Norges miljø- og biovitenskapelige universitet

Master's Thesis 2017 60 Credits
Faculty of Biosciences

Growth and flower initiation in red raspberry (*Rubus idaeus* L.) cultivars.

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i. Acknowledgments

This thesis is a part of the master program plant science at the faculty of biosciences at the Norwegian University of Life Sciences (NMBU). The study was executed in collaboration with the Norwegian Institute of Bioeconomy Research (NIBIO). It is also a part of the Norwegian project 'KLIMAFRUKT' and the European project 'GoodBerry'.

I would like to thank my supervisor Siv Fagertun Remberg and co-advisor Anita Sønsteby for their patience, encouragement and guidance. In addition, I would like to thank Professor Ola M. Heide for his help during registration and advisement. A special thank you to Rodmar Rivero Casique for helping with registrations, dissection of buds and scanning electron microscopy, this study would not be possible without you.

I would also like to thank Kari Grønnerød and Signe Hansen for their help and technical guidance during the experimental period. Thank you to Unni Myrheim Roos at NIBIO for raising the plants used in the controlled environment experiment. I would also like to express my gratitude towards Hilde Raanaas Kolstad at the Imaging Centre at NMBU for her technical support and guidance with scanning electron microscopy.

Finally, I would like to thank my family, for their support and encouragement.

Ås, May 13, 2017

Randi Hodnefjell

ii. Abstract

Growth cessation and flower initiation has been studied in different raspberry (*Rubus idaeus* L.) cultivars to better understand the factors influencing the adaptation of raspberry to a changing Nordic and European climate. Two experiments were conducted: 1. An experiment with constant temperatures (9, 15, 18 and 21°C, and ambient temperature) and natural photoperiod in controlled environment in the Ås-phytotrone with the cultivars 'Glen Ample', 'Tulameen', 'Veten', 'Vene', 'Balder', 'Anitra', 'Schöneman', 'Vajolet' and 'Lagorai Plus'; 2. A field experiment with the cultivars 'Glen Ample', 'Anitra', 'Veten', 'Cascade Delight', 'Ninni', 'Malling Juno' and the selection RU044003090 (RU90). Growth was monitored in both experiments by weekly measurements of shoot length and leaf number, and flower initiation by weekly sampling and examination of lateral bud number 5-7 from the apex of each plant. The cultivars 'Glen Ample', 'Veten', 'Vene', 'Balder', 'Anitra' and 'Ninni', which are selected for a Nordic climate, ceased growth and initiated floral primordia earlier than cultivars adapted to more southern climate. These cultivars responded earlier because they were more responsive to photoperiod at temperatures >15°C and insensitive to photoperiod at temperatures <12°C and below. By responding earlier, the plants have more time to differentiate flowers and induce strong winter hardiness. 'Malling Juno' and 'Cascade Delight' had a similar response as the Nordic cultivars, and can be suitable cultivars for Nordic production. In both experiments two cultivars tip-flowered; 'Anitra' in the field experiment and at constant 18°C in controlled environment, and 'Vene' at constant 18°C and 21°C in controlled environment. If the Nordic and European climate becomes warmer in the future as predicted, more tip-flowering could be expected.

iii. Sammendrag

Skuddvekst og blomsterknoppdanning ble studert i forskjellige bringebær (*Rubus idaeus* L.) sorter for bedre å forstå faktorene som påvirker bringebær-plantens tilpasning til et nordisk og europeisk klima i endring. To forsøk ble gjennomført: 1. Forsøk med konstant temperatur (9, 15, 18 og 21°C, og naturlig ute-temperature) og naturlig daglengde i kontrollert klima i Ås-fytotronen med sortene' Glen Ample', 'Tulameen', 'Veten', 'Vene', 'Balder', 'Anitra', 'Schöneman', 'Vajolet' og 'Lagorai Plus'; 2. Feltforsøk med sortene 'Glen Ample', 'Anitra', 'Veten', 'Cascade Delight', 'Ninni', 'Malling Juno' og seleksjonen RU044003090 (RU90). Skuddvekst ble undersøkt ved ukentlige målinger av skuddlengde og antall blad. Tidspunkt for blomsterknoppdanning ble undersøkt ved ukentlige innsamlinger og disseksjon av knopp nummer 5-7 under skuddspissen i begge forsøkene. Sortene 'Glen Ample', 'Veten', 'Vene', 'Balder', 'Anitra' og 'Ninni' er sorter spesielt selektert for et nordisk klima, og skudd av disse sortene viste en tidligere vekstavslutning og blomsterknoppdanning enn sortene selektert for et varmere klima. De nordiske sortene reagerte raskere fordi de er mer sensitive for daglengde når temperaturen er >15°C, og dagnøytrale ved temperaturer <12°C. Siden disse sortene reagerer på klimasignalene tidligere i sesongen, har de lengre tid til å differensiere blomster og indusere en sterkere vinterherdighet. Sortene 'Malling Juno' og 'Cascade Delight' kan være egnet for et nordisk klima ettersom de reagerte veldig likt som de nordiske sortene. I løpet av forsøksperioden blomstret to av sortene i toppen av årsskuddet; ' Anitra' blomstret både i felt og i kontrollert klima ved konstant 18°C, og 'Vene' blomstret i fytotronen ved 18°C og 21°C. Med en forventet klimaendring, med et varmere klima i Norden og Europa, kan blomstring i toppen på årsskudd i enkelte bringebærsorter bli mer vanlig enn i dag.

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1. Introduction

The origin of the red raspberry (*Rubus idaeus* L.) locates near the Ida Mountains of Turkey, hence the name *idaeus*. *Rubus idaeus* L. belongs to the Rosacea family and the genus *Rubus*, and is a diploid plant (2n=2x=14 chromosomes) (Funt & Hall, 2013). Other species of *Rubus* are also used for commercial production around the world. The most important types are the North American red raspberry, *R. strigosus*, the black raspberry *R. occidentalis* and the European red raspberry, *R. idaeus*, which is the main domesticated type (Hendrick, 1925; Jennings, 1988; Daubeny, 1996 as cited in Funt & Hall, 2013).

Raspberry grows in the entire northern hemisphere, from the Middle East to the Arctic Circle. It can even be found as far south as Australia (Funt & Hall, 2013). Important factors influencing growth and development in raspberry is temperature and photoperiod, but in what degree depends on the cultivar. Through extensive breeding in cultivated raspberry through many years, raspberry can be grown further south and further north than its wild relative (Funt & Hall, 2013). To breed for extreme climate means to improve the adaptability of the plant. For Nordic conditions, this means well-adapted winter hardiness and that temperature and photoperiod gives strong signals for vegetative and generative growth (Säkö & Hiirsalmi, 1980; Funt & Hall, 2013). The most important adaptability a raspberry plant growing in Nordic conditions can have is day-neutrality at temperatures lower than 15°C (Williams, 1959b; Sønsteby & Heide, 2008). Adapting plants for more southern climate includes photoperiod being a weaker signal as the photoperiod varies less throughout the year closer to the equator. Cultivars bred for southern climate must also have the ability to handle drought and high temperatures (Funt & Hall, 2013). A problem in warmer climate is lack of low temperatures in winter for breaking dormancy. Even as far north as England, lack of sufficient winter chill is experienced (Jennings and McGregor, 1986 as cited in White, Wainwright and Ireland, 1998).

