

Climatic and seasonal control of annual growth rhythm and flower formation in *Vaccinium myrtillus* (Ericaceae), and the impact on annual variation in berry production

Vidar Selås^{1,*}, Anita Sønsteby², Ola M. Heide¹ & Nina Opstad²

¹Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

²NIBIO - Norwegian Institute for Bioeconomy Research, P.O.Box 115, NO-1431 Ås, Norway

*Author for correspondence: vidar.selas@nmbu.no

Background and aims – Large variation in annual berry production occurs in *Vaccinium myrtillus* L., and the variation has been associated with population cycling of many herbivores. Because of its ecological significance, variation in berry production has frequently been related to climatic variables.

Methods – In an attempt to elucidate the possible causality of such relations, we have examined the seasonal and climatic control of growth rhythm and flowering performance of *V. myrtillus* in the field and in a controlled environment. We then reanalysed two long-running time series of berry production, with selected climatic factors as explanatory variables.

Key results – Variables retained in the regression models were maximum temperature in June and mean temperature in August–September the year before fruiting, and maximum snow depth in April, minimum temperature in May, and hydrothermal ratio in June–July the current year. These factors could all be directly linked to critical plant processes and events such as timing of floral initiation, winter hardening, avoidance of frost injury during bloom, and drought avoidance in summer. Demonstration of superficial winter dormancy explained the vulnerability of *V. myrtillus* to winter frost injury and the need for stable snow cover. A highly significant lunisolar index corresponds to oscillations in ionizing cosmic radiation that by some unknown mechanism(s) may affect plant growth and development.

Conclusions – We conclude that the explanatory variables obtained in the regression models for annual berry production are causally related to specific physiological mechanisms controlling crucial events in the annual life cycle of *V. myrtillus*.

Key words – Berry production, bilberry, climate, flower formation, growth rhythm, time series, *Vaccinium myrtillus*.

INTRODUCTION

In northern boreal forests of Eurasia, *Vaccinium myrtillus* L. (Ericaceae, European blueberry or bilberry) is an abundant dwarf shrub with substantial ecological impacts (Nestby et al. 2011). Shoots and leaves are browsed by a large number of herbivores, including insects, grouse, small rodents and cervids, and bilberries are used by several birds and mammals, with thrushes being the most important seed dispersers. There is also a long tradition for berry use by man, and the species is now becoming of horticultural interest (Nestby et al. 2014). The large annual variation in berry production (Myrberget 1982, Wallenius 1999) affects most species that use this plant, especially herbivores (Selås et al. 2011b, 2011c). A possible underlying mechanism is that there is a trade-off between reproduction and chemical defence in the

plants (Selås 1997), i.e. feeding deterrents are reallocated and used for berry production during summer. Flowering peaks, which usually occur at regular intervals (Selås 2000), are synchronized over large areas (Selås et al. 2011c), indicating that flowering is triggered by some large-scale environmental factors that act during key stages of the annual life cycle. Particular critical phases are the periods of floral initiation and development, when the environment may act as a controlling and synchronizing factor (Heide 1997).

Because of the great ecological impact of *V. myrtillus*, attempts have been made to explain annual variation in berry production in Norway using climate variables and previous reproduction (Selås 2000, 2006a, 2006b, Selås et al. 2011a). A shortcoming of the analyses conducted so far, however, is that the seasonal timing and climatic regulation of floral induction in *V. myrtillus* is unknown. A negative relationship

between annual berry yield and temperatures in August–September in the previous year was identified by Selås (2006a, 2006b), but because this is the period of frost hardening (Taulavuori et al. 2004), the mechanism is most likely that low autumn temperatures result in elevated contents of soluble sugars and starch (e.g. Lee et al. 2012), at the expense of further growth. These resources may then be reallocated to growth and reproduction in the following spring. Severe winter injuries with greatly reduced flower production have also been reported for *V. myrtillus* (Ögren 1996, Bokhorst et al. 2008), and Gjørøvoll (1949) identified the species as a snow bed plant with affinity to sites with stable snow cover. Furthermore, in a 15-year study in Sweden, years with low or zero berry yields always had a history of spring frost during bloom (Kardell & Eriksson 1990). The same was reported from the Archangel region of Russia by Puchnina (1996), who also identified adequate moisture supply in July and August as an important determinant of bilberry yields.

A general problem related to regression analyses of bilberry production time series, is that weather variables are often intercorrelated, and therefore, spurious patterns may occur. The selection of weather variables therefore needs to be causally justified. When building multiple regression models from different periods, a requirement should also be that any climate variable is obtained by similar effect in all time series, unless there are clear rationales for lack of effect in one of the periods.

In the Norwegian time series analysed so far, there has been a c. 10-year periodic signal, accounted for by enhanced sunspot numbers as an explanatory variable. The assumption has been that productivity and resource allocation in *V. myrtillus* plants may be affected by UV-B-radiation (e.g. Gwynn-Jones et al. 2012). Montefalcone et al. (2013) found that flowering peaks in the seagrass *Posidonia oceanica* correlated well with sunspot numbers, but they suggested that the zeitgeber for flowering is galactic cosmic ray fluxes, which are negatively related to solar activity. Cosmic rays should in fact be expected to result in a more regular fluctuation pattern in the plants' resource allocation than UV-B, because they are less influenced by weather. Plants are most affected by the muon component of cosmic rays (Ferrari & Szuskiewicz 2009), which at higher latitudes depends on both the 11-year solar cycle and the 9.3-year lunar nodal phase cycle (Selås 2014).

In this study we first examine the growth rhythm and flowering performance of *V. myrtillus* in the field and under controlled conditions in a phytotron in order to determine the seasonal timing of floral initiation and development. Thereafter we reanalyse two time series on annual bilberry production from southern Norway after inclusion of the resulting findings in the database.

MATERIAL AND METHODS

Plant material and cultivation

For the examination of shoot structure, growth, and timing of floral initiation, *V. myrtillus* shoots from ramets at least three years old were sampled at weekly intervals in two years during spring and early summer from a natural stand located in

an open pine forest near the Apelsvoll Experimental Centre in the central part of South Norway (60°40'N 10°52'E, 250 m a.s.l.). Plants used for the growth experiments were raised from seed extracted from ripe berries harvested at the same location. The seed was germinated in light at 20°C as described by Giba et al. (1993). After germination, the seedlings were first pricked off into plastic trays and then potted singly into 8 cm plastic pots (c. 200 cm³ volume). A coarse-textured sphagnum peat growth medium with pH 4.1 (Degernes Torvstrøfabrikk, Norway) was used throughout. The plants were fertilized weekly with a complete fertilizer solution (Superba™ Orange (14N-4P-21K plus micronutrients), Yara Norge AS, Norway) with an electric conductivity of 0.4 mS cm⁻¹. The plants were watered daily as required.

