud. In addition to the monitoring of the successive stages of floral development in selected buds, the profile of flower development along the entire length of the shoots was determined in three plants per treatment at termination of the phytotron experiment. For this purpose, the canes were cut at ground level and wrapped in a sheet of polyethylene and stored at 0 °C for later dissection and scoring of flowering stage of each bud along the entire length of the shoot.

Experimental data were subjected to analysis of variance (ANOVA) by standard procedures, and means between treatments were compared by Tukey's multiple comparison test. The Minitab® Statistical Software program package (Release 15: Minitab. Inc., State College, PA, USA)

were used for these calculations.

## 3. Results

### 3.1. Phytotron experiment

When exposed to natural photoperiods from 18 August, extension growth of all cultivars levelled off immediately at 9 °C and ceased completely within 1–2 weeks (Fig. 3). At 15 °C, all cultivars grew at constant rate until 15 September when growth started to level off in 'Glen Ample'. One week later, growth levelled off in all remaining cultivars except 'Schönemann' which grew at constant rate until 29 September. In all cultivars, growth had ceased completely one to two weeks after it started to decrease. In the plants grown outdoors at ambient temperature, growth and growth cessation followed by much the same patterns as in those grown at 15 °C in the phytotron. Exceptions were 'Tulameen' and 'Anitra' in which total growth increment was nearly twice as high at ambient temperature than at constant 15 °C, although with the same timing of growth cessation. At 18 °C, growth started to level off on 29 September in the cultivars 'Anitra', 'Balder', 'Glen Ample' and 'Vene', while plants of 'Schönemann', 'Tulameen' and 'Veten' grew at constant rate throughout the experimental period, as did all cultivars at 21 °C (Fig. 3, Table 2). 'Glen Ample' plants grown for 8 weeks at the different constant temperatures are shown in Fig. 4. A special situation occurred in some plants of 'Anitra' at 18 °C, and in 'Vene' at 18 and 21 °C, in which the terminal buds formed inflorescences and developed so-called tip flowering after 8 weeks (Fig. 5). This was associated with terminal growth cessation in these cultivars. Increment in leaf numbers followed the increment in shoot length very closely with the exception that, since new leaves were not registered before they had reached a length of 2 cm, the inflection points for this parameter was delayed by about two weeks compared with shoot growth. Table 2 shows total shoot heights and leaf numbers of all cultivars at the various temperature conditions after 8 weeks of cultivation.

Serial dissections of lateral buds revealed that the first microscopic signs of floral initiation coincided rather closely with the slowing down of shoot growth in all cultivars. However, since different buds were examined each week, the flower developments curves were not as smooth as those for shoot growth (Fig. 6). In all cultivars, the first signs of floral initiation were observed on 1 September at 9 °C, two weeks after start of the treatments, and most plants reached stage 6 within 8 weeks. The fastest development was observed in 'Anitra', 'Balder', and 'Vene' which reached stage 5 within six weeks, while the other cultivars followed one week later. At 15 °C, floral initiation stages close to 2 were usually delayed by two or more weeks compared with 9 °C. In some cultivars such as 'Tulameen' and 'Schönemann', stage 2 was not observed until 29 September, and these two cultivars were the only ones that did not reach stage 3 within 8 weeks. While growth at ambient temperature was similar to that at 15 °C, flower development generally lagged somewhat behind at ambient temperatures. However, when comparing the results from constant 15 °C in the phytotron at Ås with ambient temperature at Apelsvoll, it should be kept in mind that the photoperiod was about 40 min longer at Apelsvoll during the relevant period in September. At 18 °C, little or no flower bud formation took place. Only the cultivars 'Balder', 'Glen Ample' and 'Vene' initiated flower primordia in lateral bud positions, reaching stage 3 within 8 weeks. Since the selected lateral buds were positioned 5 to 7 nodes below the apex, the mentioned tip flowering is not reflected in the graphs of Fig. 6. Except for the tip flowering plants in 'Vene', there was no flower initiation in plants grown at 21 °C. At the end of the experiment, 80% of five plants at 18 °C, and 60% of five of this cultivar at 21 °C were tip flowering. The first observation of open flowers in 'Vene' was made after five weeks at 18 °C, and after eight weeks at 21 °C. One plant of 'Anitra' was also tip flowering at 18 °C by termination of the experiment.