Two different life cycles is recognized in raspberry. Raspberry is a woody plant with a perennial root system and short-lived shoots, which can either, be annual-fruiting or biennial-fruiting. Annual-fruiting types complete their life cycle in one season, while biennial completes its life cycle in two seasons. During their life cycle the shoots go through several seasonal phases including vegetative growth, flower initiation and differentiation, dormancy, bud burst, flowering, fruiting and senescence (Hudson, 1959; Sønsteby & Heide, 2008). The main reason for the difference between these two groups is that a biennial-fruiting cultivar has a chilling requirement to fulfil (breaking of dormancy) in order to complete its life cycle,

while annual-fruiting cultivars can complete their life cycle without chilling (Williams, 1959b, c, 1960; Sønsteby & Heide, 2008)

Other important adaptations are cold acclimation, dormancy and earliness. In a Nordic climate were temperatures easily can sink to -20°C during winter, it is important that the cultivar can withstand the freezing temperature without damaging the buds. In spring, it is very common with freezing temperatures during night in addition to thawing temperatures during the day. These conditions can have a very damaging effect on the raspberry shoot and buds. For a cultivar to cope with such conditions, it needs to respond easily to climatic signals in order to avoid damage caused by freezing temperatures. At the same time, it is important that the dormancy is not too deep, as lack of chilling then becomes a problem. The cultivars must also avoid fruiting too late. Equal to spring frost, low temperature in autumn at many locations may cause severe frost damage if the plant has not ended growth and had sufficient time for cold acclimation.

To understand better the adaptation of raspberry to different environment and a changing climate, two projects was initiated in Norway, and the current study is a part of both projects. This thesis is a collaboration between the Norwegian University of Life Sciences (NMBU) and the Norwegian Institute of Bioeconomy Research (NIBIO). NIBIO is currently working on a Norwegian project named "KLIMAFRUKT" which focuses on increasing knowledge of critical factors influencing adaptation of fruit and berries to a Nordic climate, with the main focus on raspberry. NIBIO is also involved in the European project (Horizon 2020) "GoodBerry", were the main goal is to increase production and quality of European berry production, and to understand which qualities cultivars of strawberry, blackcurrant and raspberry must have for a changing European climate.

The goals in the projects mentioned above is the motivation for the current study, which aims at better understand what factors influence the adaptation of raspberry to a changing Nordic and European climate. Cultivars for this experiment were chosen based on their well-known good adaptability to different environmental conditions. This study consists of two experiments: 1. An experiment with constant temperatures conducted in controlled environment in the Ås phytotrone with the cultivars 'Glen Ample', 'Tulameen', 'Veten', 'Vene', 'Balder', 'Anitra', 'Schöneman', 'Vajolet' and 'Lagorai Plus'. 2. A field experiment with the cultivars 'Glen Ample', 'Anitra', 'Veten', 'Cascade Delight', 'Ninni', 'Malling

Juno' and the selection RU044003090 (RU90). The objective of this study was to determine how temperature in controlled climate and field affects plant development and flower initiation in different cultivars during growth cessation. A second object was to study flower morphology in the different cultivars using Scanning electron microscopy (SEM)

2. Literature review

2.1. Morphology

Red raspberries (*Rubus idaeus* L.) are a woody plant with a long-lived perennial root system and short-lived shoots. The shoots can grow to a height of 1 to 5 m depending on cultivar, climatic conditions and cultivar group (Fig. 1) (Hudson, 1959; Sønsteby & Heide, 2008; Funt & Hall, 2013

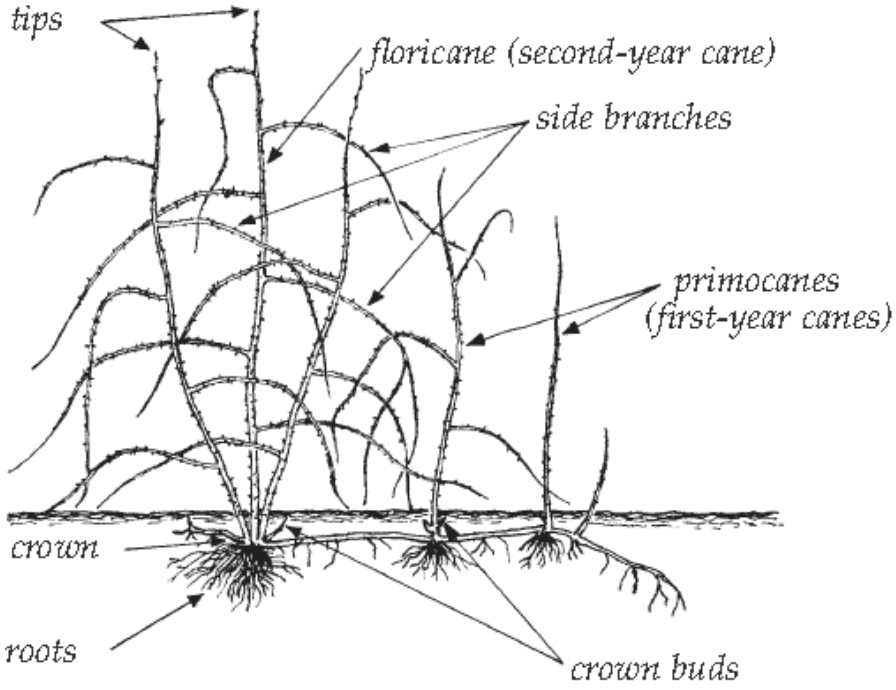

Figure 1. Raspberry plant. Showing the root system along with the annual- and biennial-canes. (Source: Funt & Hall, 2013).

In their first year, buds will form on the cane at every node. These buds will initiate flower primordia and develop inflorescence at the right climatic conditions and either flower and set fruit the first year, or they will enter a dormancy and flower and set fruit the following year. Floral primordia are a fully differentiated flower bud ready to flower at the right signals. The inflorescence is a cyme, meaning that the terminal flower develops first, followed by the secondary and tertiary further down the inflorescence axis. The complexity of the inflorescence varies down the shoot, and normally the number if inflorescence increases from the top to the base of the shoot (Sønsteby & Heide, 2008, 2009). If the buds flower and set fruit in their first or second year depends on the climatic conditions, cultivar group and cultivar (Hudson, 1959; Sønsteby & Heide, 2008; Funt & Hall, 2013). In addition to the primary bud, many cultivars also have one or two secondary or sub-axillary buds located slightly below or outside the primary axillary bud. These buds work as a replacement if the primary bud is damaged (Williams, 1959c).