The plants used in Experiment 1 were raised outdoors during summer and early autumn under natural temperature and daylength conditions at Apelsvoll, and according to dissection had formed flower buds when the experiment was started on October 1st. Those used in Experiments 2 were sown in September and raised at 20°C and 20 h photoperiod (to simulate summer conditions) in a greenhouse with supplemental lighting (SonT and incandescent lamps) at a photosynthetic photon flux (PPF) of c. 200 μmol photons m⁻² s⁻¹ during the winter. These plants were vegetative when the experiment was started on February 1st. The experiment was conducted in daylight compartments in the phytotron of the Norwegian University of Life Sciences at Ås, Norway (59°40'N 10°45'E) under conditions as described by Sønsteby & Heide (2006). Photoperiods of 10 and 20 h were established by moving the plants into adjacent growth rooms with either darkness or low intensity (7 μmol photons m⁻² s⁻¹) incandescent light for 10 h during the night (18h00–06h00). The day-length extension added less than 2% to the daily light integral, thus ensuring nearly the same total light energy in both photoperiods. Temperatures were controlled within ±1°C and a water vapour pressure deficit of 530 Pa was maintained at all temperatures.

Experimental design, data observation and analysis

Sampled shoots were visually examined for active growth, and their terminal buds dissected and examined under a stereo microscope for the presence of floral primordia. Ten shoots were examined at each sampling time, and flowering stages were scored on a 6-stage scale with 1 denoting vegetative apices and 6 denoting fully differentiated floral primordia. The growth experiments were factorial in structure with a split-plot design. Each treatment had three replications containing five plants each in Exp. 1 (n = 15), and six plants each in Exp. 2 (n = 18). Shoot elongation growth and the production of new leaves (nodes) were monitored by weekly measurements of plant height and recording the unfolded leaf numbers on the leading shoot. Data for final plant height and leaf number and the number of flowering plants (Exp. 2) were subjected to analysis of variance (ANOVA) by standard procedures using the Minitab® Statistical Software program package (Release 15; Minitab. Inc., State College, PA, USA).

Regression analyses of annual bilberry production

The regression analyses were based on two time series of annual bilberry production obtained from Aust-Agder County in southern Norway. One was calculated from annual game reports from the entire county for the period 1932–1977 (for details see Selås 2006a), and the other from counts of berries at 16 plots of 2 × 2 m in south-eastern parts of the county (58°39′–58°43′N 8°38′–8°49′E, 150–300 m a.s.l.) during 1999–2014 (see Selås et al. 2011a). The latter was square-root transformed to obtain normally distributed residuals, and plot was included as random factor in a mixed model. Only climate variables that contributed with a P-value < 0.10 in both models were maintained in the final linear multiple regression models, unless lack of effect in one of the periods could be explained.

In previous analyses (Selås 2006a, Selås et al. 2011a), factors used to explain the annual variation in bilberry production included previous berry production, mean temperature in August or September in the previous year, the interaction of snow depth and temperature in January, maximum snow depth in April, summer precipitation or hydrothermal ratio (total precipitation divided by mean temperature) of the current year, and yearly mean of sunspot numbers, with var-

ying time lags. Mean temperature in May, i.e. during flowering, usually lasting c. two weeks in a given location (Kuchko 1988), had less impact in the previous studies, so in the present study we used minimum temperature in May instead. To account for the c. 10-year signal, we used both the yearly mean of sunspot numbers and a lunar nodal phase index (for calculation see Selås 2014), with the time lag that gave the best fit in the models, as possible explanatory variables.

The summer climate during 1932–1977 was in general cooler than during 1999–2014; the mean June–September temperature in the two periods was 13.4 and 14.3°C, respectively. However, minimum temperatures in May were not as low during 1932–1977 (mean -2.6°C) as during 1999–2014 (mean -4.1°C). Snow depth in April was highest in the former period, with a mean of 35.5 cm, compared to 21.2 cm in the latter period. These differences may affect the timing of key stages during the flowering cycle. For some of the assumed climate effects, we therefore operated with two or more alternative climate variables. This was (1) mean temperature in August, September and August–September, and (2) hydrothermal ratio in June, in July and the lowest of June or July.