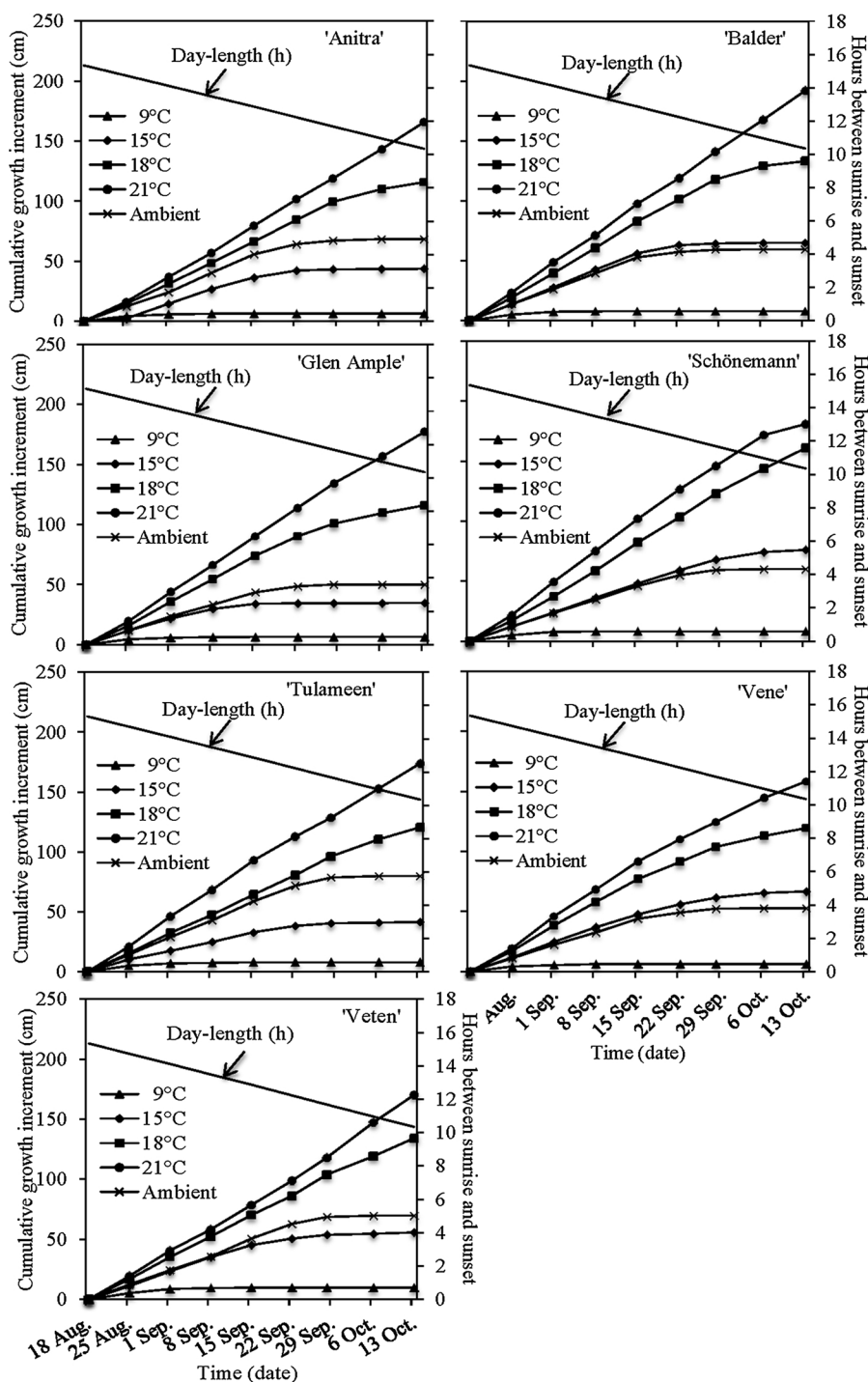


Fig. 3. Time courses of shoot elongation in seven raspberry cultivars during cultivation in the phytotron at naturally decreasing photoperiod and temperatures as indicated. The parallel results from out-door (ambient) conditions at Apelsvoll are also included. Each value is the mean of three replicates, each with five plants of each cultivar in each treatment.