The raspberry flower has five sepals and petals that together with stamens (pollen), the male production structure, encircle the receptacle known as the female production structure (Fig. 2). After pollination individual fruits known as drupelets develop around the receptacle and form an aggregate fruit that we know as a raspberry (Funt & Hall, 2013).

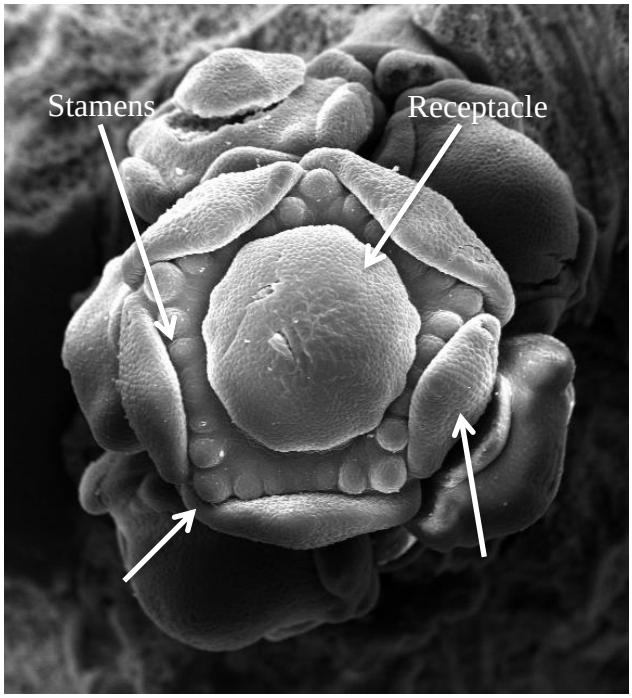

Figure 2. Scanning electron microscopy picture showing a fully differentiated raspberry flower of cultivar 'Glen Ample'. Photo Randi Hodnefjell

2.2. Cultivar groups

There are two main groups of raspberry cultivars, namely annual- and biennial-fruiting types. Annual-fruiting types are also referred to as fall bearing or primocanes, while biennial-fruiting is referred to as summer-cropping or floricanes (James Carew, Hadley, Battey, & Darby, 1998). In this thesis, the two groups of cultivar will be referred to as annual- and biennial-fruiting as suggested by Sønsteby & Heide (2008). In addition, an intermediate type that can tip-flower exists. The cultivars that can tip-flower are both annual and biennial, with a plastic trait that makes them susceptible to flowering in their first year if the temperature is high over a longer period. The rest of the shoot enters dormancy and complete its life cycle the following season (Williams, 1960; Carew et al., 2000; Carew, Mahmood, Darby, Hadley, & Battey, 2003; Sønsteby & Heide, 2009). All groups of cultivars have the same life cycle (Fig. 3), but the shoots of annual-fruiting types complete their life

cycle within one year while the shoot of biennial fruiting types requires two years (Hudson, 1959; Sønsteby & Heide, 2008).

2.3. Life cycle of a biennial raspberry plant

According to Hudson (1959), the raspberry shoot goes through several seasonal phases during its life cycle. All depends on physical, genetically and environmental factors. How the morphology of the shoot develop depends on a) if the apical meristem is producing flower primordia or leaves, b) if flower primordia expands and the following rate of expansion, or if flower primordia expands at all, and c) the internodes remain short or elongates. Hudson et al. (1959) describes the life cycle of biennial red raspberry in nine phases based on combinations of the above behaviours:

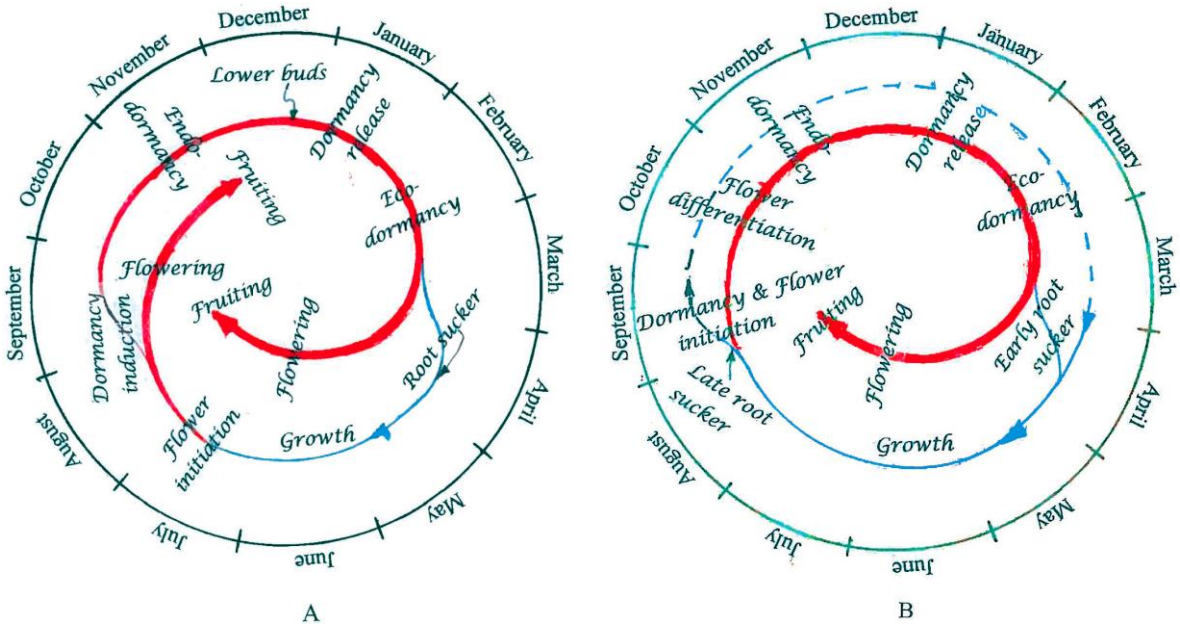

Figure 3. Illustrations of the annual-fruiting (A) and the biennial -fruiting (B) life cycle. The blue line shows the vegetative phases and the red line shows the generative phases. The stapled blue line refers to phase 4 (Source: Heide & Sønsteby, 2011)

Phase 1: Initiation of the root buds.
Buds under normal conditions arise laterally from raspberry roots.

Phase 2: Subterranean sucker.
Root buds commence to grow towards the surface due to elongation of internodes.

Phase 3: Emergent sucker.
Tip of the shoot emerge at soil level, the elongation of internodes ceases and expansion of leaves starts until a rosette of leaves is created, called the "primary rosette".

Phase 4: First winter dormancy.
Shoots emerging in late summer or autumn shed their leaves and the terminal apex become dormant. This is an anomalous phase that occurs rarely, especially in natural conditions.