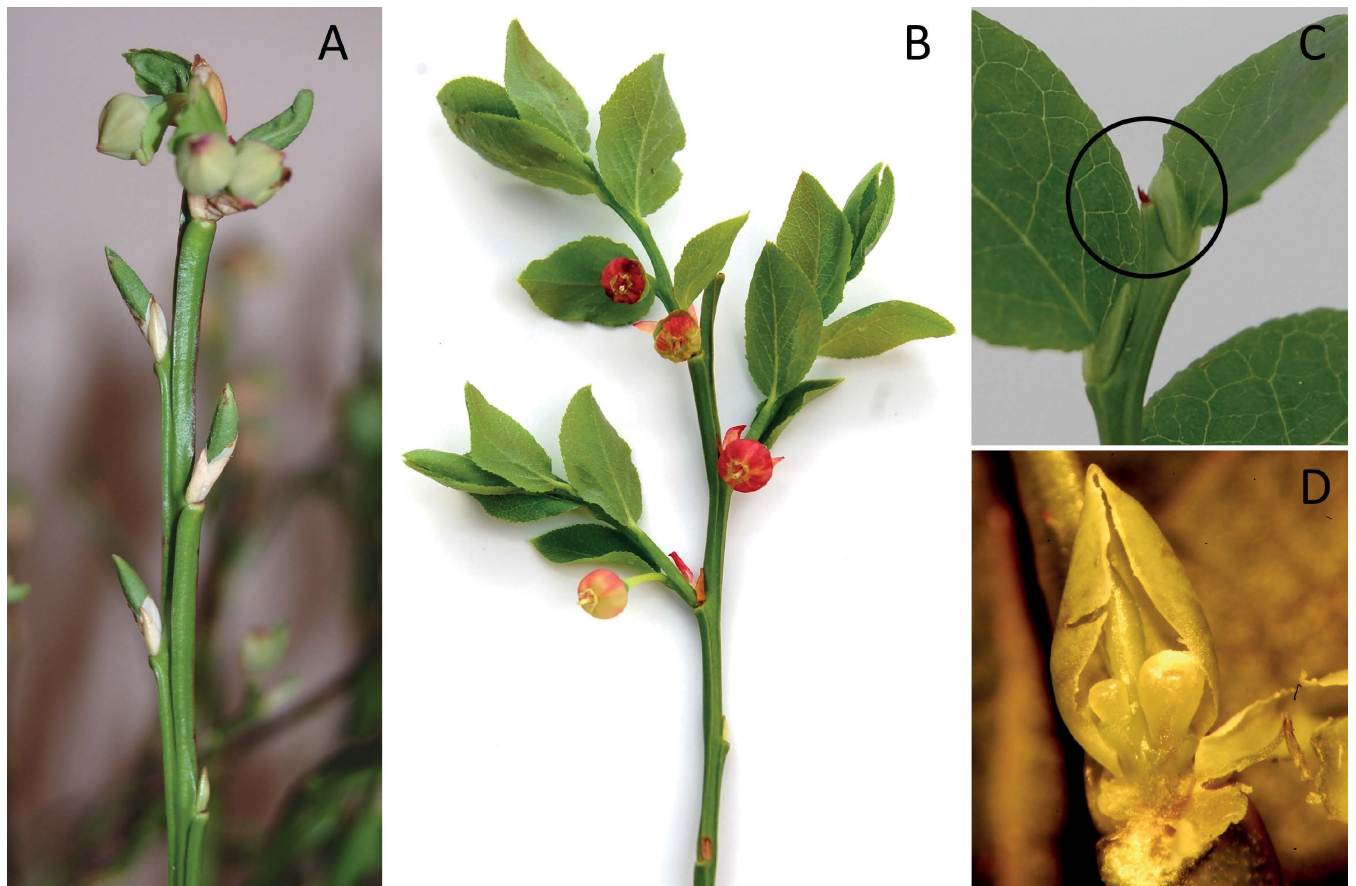


Figure 1 – Shoot and bud development of *Vaccinium myrtillus* in the field. A, sprouting of an overwintered shoot in early May. Note the early appearance of flowers at the uppermost nodes; B, an overwintered shoot with three developing lateral shoots. Note flowers at the base of the new shoots, the topmost one with two flowers; C, new shoot with aborted terminal bud; D, dissected terminal bud with two flower primordia and a terminal vegetative bud primordium with leaf primordia for next year’s growth. The most advanced flower (right) at stage 6, the other (left) at stage 4. Photographed on July 1st, 2010.

Temperature data were from the Bygland Meteorological Station (58°40'N 7°48'E, 200 m a.s.l.), and data on precipitation from the Mykland Meteorological Station (58°38'N 8°17'E, 250 m a.s.l.), all provided by the Norwegian Meteorological Institute (www.met.no). These stations were the closest that provided data throughout both study periods. The analyses were conducted in JMP, version 10 (SAS Institute, Cary, North Carolina).

RESULTS

Growth and development in the field

Bud burst and growth started as soon as the temperature began to rise after snowmelt. The first organs to appear in spring were the flowers formed in the previous season (fig. 1A), followed by unfolding of a series of leaves, which apparently also had been present as primordia in the overwintering buds (fig. 1B). The number of leaves on the shoots varied from two to nine, increasing from the base towards the tip of the overwintering shoot. Annual shoot elongation was terminated by abortion of the terminal bud at mid-summer, the sub-terminal bud then becoming terminal (fig. 1C). Examination of dissected buds demonstrated that the first visible sign of floral initiation was a flattening of the apical meristem followed by the formation of rounded protuberances on the apical flanks as described for the North American *Vaccinium angustifolium* Ait. (lowbush blueberry) by Aalders & Hall (1965). Buds at this stage were assigned flower development stage 2. Differentiation of the various flower structures then followed rapidly so that within a fortnight all flower parts were visible in dissected buds by the end of June (fig. 1D, table 1). While *V. angustifolium* forms an inflorescence with many flowers at each flowering site, *V. myrtillus* initiates only one solitary flower (sometimes two), in the lower leaf angles of the developing new shoot (fig. 1B & D).

Weekly time series of bud dissection showed that floral initiation took place during a short period in mid-June (fig. 2) shortly preceding growth cessation caused by terminal bud abortion. A calendar of growth and developmental events in the two years of investigation is presented (table 1). A three-week difference in the time of snowmelt in the two years strongly affected the timing of all plant developmental events. Examination of buds from 80 leading shoots on July 1st and 75 shoots on September 1st illustrated that nearly 100% of the terminal buds had formed flower primordia by July 1st, while the percentage of buds with flower primordia decreased rapidly in the lower, lateral buds (fig. 3). The results indicate that some flower primordia aborted during late summer. Following completion of flower differentiation, no other visible development took place, the plants apparently becoming dormant.

Examination of dormancy status

In order to test the dormancy state of the plants in autumn, a batch of potted plants were grown outdoors during summer and subjected to forcing in a heated greenhouse at 18°C and 20 h photoperiod on October 1st, without and with varying lengths of chilling in a cold store at 1°C in the dark. At this time, the plants had shed almost all leaves, and dissections

Table 1 – Calendar of developmental events in field-grown *Vaccinium myrtillus* plants in two succeeding years.

Developmental event	2010	2011
Snow melt	April 27	April 5
Green bud tip showing	May 24	April 28
Open flower (anthesis)	May 30	May 6
Shoot growth completed	June 8	May 24
Terminal abortion visible	June 16	June 1
Flower formation initiated	June 21	June 7
All flower organs differentiated	June 30	June 16
Accumulated temperature sum to flower stage 2 (degree days > 0°C)	535	618

revealed that they had flower primordia. The results indicated that the plants were in a superficial state of dormancy and were able to start growing and develop flowers without any chilling (fig. 4). However, with four or more weeks of chilling, time to budburst and anthesis was significantly reduced, while at the same time, the percentage of flowering plants increased in parallel. Further extension of chilling for up to 12 weeks resulted in only minor further advancements of bud development.

Growth and development in the phytotron

Vegetative plants were exposed to temperatures of 6, 12, and 18°C in 10 h SD and 20 h LD. These treatments were applied for 6 weeks or 10 weeks, whereupon the plants were transferred to 18°C and 20 h LD for recording of flowering performance. The results illustrated that continuous growth was maintained in LD at 18°C only (fig. 5). Plants grown continuously under these conditions for over a year grew continuously to a height of 1 m and formed only occasional flowers on subordinate, lower shoots (fig. 6). Under all the other conditions, growth levelled off and ceased completely after approximately three weeks. However, when transferred to LD at 18 °C all these plants resumed rapid growth for several weeks whereafter growth usually levelled off or ceased

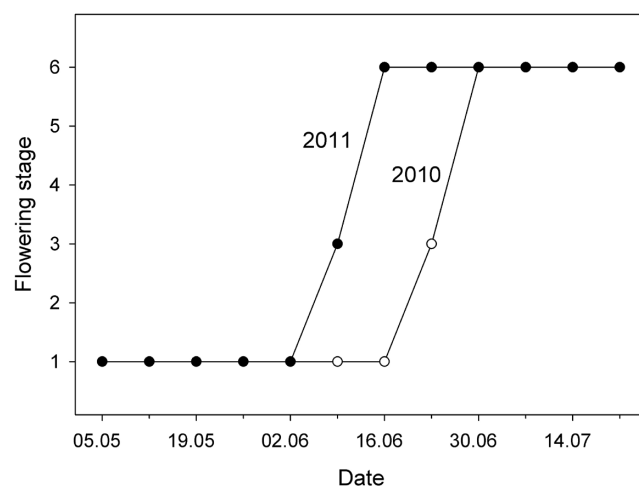


Figure 2 – Time courses of floral initiation of *Vaccinium myrtillus* in the field in the years 2010 and 2011. Data are means of 10 shoots at each sampling time.