Profiles of flower bud development along the entire lengths of the canes of all cultivars at the various temperatures are shown in Fig. 7. At 9 °C, plants of all cultivars had fully differentiated inflorescences with terminal flowers at stage 5–6 at all bud positions from approximately node 10 and upward, including the terminal bud. Only small differences were present between the cultivars, although the cultivars ‘Anitra’, ‘Balder’ and ‘Schönemann’ had initiated inflorescences a bit further down the cane than the other cultivars. At 15 °C and ambient temperature on the other hand, the profiles had a different look with the most advanced buds at the middle part of the canes and gradually decreasing development towards both the top and the base. There were

also considerable differences between the cultivars at these temperatures, ‘Tulameen’ and ‘Schönemann’ being significantly less advanced than the other cultivars. Also, while ‘Tulameen’ and ‘Schönemann’ had vegetative buds at the terminal and nearest sub-terminal positions, also the terminal buds were generative in all the other cultivars. Compared with 9 °C, floral bud initiation took place further down the cane at these higher temperatures, a tendency that was particularly marked in ‘Balder’ and ‘Veten’ which had only one or two vegetative nodes at the base of the canes. At 18 °C, flowers were initiated in cultivars ‘Balder’, ‘Glen Ample’ and ‘Vene’ only. While ‘Vene’ were tip flowering with open flowers or developing fruits at the uppermost ten bud positions,



**Table 2**  
Total shoot heights and leaf numbers of seven raspberry cultivars after 8 weeks of cultivation at the indicated temperature conditions.

Cultivar	Temperature (°C)	Shoot height (cm)	Total no. of nodes
'Glen Ample'	9	92.6	25.8
	15	120.8	31.4
	18	204.4	47.8
	21	258.4	51.4
	Ambient	132.4	32.8
<i>Mean</i>		161.7	37.8
'Tulameen'	9	86.0	26.0
	15	127.2	37.8
	18	226.6	57.4
	21	266.4	64.4
	Ambient	161.2	41.0
<i>Mean</i>		173.5	45.3
'Veten'	9	98.4	24.4
	15	145.8	32.6
	18	265.0	51.4
	21	263.4	49.8
	Ambient	171.1	37.6
<i>Mean</i>		188.8	39.2
'Vene'	9	97.0	28.6
	15	158.8	42.2
	18	220.0	54.8
	21	248.8	57.0
	Ambient	134.8	37.0
<i>Mean</i>		171.9	43.9
'Balder'	9	104.4	25.8
	15	161.0	36.0
	18	231.6	46.8
	21	289.2	52.8
	Ambient	153.6	34.6
<i>Mean</i>		189.1	39.3
'Anitra'	9	141.8	29.0
	15	186.4	37.8
	18	253.2	49.0
	21	290.6	48.6
	Ambient	199.2	36.6
<i>Mean</i>		214.2	40.2
'Schönemann'	9	107.8	22.6
	15	187.4	39.4
	18	307.6	52.2
	21	298.0	51.4
	Ambient	164.5	34.4
<i>Mean</i>		213.1	40.0
Probability levels of significance by ANOVA			
Source of variation			
Temperature (A)		< 0.001	< 0.001
Cultivar (B)		< 0.001	< 0.001
A × B		< 0.001	< 0.001

the other two cultivars were vegetative at the topmost five to ten positions and with only moderately advanced buds at the other positions.

### 3.2. Field experiment

Under field conditions, shoot growth started to slow down in all cultivars between 17 and 24 August (Fig. 8A) when the photoperiod had decreased to approximately 15 h and the mean temperature was about 17 °C and had been down to about 12 °C for two weeks shortly before (Fig. 2). This was followed by complete growth cessation 2–3 weeks later, depending on the cultivar. 'Cascade Delight', 'Glen Ample', 'Malling Juno' and 'Ninni' were the first to cease growth on 7 September, while 'Anitra' was last and continued growing until 21 September. Cessation of leaf initiation followed the same cultivar sequence, but was delayed by about two weeks due to different observational procedures as stated above (data not shown).

The first visible changes at the shoot apex were observed by the



Fig. 4. Appearance of the 'Glen Ample' plants on 13 October after eight weeks of cultivation in the phytotron under natural daylength conditions and temperatures as indicated.



Fig. 5. Tip flowering in 'Vene' after eight weeks at 18 °C and natural daylength conditions.

17–24 August to coincide with the reduction in shoot growth (Fig. 8B). The earliest changes were observed in 'Glen Ample' and 'Malling Juno', but by 24 August, all cultivars had reached development stages close to 2, except for 'Ninni' and the closely related selection which were particularly late. However, none of the cultivars developed fully differentiated flower buds before 28 September when sampling of buds was terminated in the field. At this stage, 'Malling Juno' followed by 'Cascade Delight' and 'Veten', had the most advanced floral primordia at