Phase 5: Elongating shoot.
Dormancy is broken by longer days and higher temperature before vegetative growth resumes and continues from early spring to autumn. Vegetative growth includes internode elongation and leaf expansion. At the same time as the shoots starts to elongate, the root also produces adventitious roots that will become new shoots.

Phase 6: Initiation of flower buds.
The shoot becomes generative when grown to about 15 to 20 nodes. The photoperiod has become shorter and the temperature has dropped to about a middle of 15°C. Due to these environmental signals the shoot stops elongating and the internodes at the top of the shoot will be constrained and a small secondary rosette will be formed. At the same time as the elongation ceases the axillary meristems starts to initiate flower primordia. Axillary and terminal buds develop into complete flower buds before they enter dormancy. Leaves become senescent and the shoot enters its second dormancy.

Anomalous phase 6: Tip-flowering.
Some cultivars such as 'Lloyd George' have the ability to tip-flower under the right conditions. What happens is that the apical meristem initiates flower primordia early in the fall when the shoot is still elongating, and instead of entering dormancy the buds flower and

set fruit before the shoot enters dormancy. If the shoot tip-flower or not depends on temperature, a warm autumn makes the cultivar more likely to tip-flower. It is only the tip of the shoot that flowers in these cases, while the rest of the shoot enters dormancy and flowers next season.

Phase 7: Breaking flower bud dormancy.
Most raspberry cultivars have a certain cold requirement for breaking dormancy. How long the requirement is varies between cultivars, but most cultivars that enter dormancy in early October have their requirements fulfilled by the end of December.

Phase 8: Flowering and fruiting.
When the fruit buds have met their cold requirement, they resume growth when the temperature is high enough and the days are long enough. The fruit buds will then flower and set fruits. At the same time, basal buds starts to elongate to become the replacement shoot that will repeat the biennial life cycle.

Phase 9: Senescence and death.
After the shoot has fruited, the shoot dies back and the replacement shoot grows and repeats the life cycle.

For annual cultivars the life cycle are very similar (Fig. 3), but instead of entering dormancy after flower primordia the axillary and terminal bud flower and set fruits, all in the same year.

2.4. Growth

Shoot growth in red raspberries show a typical sigmoidal pattern, with slow growth in the start of the season and increasing growth through the summer until it slows down in the end of the season (Fig. 4) (Carew et al., 2000). How much the canes grow depend on several factors, but the combination of temperature and photoperiod are the most important. Vegetative growth of red raspberries also depends on cultivar, sufficient water supply, soil temperature and solar radiation (Privé, Sullivan, Proctor, & Allen, 1993). It was discovered in a trail with ‘Malling Promise’ grown at different temperatures and photoperiods that at a temperature of 21°C, the plants grew continuously in photoperiods of both 9 hours and 14

hours. At 15.5°C, growth ceased in 9-hour photoperiods, but continued growing in 14-hour photoperiods. At 10°C, growth ceased in both photoperiods (Williams, 1959b). Sønsteby and Heide (2008) showed the same results for the cultivar 'Glen Ample' in both controlled (Fig. 4) and natural photoperiod. They also included shoot growth at 12°C and natural photoperiod, and found that the plants responded equal to plants at 10°C.

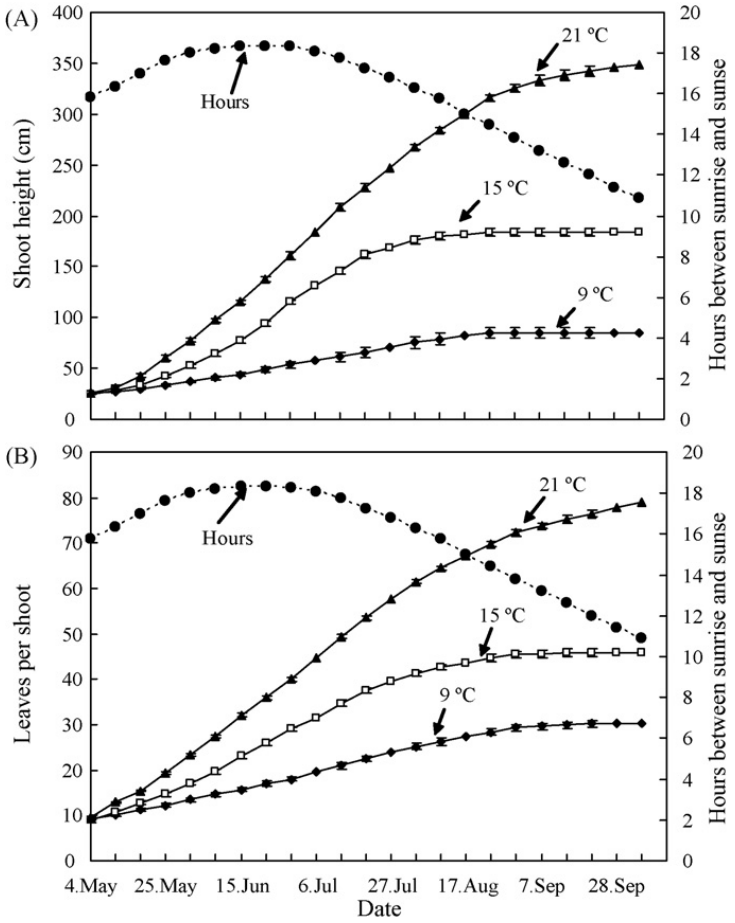

Figure 4. Increment in shoot elongation (A) and formation of leaves (B) in the cultivar 'Glen Ample'. Plants held at 9, 15 and 21°C and natural photoperiod. (Source: Sønsteby and Heide, 2008).

2.5. Flower initiation

If the right temperature and photoperiod signals are present, the plant can initiate flower primordia. The bud will then start broadening its apical meristem and elongate the growing point (Williams, 1959c). At the same time, inflorescence consisting of several

flowers will start to develop. The time of year when this occurs, differ between annual-fruiting and biennial-fruiting (Williams, 1959c; Sønsteby & Heide, 2008).

2.5.1. Annual-fruiting cultivars

In research done on annual-fruiting cultivars flower initiation have been studied by measuring flower emergence, because it is assumed that both processes are controlled by the same signals (Carew et al., 2000). The signals for flower initiation in annual-fruiting cultivars include high solar radiation, long photoperiod and high temperature, which is the main signal (Privé et al., 1993). An experiment done under semi-controlled conditions by Carew et al. (1998) with the cultivar 'Autumn Bliss' showed an optimum temperature for flower initiation at approximately 22°C, temperature below and above this resulted in an decreased rate of development. Flower initiation starts developing to flower primordia in the terminal (apical) bud and spreads basipetally (Sønsteby & Heide, 2009), at the same time shoot growth ceases.