Table 2 – Growth and flowering responses of *Vaccinium myrtillus* plants after exposure to 10 h or 20 h photoperiod at 6, 12 or 18°C for 6 or 10 weeks.

Length of treatment (weeks)	Photoperiod (h)	Temperature (°C)	Shoot growth increment at end of treatment (cm)	Leaf no. increment at end of treatment	Shoot growth increment during LD forcing (cm)	Leaf no. increment during LD forcing	Flowering plants (%)	Days to anthesis	
6	10	6	0.7	1.4	2.6	6.4	38.9	60.3	
		12	1.0	1.8	2.9	6.1	66.7	71.8	
		18	1.3	2.5	3.2	7.9	55.6	73.3	
		<i>Mean</i>	<i>1.0</i>	<i>1.9</i>	<i>2.9</i>	<i>6.8</i>	<i>53.7</i>	<i>69.5</i>	
	20	6	0.8	2.0	2.1	5.6	61.1	68.7	
		12	1.3	2.2	3.4	6.6	72.2	57.2	
		18	2.6	3.6	3.6	6.8	77.8	67.7	
		<i>Mean</i>	<i>1.6</i>	<i>2.6</i>	<i>3.0</i>	<i>6.3</i>	<i>70.4</i>	<i>64.4</i>	
	10	10	6	0.6	1.2	2.1	7.1	33.3	61.7
			12	0.7	1.6	2.3	7.4	27.8	49.2
			18	1.2	2.7	2.5	7.8	38.9	62.1
			<i>Mean</i>	<i>0.8</i>	<i>1.8</i>	<i>2.3</i>	<i>7.4</i>	<i>33.3</i>	<i>58.4</i>
20		6	1.0	1.8	2.3	6.6	27.8	54.8	
		12	2.0	4.2	2.4	7.3	66.7	48.1	
		18	4.2	6.9	4.6	8.2	50.0	44.9	
		<i>Mean</i>	<i>2.4</i>	<i>4.3</i>	<i>3.1</i>	<i>7.3</i>	<i>48.1</i>	<i>48.3</i>	
Probability level of significance (ANOVA)									
Source of variation									
Temperature (A)			0.001	0.002	0.06	NS	NS	NS	
Photoperiod (B)			< 0.001	< 0.001	0.01	NS	0.02	0.04	
Length of treatment (C)			0.02	0.004	NS	0.02	0.003	0.001	
A × B			< 0.001	0.01	0.01	NS	NS	NS	
A × C			NS	0.02	NS	NS	NS	NS	
B × C			0.001	0.002	0.04	NS	NS	NS	
A × B × C			NS	NS	NS	NS	NS	NS	

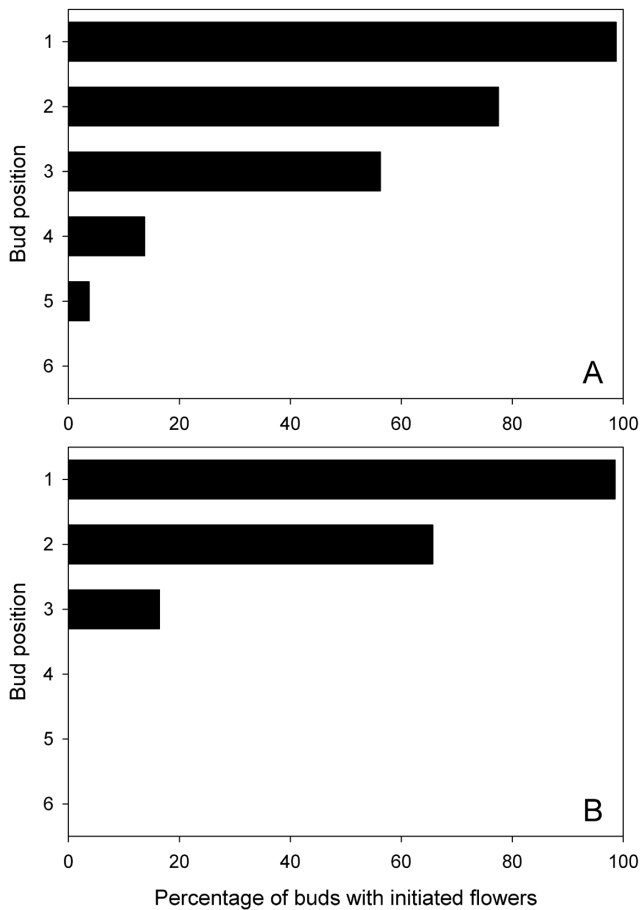


Figure 3 – Percentage of buds with floral primordia at the various bud positions on: A, July 1st; and B, September 1st. Position 1 is the terminal bud. Data are means of 80 and 75 shoots, respectively for A and B.

again. Although flowering was sparse under all conditions, the number of flowering plants was significantly ($P < 0.02$) higher in LD, while a small increase by increasing temperature was non-significant (table 2). No significant interaction of temperature and photoperiod was present. Extension of the treatment period from six to 10 weeks did not increase flowering as expected, but reduced it significantly ($P < 0.003$) under all conditions. The number of days to anthesis was significantly reduced by LD and extended treatment time, but was not significantly affected by temperature conditions (table 2).

Regression analyses of annual bilberry production

In the time series analyses on annual berry production, mean and maximum temperature in June, as well as in the second half of June (16–30), were used as possible explanatory variables. For both study periods (1932–1977 and 1999–2014), there was a significant or almost significant positive relationship between the berry indices and maximum temperature in June in the previous year, when other factors known or assumed to affect berry production in *V. myrtillus* were accounted for (table 3). Mean temperature in June, or mean or maximum temperature in late June, gave a poor fit in both models.

Other factors that contributed to explain the annual variation in berry production of *V. myrtillus* in both periods were the lunar nodal phase index of the previous year, autumn temperatures in the previous year (August + September in 1932–1977, September only in 1999–2014), maximum snow depth in April and the hydrothermal ratio in summer (June or July in 1932–1977, June in 1999–2014) (table 3). Minimum temperature in May contributed with a significant positive effect only in the 1999–2014-period, when the mean minimum May temperature was 1.5°C lower than during 1932–1977. There was no additional relationship with Janu-