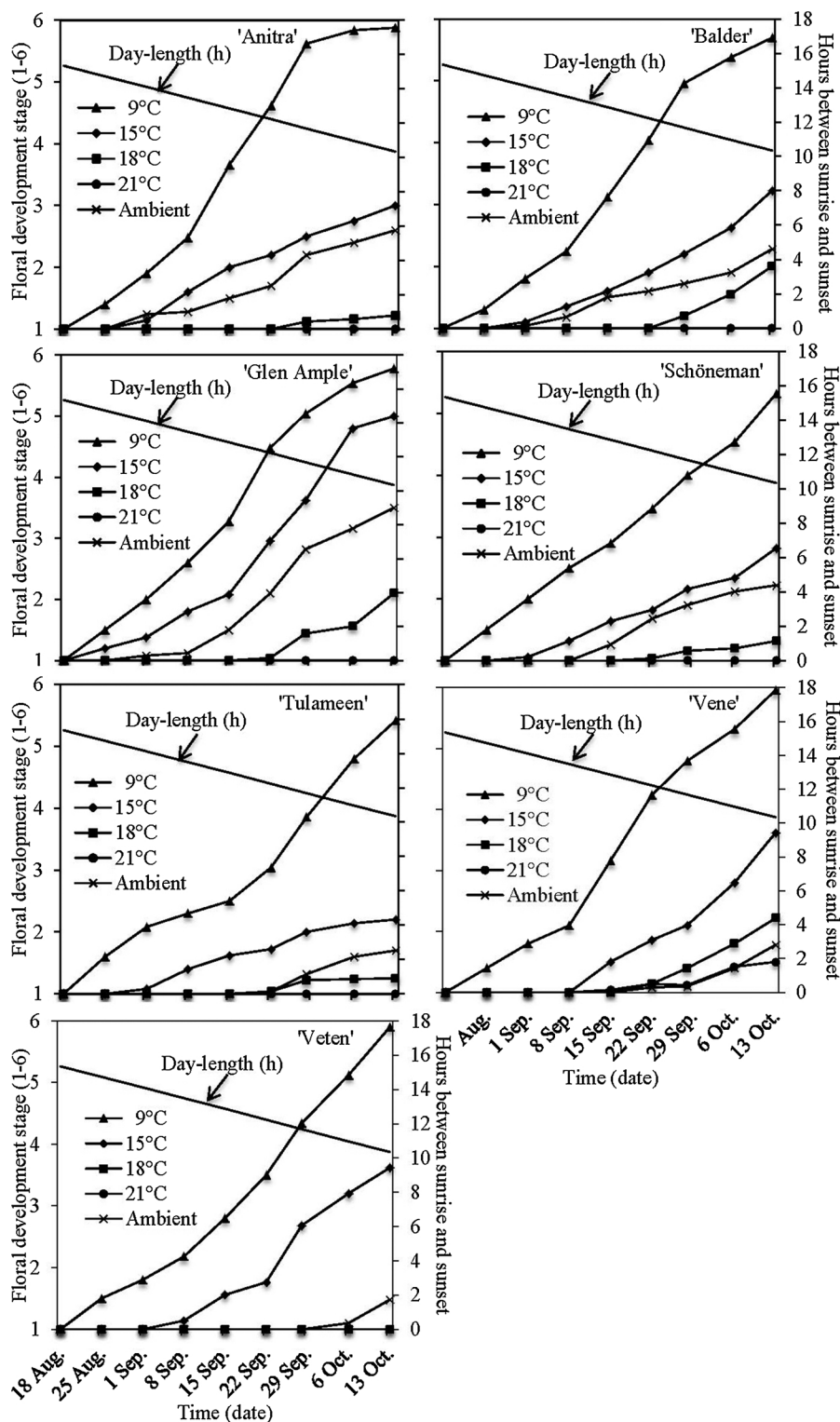


Fig. 6. Progression of flower bud development in dissected buds of seven raspberry cultivars cultivated in the phytotron at naturally decreasing photoperiod and temperatures as indicated. The parallel results from out-door (ambient) conditions at Apelsvoll are also included. Each value is the mean of three replicates, each with five plants of each cultivar in each treatment.

stages 4–4.5, whereas the selection RU044003090 was least advanced at approximately stage 2.5.

### 3.3. Scanning electron microscopy

Shape and structure of the differentiated floral primordia did not vary much between the cultivars (Fig. 9). However, as shown by

Woznicki et al. (2016), the primordia of ‘Glen Ample’ typically had an angular, pentagonal shape, whereas ‘Anitra’, representing the opposite extreme, typically had globular (rounded) primordia. The other cultivars, as represented by ‘Veten’ here, had flower primordia of an intermediate shape. Otherwise, there were only minor cultivar differences in the morphology of the floral primordia.