The major difference between annual- and biennial-fruiting cultivars is that biennial-fruiting cultivars have a cold requirement to fulfil between flower initiation and flowering, while annual-fruiting cultivars flower directly after flower initiation. However, performance of annual-fruiting cultivars is promoted by a chilling period (vernalization). As proved by Carew et al. (2001) and later confirmed by Sønsteby and Heide (2009). Plants with five nodes of the cultivar 'Polka' were exposed to 6°C for 7 weeks, then transferred to 24°C or grown continuously at 24°C. Both treatments included either long days (24 hours) or short days (10 hours). The experiment showed that plants exposed to chilling had a decreased shoot height and number of leaves at flowering compared with plants grown at continuously 24°C. In addition, number of days until anthesis, were reduced (Sønsteby & Heide, 2009). When the plants were exposed to 6°C, the vegetative growth was minimal, but when transferred to 24°C, shoot growth resumed to the same rate as plants grown continuously at 24°C indicating a non-dormant state. Considering that the plants of only five nodes managed to respond to the chilling, Sønsteby and Heide (2009) concluded that annual-fruiting cultivars do not have a juvenile phase like biennial-fruiting cultivars have.

2.5.2. Biennial-fruiting cultivars

Biennial-fruiting cultivars have a juvenile phase and need a certain amount of nodes to be able to initiate flower primordia, in 'Glen Ample' and 'Malling 'Promise' this number as found to be 15-20 nodes (Williams, 1960; Sønsteby & Heide, 2008). The signals for

flower initiation are shorter photoperiod and lower temperature (Williams, 1959c; Sønsteby & Heide, 2008), and occur at the same time as shoot growth ceases (Sønsteby & Heide, 2008). Flower initiation will start in the region five to ten nodes below the apex and develops further down and up the shoot (Williams, 1959c; Sønsteby & Heide, 2008; Woznicki, Heide, Remberg, & Sonsteby, 2016). Sønsteby & Heide (2008) showed that floral initiation in the cultivar 'Gel Ample' took place after 3 or more weeks after being exposed to 9°C and 10 hour photoperiod for 2, 3, 4, 5, and 6 weeks. In the same experiment, Sønsteby and Heide (2008) found that a photoperiod of 15 hours and a temperature of 15°C were critical for growth cessation and flower initiation. However, if the temperature is lower than 15°C the short photoperiod will not have any effect on either growth cessation or flower initiation. Because of this effect, flower initiation can occur even if the photoperiod is longer than 15 hours (Sønsteby & Heide, 2008). After flower initiation, the buds will further differentiate into fully developed flowers. In Southeast England, Williams (1959b) showed that axillary buds contained flower primordia by December, except the ten to twelve buds closest to soil level. In January when dormancy was broken and the photoperiod increasing, flower primordia was found in buds close to the soil level as well. Buds closest soil level and immediately under soil level were still dormant (Williams, 1959c).

2.6. Dormancy

The onset of dormancy is a gradual process and is induced by the same climatic conditions as flower initiation (Williams, 1959b). Dormancy is according to Lang et al. (1987) (as cited in (White, Wainwright, & Ireland, 1998) "a temporary suspension of visible growth". There are three types of dormancy: 1. Endodormancy, is controlled from within the bud and requires a chilling period to be removed. Endodormancy is also known as true dormancy or winter dormancy. 2. Paradormancy, dormancy due to apical dominance or adjacent leaves. 3. Ecodormancy, dormancy because of unfavourable conditions. Unfavourable conditions include too cold and too hot temperature, insufficient water supply and other climatic conditions that inhibit growth (White et al., 1998; Carew et al., 2000) True dormancy is most important in this thesis and will have more focus then the other types of dormancy.

The initiation of endodormancy requires decreasing temperature and shorter photoperiod over time, and if this process is erupted in its early stages by high temperature, the plant can resume elongation. But if the induction of dormancy prevails the plant will go

into a deep state of dormancy (Williams, 1960). Internal physiological factors within the bud will keep the plant in a state of true dormancy until a certain amount of chilling is met; only then can the plant resume growth (Williams, 1959b).

The chilling period requires low temperature over a certain amount of time, how long depends on the cultivars grown and the temperature during the dormancy. For 'Malling Promise' a temperature of 3.3°C for six weeks was enough to resume growth (Williams, 1959b). In later research, temperatures between 0°C and 7°C (Lamb, 1948) for a period of 800 to 1 500 hours were found to be sufficient to break bud dormancy (Lamb, 1948; White et al., 1998). However, breaking of dormancy in isolated buds and canes has different requirements. White et al. (1998) showed that 80% bud-burst in isolated nodes, independent on position on the cane, required a temperature of 4°C for approximately 1 500 hours. For intact canes the same treatment gave only 60% bud-burst on the uppermost part of the cane, with a decrease in bud-burst in lower positions. For a 100% bud-burst on the uppermost part of the cane, a period of 2 500 hours at a temperature of 4°C were sufficient (Mazzitelli et al., 2007). The difference in breaking of dormancy along the cane can be explained by paradormancy, buds inhibit other buds to burst because of apical dominance or adjacent leaves (White et al., 1998).

When true dormancy is on its deepest, depends on the climatic conditions were the plants grow. In USA, the cultivar 'Latham' reached its deepest state of dormancy in mid-October, while in the UK, cultivars 'Glen Moy' and 'Glen Cova' reached its deepest state of dormancy in the end of October (Carew et al., 2001). In a 6 year study with 12 cultivars grown in Norway and Sweden, the cultivars reach their deepest state of dormancy in October (Måge, 1975 as cited in Heide & Sønsteby, 2011). At the same time as dormancy is induced, the plants also undergo cold acclimation in order to survive freezing temperatures in the winter. The earlier cultivars ripen and enter dormancy, the better it will survive winter (Säkö & Hiirsalmi, 1980). Both processes are controlled by decreasing temperature and shorter photoperiods (Hudson, 1959; Williams, 1959b; Säkö & Hiirsalmi, 1980). After the chilling requirement is met, the plants slowly come out of dormancy. Most cultivars are ready to resume growth in January, but because of unfavourable conditions, the plant will stay in a state of dormancy, ecodormancy. The end of dormancy is, however, not equal to the duration of winter hardiness. Winter hardiness can last up to two months longer than dormancy, and will not decrease until conditions are more favourable. Strong winter hardiness is, nonetheless, depending on a deep and prolonged dormancy (Säkö & Hiirsalmi, 1980).

2.7. Flowering and fruiting

Once temperature and photoperiod are favourable, the flowers will emerge. The flowers will then pollinate and start the ripening process. Wild raspberries are often self-infertile and need to be pollinated, but cultivated raspberries pollinate within the flower (self-fruitful) (Funt & Hall, 2013). Just as climatic conditions in the vegetative phase affects the length and depth of the dormancy phase, climatic conditions in earlier phases and present phases also affects flowering and fruiting.