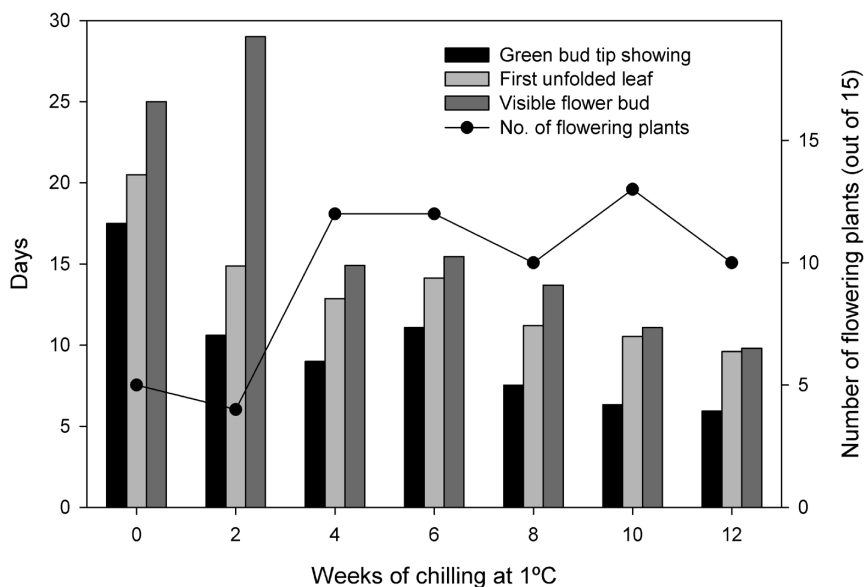


Figure 4 – Growth and flowering performance of *Vaccinium myrtillus* plants forced at 18°C in 20 h photoperiod without preceding chilling and with varying durations of chilling at 1°C. During the preceding growing season, the plants were grown outdoors under natural temperature and day-length conditions until October 1st, when the experiment was started. Data are means of three replications containing five plants each.

ary temperature or snow depth, or the interaction of these variables, in the selected models. There was, however, an additional negative effect of the berry index of year $t-1$ in the 1932–1977-period, but inclusion of this alternate bearing factor gave only minor changes in the significance level of the selected climate variables.

DISCUSSION

The results indicated that in the natural environment in the field, *V. myrtillus* plants ceased elongation growth and initiated flower primordia under LD conditions during a short pe-

riod in June. The growth cessation was associated with shoot tip abortion (table 1), a phenomenon that is well known also in some other temperate woody plants such as *Betula* and *Salix* (e.g. Junttila 1976). No further growth took place during late summer. Similar developmental patterns have been reported for *V. angustifolium* in Canada. Bell & Burchill (1955) found that in various parts of Nova Scotia floral initiation took place in late June and early July, immediately following growth cessation, and Barker & Collins (1963) observed that growth cessation was directly associated with shoot tip abortion. However, later investigations have shown

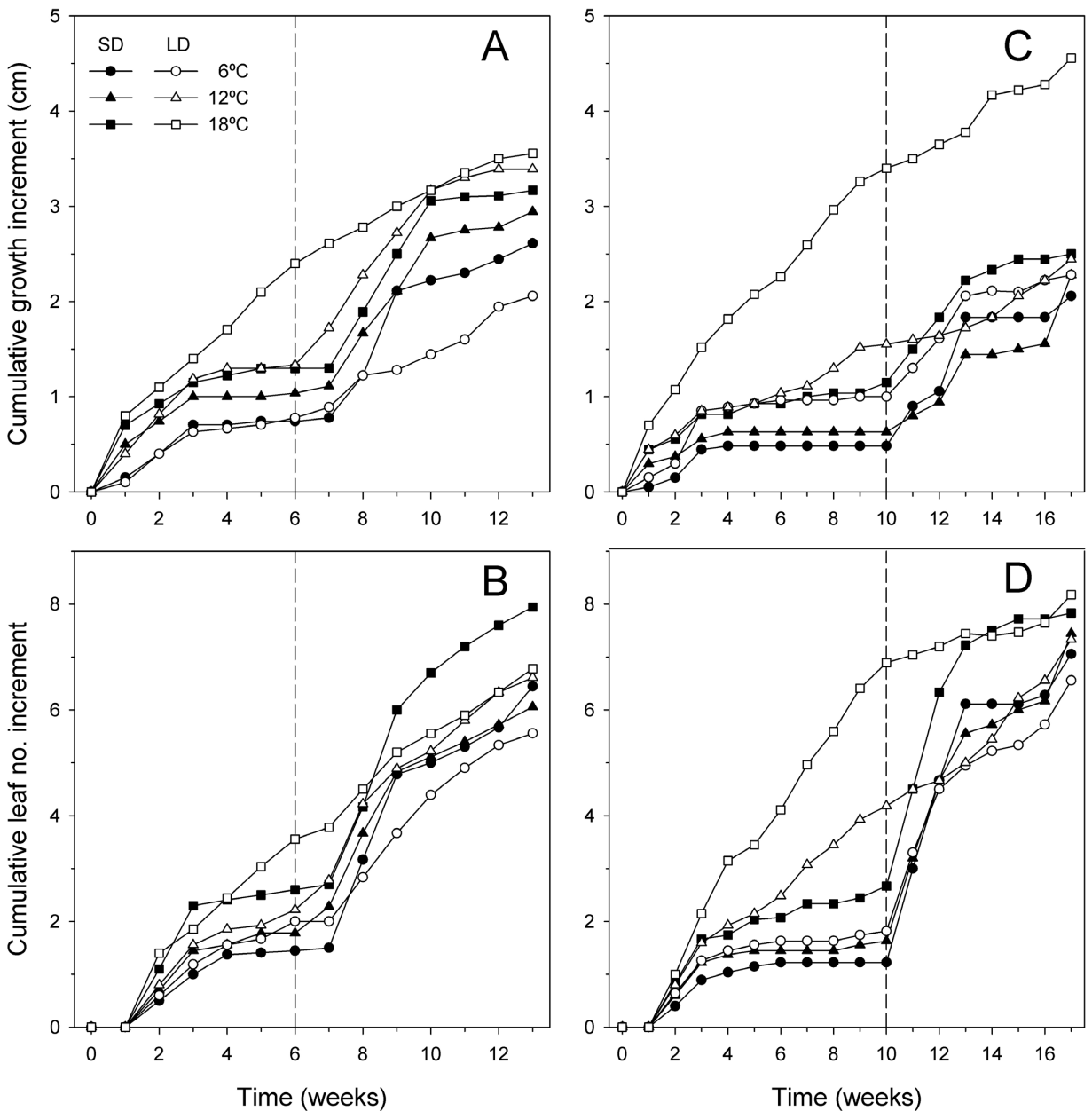


Figure 5 – Time courses of leading shoot elongation growth and production of new leaves in *Vaccinium myrtillus* plants as affected by temperature and photoperiod as indicated. The treatments were applied for 6 weeks (A, B) or 10 weeks (C, D). During the succeeding 7 weeks, all plants were grown at 18°C and 20 h photoperiod for recording of flowering performance. Data are means of three replications, each containing six plants.

Table 3 – Linear multiple regression models with an index of berry production of *Vaccinium myrtillus* in southern Norway in year *t* as response variable, and selected physical factors as explanatory variables.