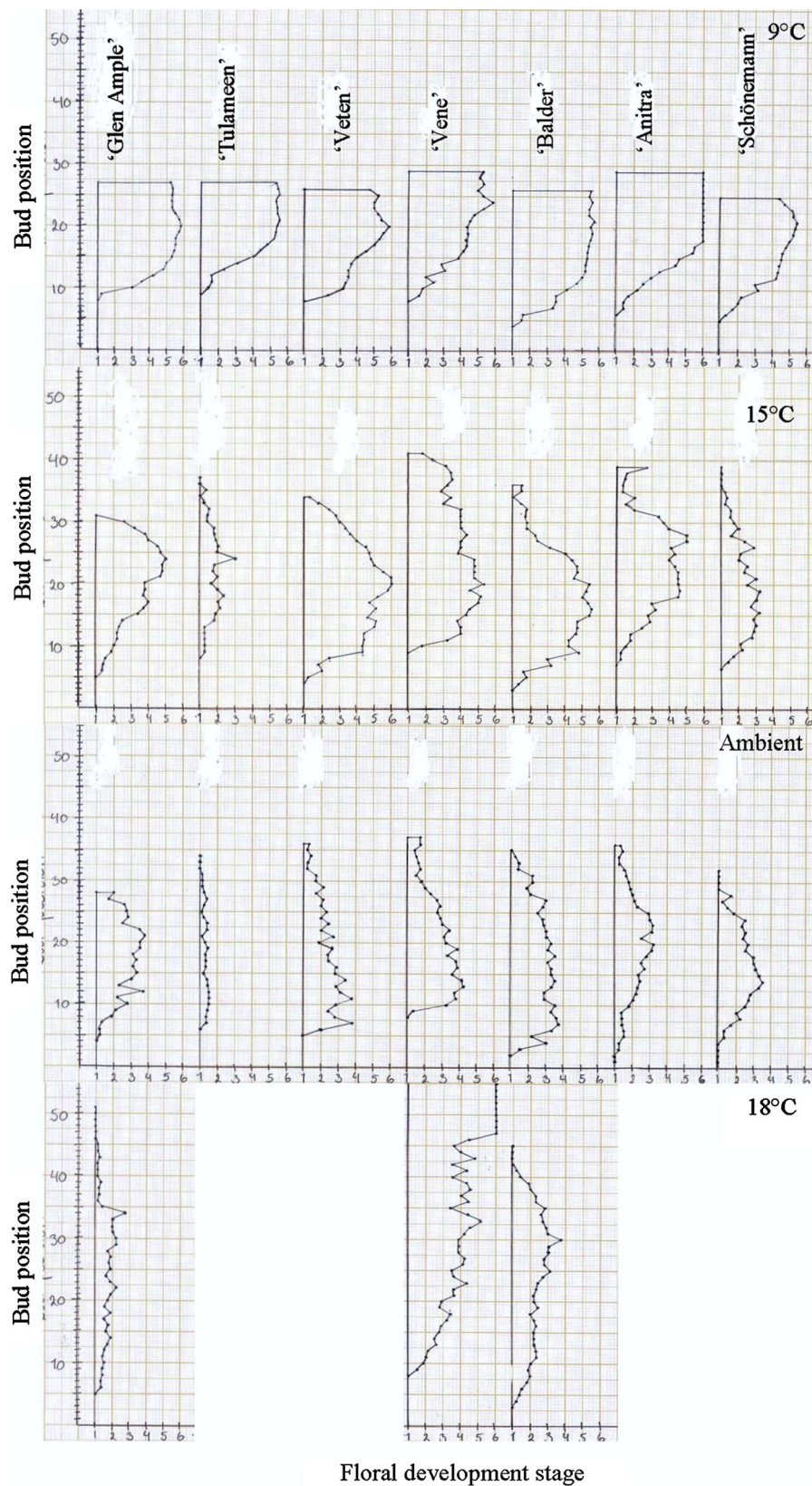


Fig. 7. Profiles of flower bud development stages along the entire length of the canes in plants grown for eight weeks in naturally decreasing photoperiod and different temperatures as indicated. Each value is the mean of three replicates, each with three plants of each cultivar in each treatment.