2.7.1. Annual-fruiting cultivars

Time of flowering in annual-fruiting cultivars is usually correlated to number of nodes (Funt & Hall, 2013). How many nodes needed for flowering is determined by the amount of chilling the shoots to. In the annual-fruiting 'Heritage', plants receiving more than 750 chilling units flowered when the shoot had 25 to 30 nodes. While plants receiving 500 chilling units and less flowered when the shoot had 67 to 81 nodes or more (Takeda, 1993). The period from flowering to harvest highly depend on climatic factors, in the cultivars 'Autumn Bliss', Carew et al. (1999a) (as cited in Carew et al., 2000) showed that an optimal temperature of 22°C during flower initiation accelerated the harvest date by two months compared with plants grown at 15°C. These results were confirmed by Sønsteby and Heide (2009) with the cultivar 'Polka' under controlled conditions, were days to anthesis was decreased with an increasing temperature up to 21°C. They also demonstrated that long days promote flowering, both on number of flowers, flowers per lateral, flowering laterals and on days until anthesis (Sønsteby & Heide, 2009). Carew et al. (1999a) (as cited in Care et al., 2000) also showed that higher light intensity results in a shorter time between planting and fruiting.

2.7.2. Biennial-fruiting cultivars

When the temperatures starts to get warmer and the days get longer, the fully differentiated raspberry buds will burst and flowers of biennial-fruiting cultivars will bloom. All buds on the cane that has initiated flower primordia will then emerge at the same time. Time from flowering to first harvest varies between locations and cultivars. In Pennsylvania, US it takes between 21 and 43 days from the first flower to bloom until first harvest (Funt & Hall, 2013). In Norway, it takes about 70 to 80 days (A. Sønsteby, personal communication, May 10, 2017). Production of fruits is usually higher in the top of the plant and decreasing

towards the base of the plant, but in common raspberry production, the top is removed in order to make the harvest easier. The consequence of this practise is that most fruits are found in the mid-part of the shoot. The size of the laterals, however, increases towards the lower half of the shoot (Dale, 1979 as cited in Carew et al., 2000). Fruiting in biennial-fruiting cultivars is highly dependent on climatic conditions in earlier phases equal to that of annual-fruiting cultivars. Sønsteby and Heide (2009) concluded that an optimal summer temperature followed by exposure to low temperature for a longer period, resulted in a fruiting plants optimal architecture. The optimal summer temperature will give optimal vegetative shoot growth and the low temperature will initiate flowers and dormancy in an ideal time before temperatures become freezing.

3. Materials and methods

3.1. Plant material and cultivation

3.1.1. Plant material

The different cultivars (Table 1) were chosen based on their known adaptation to different environmental conditions. ‘Glen Ample’ was chosen because it is the main cultivar in Norway and show high adaptability to the Nordic climate. It is also much grown in other European countries. ‘Vene’ was chosen because of its earliness, while ‘Veten’ has been the main cultivar for industry production in Norway for many years. It is also used in several European countries with good results and good adaptability. ‘Balder’ show very good winter hardiness and is bred for Nordic condition. ‘Anitra’ and ‘Ninni’ are both new, promising cultivars bred and released by the Norwegian breeding company Graminor. ‘Ninni’ was chosen because of its good taste, firm fruits and long shelf life. RU90 is a selection and not introduced to the market yet, but was included in the study because of its firm fruits and potential for fresh consumption production. ‘Tulameen’ and ‘Schöneman’ are established cultivars much used in Europe, initially because they both show good adaptability to the local climate. ‘Vajolet’ and ‘Lagorai Plus’ are new cultivars from Italy, and were chosen because they originate from a country further south and because they show good adaptability to local climate. The Norwegian raspberry industry is looking for early cultivars to stretch the season, and one of the possible cultivars is ‘Malling Juno’, which is an early cultivar in England. ‘Cascade Delight’ was chosen because of its long shelf life and firm fruits.

Table 1. An overview of the cultivars used in this thesis, including name and year they were released on the marked, parents, country of origin, some general and special cultivar traits and source of information.

Cultivar	Parents	Country of origin	Cultivar trait	Source
‘Glen Ample’ 1994	‘Glen Prosen’ x ‘Meeker’ among others	Scotland	Mid-summer Good disease resistance High yielding and good taste Light –red colour **Main cultivar in Norway**	(RoyalHorticulturalSociety, 2017) (Sagaplant, 2017)
‘Tulameen’ 1989	‘Nootka’ x ‘Glen Prosen’	Canada	Late summer Red –red-violet colour High yielding	(Sagaplant, 2017)

'Veten' 1961	'Preussen' x 'Lloyd George'	Norway	Mid-summer Dark violet colour **Suitable for conserving**	(Sagaplant, 2017)
'Vene' 1987	'Veten' x 'Newburgh'	Norway	**Early summer** **Suitable for Northern-Norway** Dark red colour Small berries Good taste Small yield	(Sagaplant, 2017)
'Balder' 1988	'Norna' x 'Malling Jewel'	Norway	Mid/late-summer Moderate yielding Sour taste Winter hardy Dark red colour **Bred for Nordic conditions**	(Sagaplant, 2017)
'Anitra' 2015	(N-91-63-1) x (N-92-68-3)	Norway	Mid-season Medium red colour High yielding Large fruits	(D. Røen, personal communication, March 3, 2017)
'Schöneman' 1950	'Loyd George' x 'Preussen'	Germany	Late Moderate to high yielding Sweet and strong aromatic Dark-red colour Less susceptible for cane diseases, medium for Botrytis	(Bundessortenamt.de, 2006)
'Vajolet' 2012	A result of free pollination of 'Polka'	Italy	Reddish orange coloured Annual: early-medium Biennial: late Average yielding **Annual-fruiting**	(CommunityPlantVarietyOffice, 2012) (Telch, 2015b)
'Lagorai pluss' 2016	A result of open pollination of 'Tulameen'	Italy	Late Medium red colour Large fruits **Annual-fruiting**	(CommunityPlantVarietyOffice, 2012, 2016) (Telch, 2015a)
'Cascade Delight' 2003	'Chilliwack' x WSU 994	USA	Late summer Fresh flavour High yielding firm fruits	(Moore, 2004)
'Ninni' 2015	'Varnes' x RU00403067	Norway	Late season Red-redviolet colour Very firm Sweet taste **Long shelf life**	(D. Røen, personal communication, March 3, 2017)

‘Malling Juno’ 1998	Cross between two early EM selections (EM = East Malling	UK	Early season Mid red colour Good flavour Good shelf life	(Knight & Fernandez Fernandez, 2005) (MEIOSIS, 2016)
RU044003090 (RU90) Not introduced to the marked	‘Varnes’ x RU00403067	Norway	Late season Red-redviolet colour Firm fruits	(D. Røen, personal communication, March 3, 2017)