Only variables with $p < 1.0$ in both models were included, unless there were clear rationales for lack of effect in one of the periods. For the second period, plot ($n = 16$) was included as random factor.

Explanatory variable	Estimate	SE	df	t-ratio	P-value
A) 1932–1977-period, $R^2 = 0.46$					
Intercept	1.0423	1.1177	1		
Lunar index, $t-1$	0.1730	0.0517	1	3.35	0.0018
Maximum temp June, $t-1$	0.0760	0.0406	1	1.87	0.0687
Mean temp August-September, $t-1$	-0.2376	0.0811	1	-2.93	0.0056
Maximum snow depth April, t	0.0043	0.0018	1	2.39	0.0214
Hydrothermal ratio, June or July, t	0.1598	0.0372	1	4.30	0.0001
B) 1999–2014-period, mixed model, $R^2 = 0.53$					
Intercept	6.9974	8.4178	1		
Lunar index, $t-1$	1.9314	0.2434	1	7.94	<0.0001
Maximum temp June, $t-1$	0.8764	0.2633	1	3.33	0.0010
Mean temp September, $t-1$	-1.3890	0.4186	1	-3.32	0.0011
Maximum snow depth April, t	0.0638	0.0172	1	3.72	0.0002
Hydrothermal ratio June, t	0.5071	0.1395	1	3.64	0.0003
Minimum temperature May, t	0.8214	0.3080	1	2.97	0.0082



that in *V. angustifolium*, late-season vegetative growth may occur (e.g. Kaur et al. 2012, White et al. 2012).

Under greenhouse conditions, Hall & Ludwic (1961) and Aalders & Hall (1965) reported that *V. angustifolium* grown at 16-h photoperiod grew continuously and remained vegetative, whereas in 8-h SD it ceased growing and initiated flower buds. The critical photoperiod was 12–14 h, depending on the origin of the clones, and 8 weeks of exposure was sufficient for full flowering (Hall & Ludwic 1961). In our study on *V. myrtillus* plants, we found similar conflicting photoperiodic responses in the field and under controlled conditions in the phytotron. The exception observed in this study with *V. myrtillus* was sparse flowering initiated also under LD conditions (tables 1 & 2, fig. 5). This suggests that growth cessation is not an absolute prerequisite for flower initiation. However, while our height measurements were confined to the leading shoot of each plant, all flowers appeared on inferior shoots that often had an intermittent growth pattern. The same was also observed in plants maintained for over a year in LD at 18°C (fig. 6). We therefore conclude that floral initiation in both *V. myrtillus* and *V. angustifolium* (Aalders & Hall 1965) is usually associated with growth cessation.

The physiological mechanisms underlying these contrasting responses of plants in the field and in greenhouses are not understood, and the phenomenon remains an enigma. However, Barker & Collins (1963) concluded that growth cessation and floral initiation in the field at mid-summer are not direct photoperiodic responses. In *V. myrtillus* plants growing in the field, all leaves of the annual shoot seem to be formed in the preceding season and contained in the over-wintering bud. This confers a determinate growth pattern on the plants that results in growth cessation and floral initiation after ex-

◀ **Figure 6** – *Vaccinium myrtillus* plant grown continuously at 18°C and 20 h photoperiod for 14 months.

pansion of the pre-formed shoot, regardless of photoperiodic conditions. After such a shift, the plants are trapped in a perpetuating cycle of predetermined closed growth. Under these conditions, each flowering shoot gets a biennial life history. This is consistent with the finding that severe pruning in the field in autumn (cutting to the ground) reduced fruit yields to nearly zero, not only in the first but also in the second year after pruning (Nestby et al. 2014). Contrary to this, the continuously growing *V. myrtillus* plants in LD at 18°C (fig. 5) had an undetermined (open) growth modus where new leaves were initiated concurrently with shoot elongation. We suggest that the basically different modes of growth of plants in the field and of vegetative plants in the greenhouse are the main explanation for the different developmental patterns observed in plants under these conditions.

Significant advancements in the molecular understanding of the mechanisms underlying floral initiation have recently been obtained also in woody perennial plants, including blueberries. In the winter-annual model plant *Arabidopsis thaliana*, photoperiodic control of flowering is mediated by a genetic pathway in which *CONSTANS (CO)* and *FLOWERING LOCUS T (FT)* are major regulatory genes (Searle & Coupland 2004). In perennial woody plants, however, the regulation of flowering is usually closely integrated with the annual growth cycle as reviewed by Kurokura et al. (2013). Thus, in aspen trees (*Populus* spp.), genes homologous to the CO/FT regulatory module control the timing of both flowering and seasonal growth cessation (Böhlenius et al. 2006, Hsu et al. 2011). Central involvement of FT-like genes in these processes was also recently demonstrated even in *Vaccinium corymbosum* L. (highbush blueberry) (Song et al. 2013). The observed close connection between growth cessation and floral initiation in *V. myrtillus* suggests that a similar dual genetic control mechanism is involved also in this species. Mediation of seasonal growth rhythm and flowering by growth regulators such as gibberellins is also well documented in many woody perennials (Moritz et al. 1990, Kurokura et al. 2013). Although a detailed discussion of these mechanisms is beyond the scope of this paper, it is interesting to note that application of growth-promoting gibberellin has been reported to influence floral initiation in *V. corymbosum* (Lindberg et al. 2014).

A main objective of our study was to determine whether the regression indices for annual berry production have a causal basis and can be explained in terms of known plant physiological processes and mechanisms (table 3). Snow depth in April is a measure of duration and stability of snow cover, which is important for winter survival of *V. myrtillus*. Experiment 1 (fig. 4) demonstrated that *V. myrtillus* plants are in a superficial state of dormancy in autumn, and thus have only a minor chilling requirement for complete dormancy release. Both natural (Ögren 1996) and artificially established warm spells (Bokhorst et al. 2008) during the winter resulted in severe winter damage that is causally related to lack of deep dormancy in the species. Such results also explain the affinity of *V. myrtillus* to snow-bed sites (cf. Gjærevoll 1949). Besides, much snow in April will usually delay the development in spring, and thus reduce the risk of frost injury during flowering. The fact that minimum temperature in

May contributed with a significant effect only during 1999–2014 could be due to lower minimum temperatures during this period, but also to earlier floral development because of less snow cover in April. Such results also suggest that the predicted and ongoing climate change has significantly increased the susceptibility of *V. myrtillus* to frost injury during flowering in spring (Gerdol et al. 2013, Wheeler et al. 2014).

The negative regression index for the preceding September temperature can be related to the general need for temperatures < 10°C for winter hardening and accumulation of freeze protectants in *Vaccinium* spp. and other boreal plants (Larcher 1995, Taulavuori et al. 2004, Lee et al. 2012). Furthermore, decreasing temperature and photoperiod in late summer are also important for induction of bud dormancy and prevention of premature flowering in late summer in plants with preformed flower primordia (Larcher 1995). Likewise, the index for hydrothermal ratio in June and July is obviously linked to the vulnerability of *V. myrtillus* plants to summer drought (Kuchko 1988, Puchnina 1996). Also, the positive index for June temperature in the preceding season can be related to the thermal time (heat-sum) requirements for floral initiation in the year preceding berry production (table 1). Elevated temperature in June would then act as a synchronizing factor to ensure timely initiation of floral primordia before midsummer (cf. table 1, fig. 2).