#### 4. Discussion

The results confirm and extend the present knowledge base for the environmental control of growth and floral induction in biennial-

fruiting red raspberry (cf. Williams, 1960; Sønsteby and Heide, 2008; Heide and Sønsteby, 2011). In all the seven cultivars tested under controlled temperature conditions, growth cessation and floral initiation were jointly controlled by an interaction of low temperature and



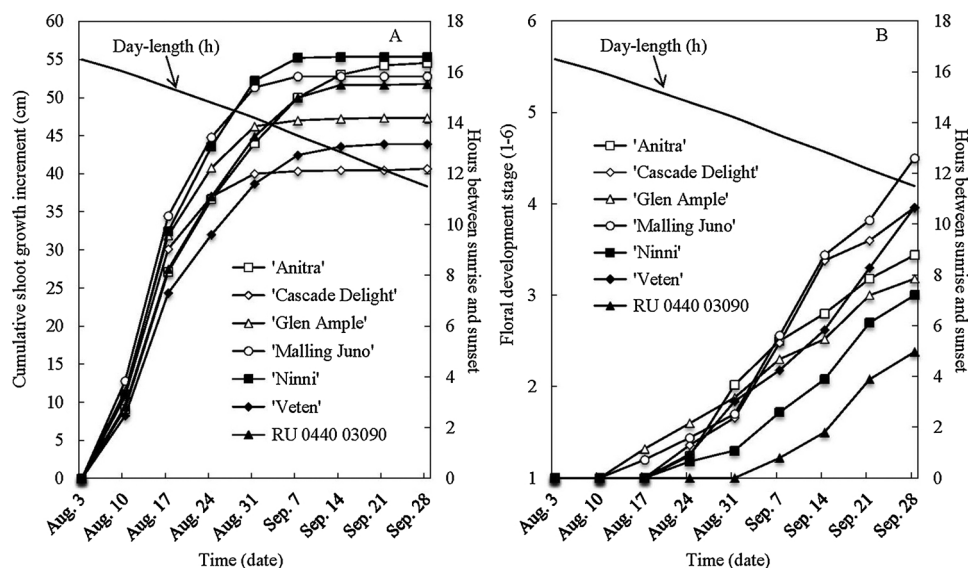


Fig. 8. Time courses of shoot elongation (Panel A) and the parallel progression of floral bud development (Panel B) of seven raspberry cultivars grown in field at Ås. Each value is the mean of three replicates, each with three plants of each cultivar in each treatment.

SD conditions. On 18 August, when the experiment was started at a photoperiod close to 16 h, the plants abruptly ceased growing and initiated floral primordia within 2 weeks at 9 °C, whereas at 15 °C, the plants continued growing and remained vegetative for another 2–4 weeks, at which time the photoperiod had decreased to less than 13 h (Figs. 4 and 6). With the necessary time lag involved between start of induction and the appearance of visible primordia, this agrees well with the critical photoperiod of 15 h at 15 °C previously reported by Sønsteby and Heide (2008). The results also confirmed that at 18 °C, floral induction is marginal even at photoperiods down to 10 h, and takes place only in a few cultivars such as ‘Balder’, ‘Glen Ample’ and ‘Vene’. These results fully confirm that biennial-fruiting raspberries are day-neutral at low temperatures ( $\leq 10$  °C), but require SD conditions ( $< 15$  h) for growth cessation and floral initiation at 15–18 °C. At higher temperatures, they do grow vegetatively regardless of daylength conditions.

The results also show that there are only minor differences in the environmental requirements for growth cessation and floral induction among raspberry cultivars of varying geographic origin. However, the cultivars ‘Tulameen’ and ‘Schönemann’ which are of relatively low latitude origin in Canada and Germany, respectively, were only weakly induced in natural September photoperiods at 15 °C, whereas ‘Glen Ample’, ‘Balder’ and ‘Vene’, which are of Scottish and Norwegian origin, were the only cultivars that initiated flower buds at 18 °C (Fig. 7). Nevertheless, these differences are relatively minor and did not represent a latitudinal cline, as was the case with black currant cultivars of varying latitudinal origin (Sønsteby and Heide, 2013). An important reason for the difference between the two genera in this respect is apparently that the origins of the black currants were spanning a much wider range of latitudes. The present results thus indicate that cultivated red raspberries represent a relatively narrow gene pool for these characters. An interesting result was the phenomenon of tip flowering at high temperatures in some cultivars, especially ‘Vene’. The phenomenon is well known in some cultivars such as the old ‘Lloyd George’ (Williams, 1960) and the more recent ‘Glen Moy’ under warm autumn temperature or tunnel production conditions (Heiberg et al., 2008). Apparently, this behaviour is related to the physiological responses of annual-fruiting cultivars in which floral induction can take place in LDs at temperatures as high as 30 °C, and where in addition, the initiated primordia proceed directly to anthesis instead of going dormant (Sønsteby and Heide, 2009). In tip flowering cultivars, initiation can take place at temperatures higher than normal for biennial-fruiting cultivars, and furthermore, the initiated terminal and uppermost buds develop directly to anthesis without chilling, whereas buds further

down the cane become dormant and biennial-fruiting (Williams, 1960; Heide and Sønsteby, 2011). Thus, the tip flowering genotypes carry some physiological characteristics that are known in both annual- and biennial-fruiting cultivars. The available evidence indicate that tip flowering is controlled by both genetic and environmental factors, in particular temperature conditions (cf. Heide and Sønsteby, 2011).