3.1.2. Controlled environment experiment

Long cane plants in pots of the cultivars ‘Glen Ample’, ‘Tulameen’, ‘Veten’, ‘Vene’, ‘Balder’, ‘Anitra’, ‘Schöneman’, ‘Vajolet’ and ‘Lagorai Plus’ where propagated at NIBIO Apelsvoll Experimental Centre in South-East Norway (60°40´N; 10°52´E) in 2016. The plants were propagated from root systems stored at 2°C during winter until early May, where pieces of maximum 15cm were cut from the root systems. The root cuttings were left to sprout in peat substrate in a greenhouse with natural light conditions and 18°C. Emerging shoots where from early-June cut at the base and planted in a peat-based soil mixture either in 4cm x 4cm Jiffy pots or in plastic trays. Throughout the propagation period, the plants had a minimum temperature of 20 °C and a natural long day (16-19hours). The plants were covered with a white plastic sheet to provide a water-saturated atmosphere until the plants started rooting. When the plants reached a height of about 20cm they were transplanted into 2.5l pots with a mixture of 75% coarse textured sphagnum peat and 25% chipped spruce bark with a pH of 5.8. The plants were in early-June moved out-doors and further cultivated under ambient temperature and day-length until mid-August. The plants were spaced with five plants per running meter within a row and two meters between the rows. Only one cane per plant was allowed to grow, additional emerging shoots where removed by weekly pruning throughout the experiment. The plants were automatically fertigated throughout the summer with a fertilizer solution consisting of a 2:3 mixture of Superba Red^{TM} (7-4-22% NPK – micronutrients) and Calcinite^{TM} (15.5% N and 19% Ca), both from Yara International (Oslo, Norway) and having an electric conductivity (EC) of 1.5 m S cm^{-1}.

On august 18, a majority of the plants where moved to the phytotrone at the Norwegian University of Life Science in Ås (59°40N; 10°45´E). Five plants (with one cane per plot) of each cultivar were contained in natural daylight compartments with natural decreasing photoperiod, and exposed to temperatures of 9 °C, 15 °C and 21°C for 8 weeks,

and 18 °C for 10 weeks. Temperatures were controlled within ± 1.0 °C, and water vapour saturation deficit of 530 Pa was maintained at all temperatures. Whenever the photosynthetic photon flux density (PPFD) fell below approx. 150 μmol quanta $m^{-2}s^{-1}$ as on cloudy days, an additional 125 μmol quanta $m^{-2}s^{-1}$ were added automatically using Philips HPT-I 400 W lamps. The plants were fertigated one to two times per day with a complete fertilizer solution consisting of a 2:3 mixture of Superbra Red™ (7-4-22% NPK + micronutrient) and Calcinite™ (15.5% N and 19% Ca), both from Yara International (Oslo, Norway), and having an electric conductivity (EC) of 1.5 mS cm^{-1}. Biological pest control where installed when the plants were moved to the phytotrone. In the same period, an additional group of plants were kept at NIBIO Apelsvoll under ambient temperature and day-length conditions for weekly registration of shoot and leaf growth. All cultivars are presented in Fig. 5, before they were grown at different temperatures. Temperature and photoperiod during the experimental period shows in Fig. 6 and 7.

Figure 5. Plants of all cultivars before at the start of the phytotron, on August 18. From the left 'Lagorai Plus', 'Vajolet', 'Schöneman', 'Anitra', 'Balder', 'Vene', 'Veten', 'Tulameen' and 'Glen Ample' Photo: Randi Hodnefjell

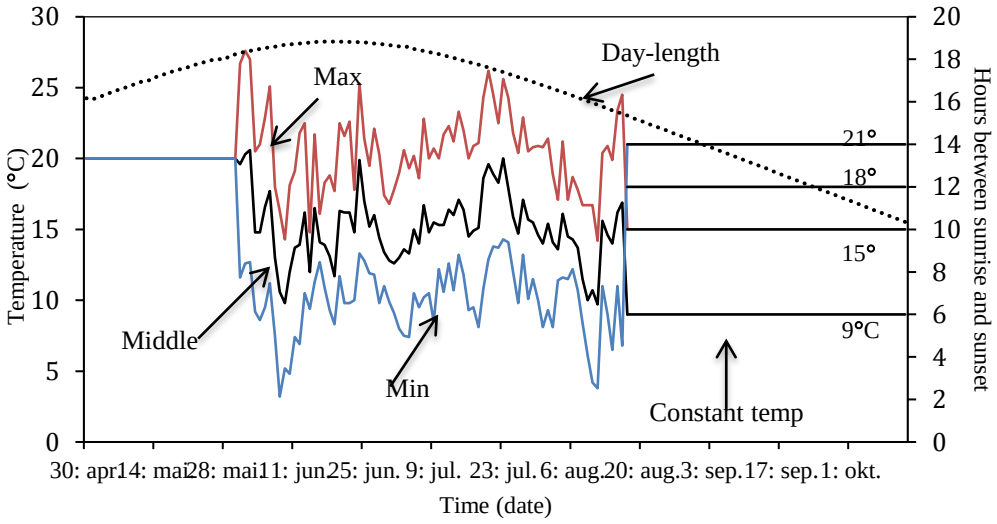

Figure 6. Temperature and photoperiod conditions during plant raising and experimentation in constant temperature in controlled environment experiment. Red shows maximum temperature, black show middle temperature and blue show minimum temperature. The dotted line shows the photoperiod. Data collected from (timeanddate.no, 2017) and (Kroken, 2017)

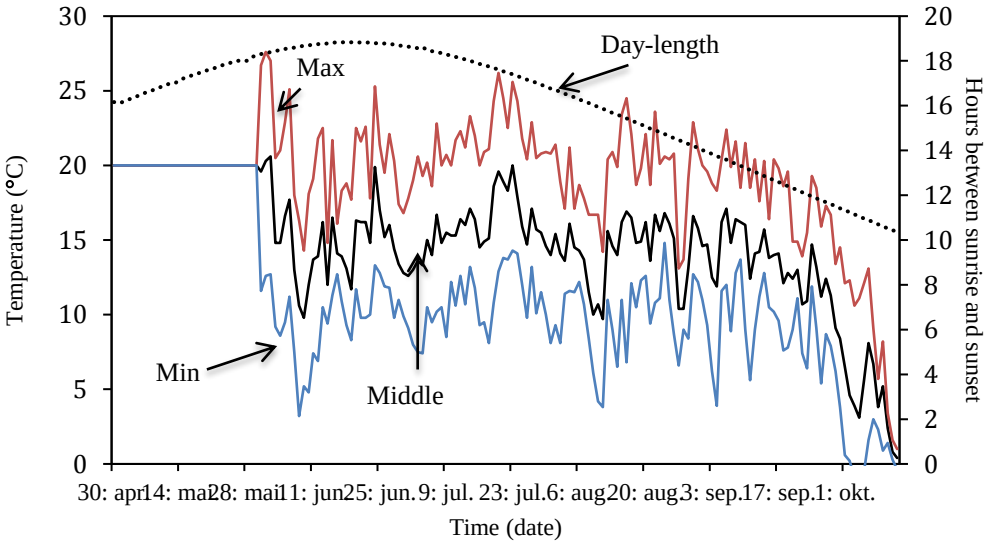

Figure 7. Temperature and photoperiod conditions during plant raising and experimentation in ambient temperature in controlled environment experiment. Red shows maximum temperature, black show middle temperature and blue show minimum temperature. The dotted line shows the photoperiod. Data collected from (timeanddate.no, 2017) and (Kroken, 2017)