The 9.3-year lunar index is of a different nature, and the positive relationship with berry production must be mediated via indirect effects on the vegetation. The lunar index is positively related to cosmic rays, and also indices of herbivore populations have been better explained by this index than by the 11-year solar index (Selås 2014). Possible impacts of cosmic rays on plants has so far received little attention, but Dengel et al. (2009) found a significant positive correlation between the growth of *Picea sitchensis* Bong. (Sitka spruce) and cosmic rays in Scotland. They hypothesised that photosynthesis is affected by changes in the ratio between direct sunlight and diffuse solar radiation as a result of increased amounts of clouds in periods with high levels of cosmic rays. However, they also suggested that cosmic rays somehow directly influence plant growth.

With reference to the present and earlier investigations, we conclude that the indices for annual berry production of *V. myrtillus* presented, to a large extent are causally related to specific and well known plant eco-physiological processes and mechanisms in the bilberry plant.

ACKNOWLEDGEMENTS

We are grateful to David Percival and an anonymous referee for valuable comments on the manuscript, and to Ole Wiggo Røstad for the technical presentation of the figures. NIBIO acknowledges financial support of this work by the Norwegian Research Council through project #: 184797/I10. Nina Opstad is grateful to Inger Martinussen, NIBIO, for fruitful discussions, and to Hans Gunnar Espelien and Unni Myrheim Roos at NIBIO, for excellent technical assistance.

REFERENCES

- Aalders L.E., Hall I.V. (1965) A comparison of flower-bud development in the lowbush blueberry, *Vaccinium angustifolium* Ait. under greenhouse and field conditions. *Proceedings of the American Society for Horticultural Science* 85: 281–284.
- Barker W.G., Collins W.B. (1963) Growth and development of the lowbush blueberry: apical abortion. *Canadian Journal of Botany* 41: 1319–1324. <http://dx.doi.org/10.1139/b63-112>
- Bell H.P., Burchill J. (1955) Flower development in the lowbush blueberry. *Canadian Journal of Botany* 33: 251–258.
- Böhlenius H., Huang T., Charbonnel-Campaa L., Brunner A.M., Jansson S., Strauss S.H., Nilsson O. (2006) CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* 312: 1040–1043. <http://dx.doi.org/10.1126/science.1126038>
- Bokhorst S., Bjerke J.W., Bowles F.W., Melillo J., Callaghan T.V., Phoenix G.K. (2008) Impacts of extreme winter warming in the sub-Arctic: growing season responses of dwarf shrub heathland. *Global Change Biology* 14: 2603–2612. <http://dx.doi.org/10.1111/j.1365-2486.2008.01689.x>
- Dengel S., Aeby D., Grace J. (2009) A relationship between galactic cosmic radiation and tree rings. *New Phytologist* 185: 545–551. <http://dx.doi.org/10.1111/j.1469-8137.2009.03026.x>
- Ferrari F., Szuskiewicz E. (2009) Cosmic rays: a review for astrobiologists. *Astrobiology* 9: 413–436. <http://dx.doi.org/10.1089/ast.2007.0205>
- Gerdol R., Siffi C., Iacumin P., Gualmini M., Tomaselli M. (2013) Advanced snowmelt affects vegetative growth and sexual reproduction of *Vaccinium myrtillus* in a sub-alpine heath. *Journal of Vegetation Science* 24: 569–579. <http://dx.doi.org/10.1111/j.1654-1103.2012.01472.x>
- Giba Z., Grubisic D., Konjevic R. (1993) The effect of white light, growth regulators and temperature on the germination of blueberry (*Vaccinium myrtillus* L.) seeds. *Seed Science and Technology* 21: 521–529.
- Gjærevoll O. (1949) Snøleivevegetasjonen i Oviksfjellene. *Acta Phytogeographica Suecica* 25: 1–108. [In Norwegian, with English summary].
- Gwynn-Jones D., Jones A.G., Waterhouse A., Winters A., Comont D., Scullion J., Gardias R., Graae B.J., Lee J.A., Callaghan T.V. (2012) Enhanced UV-B and elevated CO₂ impacts sub-arctic shrub berry abundance, quality and seed germination. *Ambio* 41: 256–268. <http://dx.doi.org/10.1007/s13280-012-0311-4>
- Hall I.V., Ludwic R.A. (1961) The effects of photoperiod, temperature and light intensity on the growth of the lowbush blueberry (*Vaccinium angustifolium* Ait.). *Canadian Journal of Botany* 39: 1733–1739. <http://dx.doi.org/10.1139/b61-151>
- Heide O.M. (1997) Environmental control of flowering in some northern *Carex* species. *Annals of Botany* 79: 319–327. <http://dx.doi.org/10.1006/anbo.1996.0343>
- Hsu C.-Y., Adams J.P., Kim H., No K., Ma C.P., Strauss S.H., Drnevich J., Vandervelde L., Ellis J.D., Rice B.M., Wickett N., Gunter L.E., Tuskan G.A., Brunner A.M., Page G.P., Barakat A., Carlson J.E., dePamphilis C.W., Luthe D.S., Yuceer C. (2011) FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar. *Proceedings of the National Academy of Sciences of the United States of America* 108: 10756–10761. <http://dx.doi.org/10.1073/pnas.1104713108>
- Junttila O. (1976) Apical growth cessation and shoot tip abscission in *Salix*. *Physiologia Plantarum* 38: 278–286. <http://dx.doi.org/10.1111/j.1399-3054.1976.tb04004.x>
- Kardell L., Eriksson L. (1990) Skogsskötselmetodernas inverkan på blåbær och lingon. Resultat av en tioårig försöksserie. SLU Rapport no. 47. Swedish University of Agricultural Sciences, Department of Environmental Forestry. [In Swedish].
- Kaur J., Percival D., Hainstock L.J., Privé J.-P. (2012) Seasonal growth dynamics and carbon allocation of the wild blueberry plant (*Vaccinium angustifolium* Ait.). *Canadian Journal of Plant Science* 92: 1145–1154. <http://dx.doi.org/10.4141/cjps2011-204>
- Kuchko A.A. (1988) Bilberry and cowberry yields and the factors controlling them in the forest of Karelia, USSR. *Acta Botanica Fennica* 136: 23–25.
- Kurokura T., Mimida N., Battey N.H., Hytönen T. (2013) The regulation of seasonal flowering in the Rosaceae. *Journal of Experimental Botany* 64: 4131–4141. <http://dx.doi.org/10.1093/jxb/ert233>
- Larcher W. (1995) *Physiological Plant Ecology*. 3rd Ed. Berlin, Heidelberg & New York, Springer-Verlag.
- Lee J.H., Yu D.J., Kim S.J., Choi D., Lee H.J. (2012) Intraspecific differences in cold hardiness, carbohydrate content and β -amylase gene expression of *Vaccinium corymbosum* during cold acclimation and deacclimation. *Tree Physiology* 32: 1533–1540. <http://dx.doi.org/10.1093/treephys/tps102>
- Lindberg W., Hanson E., Lobos G.A. (2014) Partial inhibition of flowering in young highbush blueberries with gibberellins. *Ciencia e Investigación Agraria* 41: 349–356. <http://dx.doi.org/10.4067/S0718-16202014000300007>
- Montefalcone M., Giovannetti E., Morri C., Peirano A., Bianchi C.N. (2013) Flowering of the seagrass *Posidonia oceanica* in NW Mediterranean: is there a link with solar activity? *Mediterranean Marine Science* 14: 416–423. <http://dx.doi.org/10.12681/mms.529>
- Moritz T., Philipson J., Odén, P.C. (1990) Quantitation of Gibberellins A₁, A₃, A₉, and A₉ and a putative A₉-conjugate in grafts of Sitka spruce (*Picea sitchensis*) during the period of shoot elongation. *Plant Physiology* 93: 1476–1481. <http://dx.doi.org/10.1104/pp.93.4.1476>
- Myrberget S. (1982) Production of some wild berries in Norway. *Fauna och Flora* 77: 261–268. [In Swedish, with English summary].
- Nestby R., Percival D., Martinussen I., Opstad N., Rohloff J. (2011) The European blueberry (*Vaccinium myrtillus* L.) and the potential for cultivation. A review. *The European Journal of Plant Science and Biotechnology* 5: 5–16.
- Nestby R., Martinussen I., Krogstad T., Uleberg E. (2014) Effect of fertilization, tiller cutting and environment on plant growth and yield of European blueberry (*Vaccinium myrtillus* L.) in Norwegian forest fields. *Journal of Berry Research* 4: 79–95. <http://dx.doi.org/10.3233/JBR-140070>
- Ögren E. (1996) Premature dehardening in *Vaccinium myrtillus* during a mild winter: a cause for winter dieback? *Functional Ecology* 10: 724–732. <http://dx.doi.org/10.2307/2390507>
- Puchnina L.V. (1996) Crop yield of fruits of *Vaccinium myrtillus* L. in the Pinega national forest. *Rastitel nye Resursy* 32: 29–32.
- Searle I., Coupland G. (2004) Induction of flowering by seasonal changes in photoperiod. *The EMBO Journal* 23: 1217–1222. <http://dx.doi.org/10.1038/sj.emboj.7600117>
- Selås V. (1997) Cyclic population fluctuations of herbivores as an effect of cyclic seed cropping of plants: the mast depression hypothesis. *Oikos* 80: 257–268. <http://dx.doi.org/10.2307/3546594>
- Selås V. (2000) Seed production of a masting dwarf shrub, *Vaccinium myrtillus*, in relation to previous reproduction and weather. *Canadian Journal of Botany* 78: 423–429. <http://dx.doi.org/10.1139/b00-017>