The time-consuming dissections of all the buds in three canes of each cultivar proved very useful by providing profiles of floral bud initiation along the entire length of the canes. By this technique it was possible to detect how far down the shoot the initiation took place, and it also clearly detected the tip flowering tendency of some cultivars. Furthermore, the results in Fig. 7 also revealed interesting differences in the strength and duration of the flower-inducing signal at the different environmental conditions. Thus, when the plants were abruptly transferred from non-inductive to optimally inductive conditions of SD and 9 °C, the plants responded with an immediate but short-lasting induction pulse that did not bring about initiation in the buds situated near the base. This response pattern differed clearly from the slower and weaker, but more lasting response at 15 °C, (and at 18 °C in the responding cultivars), that brought about initiation almost all the way down to the ground in some cultivars (Fig. 7). Since at 9 °C, flowering was induced by low temperature under LD conditions while at 15 °C only under SD conditions, these results show that SDs are required for extensive floral initiation along the entire length of the raspberry cane. This may be an important reason why long canes with yield potentials of more than 3 kg fruit per cane were only obtained in plastic tunnels at elevated temperatures where induction takes place in SD only (Sønsteby et al., 2009; 2013).

Generally, there were close agreements in the critical temperatures and photoperiods for growth cessation and floral initiation that were obtained in controlled and natural environments. In the field experiment at Ås, where the mean daily temperature was well above 15 °C until about mid-September (Fig. 2), the plants did not cease growth and initiate floral primordia until well after this date, when temperature and daylength had decreased to about 15 °C and 15 h, respectively. This agrees closely with the critical values obtained in the phytotron under the same photoperiodic conditions at 15 °C (Figs. 3 and 6). Likewise, the potted plants grown in the phytotron at 15 °C and those grown outdoors at Apelsvoll at similar temperature conditions, responded quite similarly, although with some delayed flower initiation at the slightly longer photoperiods at Apelsvoll (Fig. 6). This was particularly the case with the cultivars ‘Veten’ and ‘Vene’. Such results demonstrate that a difference of 40 min in the length of day can have a significant effect on the timing of floral induction in some cultivars. This as well as the other results