- Selås V. (2006a) Explaining bank vole cycles in southern Norway 1980–2004 from bilberry reports 1932–1977 and climate. *Oecologia* 147: 625–631. <http://dx.doi.org/10.1007/s00442-005-0326-7>
- Selås V. (2006b) Patterns in grouse and woodcock *Scolopax rusticola* hunting yields from central Norway 1901–24 do not support the alternative prey hypothesis for grouse cycles. *Ibis* 148: 678–686. <http://dx.doi.org/10.1111/j.1474-919X.2006.00566.x>
- Selås V., Holand Ø., Ohlson M. (2011a) Digestibility and N-concentration of bilberry shoots in relation to berry production and N-fertilization. *Basic and Applied Ecology* 12: 227–234. <http://dx.doi.org/10.1016/j.baae.2011.01.004>
- Selås V., Sonerud G.A., Framstad E., Kålås J.A., Kobro S., Pedersen H.B., Spidsø T.K., Wiig Ø. (2011b) Climate change in Norway: warm summers limit grouse reproduction. *Population Ecology* 53: 361–371. <http://dx.doi.org/10.1007/s10144-010-0255-0>
- Selås V., Sonerud G.A., Hjeljord O., Gangsei L.E., Pedersen H.B., Framstad E., Spidsø T.K., Wiig Ø. (2011c) Moose recruitment in relation to bilberry production and bank vole numbers along a summer temperature gradient in Norway. *European Journal of Wildlife Research* 57: 523–535. <http://dx.doi.org/10.1007/s10344-010-0461-2>
- Selås V. (2014) Linking “10-year” herbivore cycles to the lunisolar oscillation: the cosmic ray hypothesis. *Oikos* 123: 194–202. <http://dx.doi.org/10.1111/j.1600-0706.2013.00716.x>
- Song G.-Q., Walworth A., Zhao D., Jiang N., Hancock J.F. (2013) The *Vaccinium corymbosum* FLOWERING LOCUS T-like gene (VcFT): a flowering activator reverses photoperiodic and chilling requirements in blueberry. *Plant Cell Reports* 32: 1759–1769. <http://dx.doi.org/10.1007/s00299-013-1489-z>
- Sønsteby A., Heide O.M. (2006) Dormancy relations and flowering of the strawberry cultivars Korona and Elsanta as influenced by photoperiod and temperature. *Scientia Horticulturae* 110: 57–67. <http://dx.doi.org/10.1016/j.scienta.2006.06.012>
- Taulavuori E., Tahkokorpi M., Taulavuori K., Laine K. (2004) Anthocyanins and glutathione S-transferase activities in response to low temperature and frost hardening in *Vaccinium myrtillus* (L.). *Journal of Plant Physiology* 161: 903–911. <http://dx.doi.org/10.1016/j.jplph.2003.12.001>
- Wallenius T.H. (1999) Yield variations of some common wild berries in Finland in 1956–1996. *Annales Botanici Fennici* 36: 299–314.
- Wheeler J.A., Hoch G., Cortés A.J., Sedlacek J., Wipf S., Rixen C. (2014) Increased spring freezing vulnerability for alpine shrubs under early snowmelt. *Oecologia* 175: 219–229. <http://dx.doi.org/10.1007/s00442-013-2872-8>
- White S.N., Boyd N.S., Van Acker R.C. (2012) Growing degree-day models for predicting lowbush blueberry (*Vaccinium angustifolium* Ait.) ramet emergence, tip dieback, and flowering in Nova Scotia, Canada. *HortScience* 47: 1014–1021.

Manuscript received 3 Feb. 2015; accepted in revised version 6 Oct. 2015.

Communicating Editor: Renate Wesselingh.