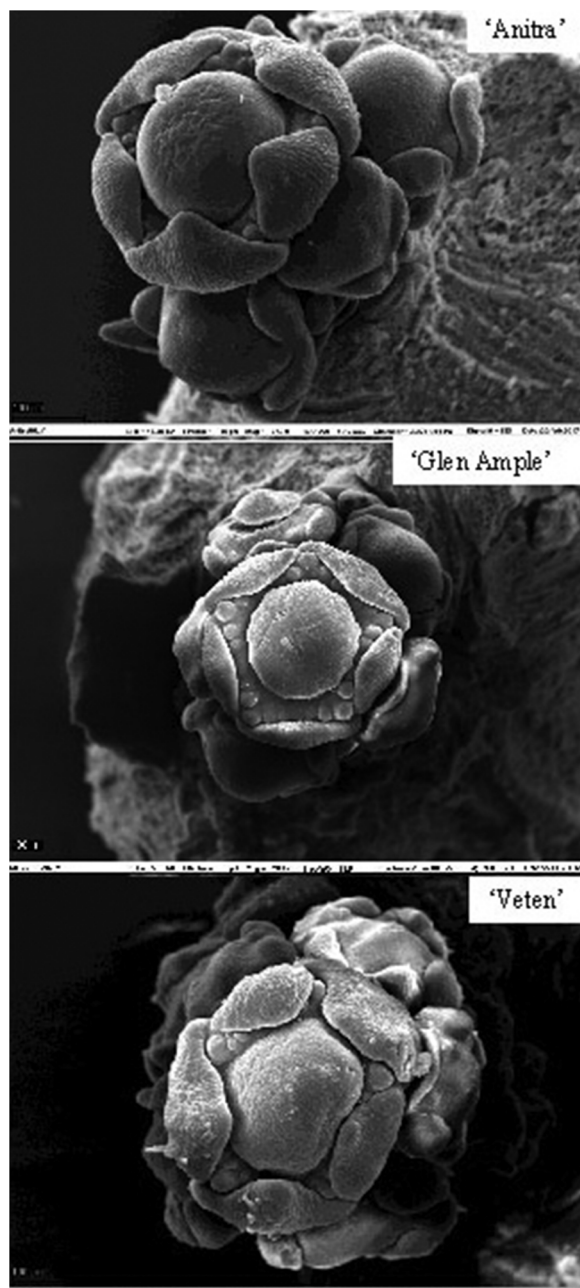


Fig. 9. SEM imaging of fully differentiated flowers (Stage 6) of the cultivars 'Anitra', 'Glen Ample' and 'Veten'.

presented, demonstrates the high precision with which raspberry plants control their seasonal timing of growth cessation and the associated floral bud formation in relation to the decreasing photoperiod and temperature during late summer and autumn. We conclude that results obtained with raspberry in properly controlled daylight phytotron experiments are generally applicable to field conditions.

#### Acknowledgements

We thank Unni M. Roos, Signe Hansen and Kari Grønnerød for excellent technical assistance. R.H. was supported through the MSc. program at the Norwegian University of Life Sciences. A.S., R.R. and O.M.H. acknowledge financial support from the European Union's Horizon 2020 research and innovation programme (grant number 679302) and from the Norwegian Agriculture Agency (grant number 15/58579).

#### References

- Heiberg, N., Lunde, R., Nes, A., Hageberg, B., 2008. Long cane production of red raspberry plants and effect of cold storage. *Acta Hort.* 777, 225–229.
- Heide, O.M., Sønsteby, A., 2011. Physiology of flowering and dormancy regulation in annual- and biennial-fruiting red raspberry (*Rubus idaeus* L.) – a review. *J. Hortic. Sci. Biotechnol.* 86, 433–442.
- Hudson, J.P., 1959. Effects of environment on *Rubus idaeus* L. I. Morphology and development of the raspberry plant. *J. Hortic. Sci.* 34, 163–169.
- Måge, F., Kvam, G., Byrkjenes, K., 1989. Blomsterknoppar hos solbær og bringebær. *Frukt og Bær* 7–16 (In Norwegian).
- Mathers, B.A., 1952. A study of fruit-bud development in *Rubus idaeus*. *J. Hort. Sci.* 27, 266–272.
- Moore, J.N., Caldwell, J.D., 1985. *Rubus*. In: Halevy, A.H. (Ed.), *CBC Handbook of Flowering*, vol. IV. CBC Press, Boca Raton, Florida, pp. 226–238.
- Robertson, M., 1957. Further investigations on flower bud development in the genus *Rubus*. *J. Hortic. Sci.* 32, 265–273.
- Sønsteby, A., Heide, O.M., 2008. Environmental control of growth and flowering of *Rubus idaeus* L. cv. Glen Ample. *Sci. Hortic.* 117, 249–256.
- Sønsteby, A., Heide, O.M., 2009. Effects of photoperiod and temperature on growth and flowering of the annual (primo-cane) fruiting raspberry (*Rubus idaeus* L.) cultivar Polka. *J. Hortic. Sci. Biotechnol.* 84, 439–446.
- Sønsteby, A., Heide, O.M., 2013. Variation in seasonal timing of flower bud initiation in black currant (*Ribes nigrum* L.) cultivars of contrasting geographic origin. *J. Hortic. Sci. Biotechnol.* 88, 403–408.
- Sønsteby, A., Myrheim, U., Heiberg, N., Heide, O.M., 2009. Production of high-yielding red raspberry long canes in a Northern climate. *Sci. Hortic.* 121, 289–297.
- Sønsteby, A., Stavang, J.A., Heide, O.M., 2013. Production of high-yielding raspberry long canes: The way to 3 kg of fruit per cane. *J. Hortic. Sci. Biotechnol.* 88, 591–599.
- Williams, I.H., 1960. Effects of environment on *Rubus idaeus* L. V. Dormancy and flowering in the mature shoot. *J. Hortic. Sci.* 335, 214–220.
- Woznicki, T.L., Heide, O.M., Remberg, S.R., Sønsteby, A., 2016. Effects of controlled nutrient feeding and different temperatures during floral initiation on yield, berry size and drupelet numbers in red raspberry (*Rubus idaeus* L.). *Sci. Hortic.* 212, 148–154.