3.1.3. Field experiment

The experimental field located in Åsbakken at the Norwegian University of Life Science in Ås (59°40N; 10°45´E) was established in summer 2015. It was not established for the purpose of this study, but contained many new, interesting cultivars, and was included to see how they are adapted to natural out-door conditions in our climate. The cultivars chosen for the experiment was 'Glen Ample', 'Veten', 'Ninni', 'Malling Juno' and 'Cascade Delight', 'Anitra' and selection RU90. The cultivars were planted in open soil without mulching in three single rows with spacing of 50cm between plants in the row and 4m between the rows. The experimental field comprised three randomized blocks, each with six plants of each cultivar and three replications. In spring 2016 every square was fertilized with 200g Fullgjødsel™ (12-4-18% NPK - micronutrients) and 100g Nitrabor™ (15.4% N, 18.5% Ca and 0.3% B) both from Yara International (Oslo, Norway). The field was also fertilized during flowering with 100g YaraMila Fullgjødsel™ (12-4-18% NPK - micronutrients) (Yara International, Oslo, Norway). Registration of shoot length and number of nodes started on August 3. Three representative canes were selected for each cultivar per plot, giving a total of nine shoots per cultivar. The canes selected were marked with a ribbon for later recognition.

Temperature (Kroken, 2017) and photoperiod (timeanddate.no, 2017) during the experimental period was collected and presented in Fig. 8. Photoperiod shows on the secondary axis, while temperature shows on the primary axis. Red line presents the maximum temperature, black line presents the middle temperature and blue presents minimum temperature.

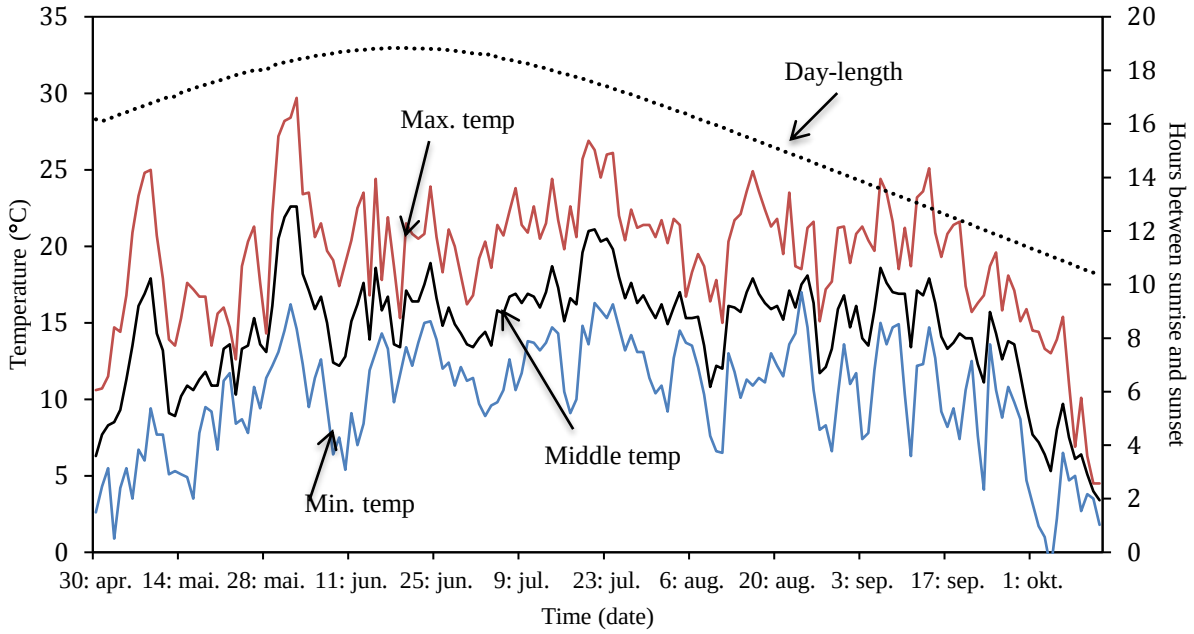

Figure 8. Temperature and photoperiod conditions during the field experiment. Red shows maximum temperature, black show middle temperature and blue show minimum temperature. The dotted line shows the photoperiod. Data collected from (timeanddate.no, 2017) and (Kroken, 2017)

3.2. Growth measurements

3.2.1. Controlled environment experiment

Registration of shoot length and number of nodes started on August 18 and was done weekly for eight (9, 15 and 21 °C) and ten weeks (18 °C). Flower bud collecting started the following week, and buds were collected weekly throughout the experiment. Registration of shoot length and counting of leaves was done every week throughout the experiment period in order to look at growth increment and development of nodes in relation to growth cessation. The registration of shoot length was performed using a folding rule and counting of nodes was done visually. The vertical shoot length was measured from the base of the shoot to the apical meristem. To make the weekly registration easier a marking was done with a soft marking pen on the cane for every meter height. The nodes were counted from the base of the shoot and up to the apical meristem, and every five leaf was marked as they were developed, to make the weekly counting easier. Axillary bud number 5-7 from the apex of each cane was slit off by a shallow longitudinal slit with a scalpel and stored on 70% rectified ethanol until later dissection and examination under a stereo microscope. The sampling technique did not affect the continued growth of the cane, and since new nodes were initiated weekly, a new bud was available every week. When the registration was finished three shoots per cultivar from ambient temperature (Apelsvoll), 9 °C, 15 °C, and three shoots of the

cultivars ‘Glen Ample’, ‘Vene’ and ‘Balder’ from 18 °C were wrapped in plastic and stored at 0 °C for later dissection.

3.2.2. Field experiment

The registrations were done every week for ten weeks, from August 3 to October 12. Flower bud collecting started the following week, and buds were collected throughout the experimental period. All registration in the field experiment was done in the same way as for the registration in the phytotrone. However, in the field experiment, five random shoots were chosen from each cultivar every week to collect axillary buds from.

3.3. Dissection of buds

Flower initiation and differentiation in the collected buds were dissected and examined under a stereo microscope, starting with the latest collected buds and working backwards to the first collected bud. The leaves and scales surrounding the apex were removed to reveal the shoot apex. The morphological development was determined and scored according to the scale developed by Woznicki et al. (2016) (Fig. 9). The scale involves 6 stages were stage 1 is a vegetative bud, stage 2 shows the first visible sign of floral initiation and stage 6 is a fully differentiated flower bud. All buds on the whole shoots collected were dissected from top to bottom and classified after the same scale (Fig. 9).

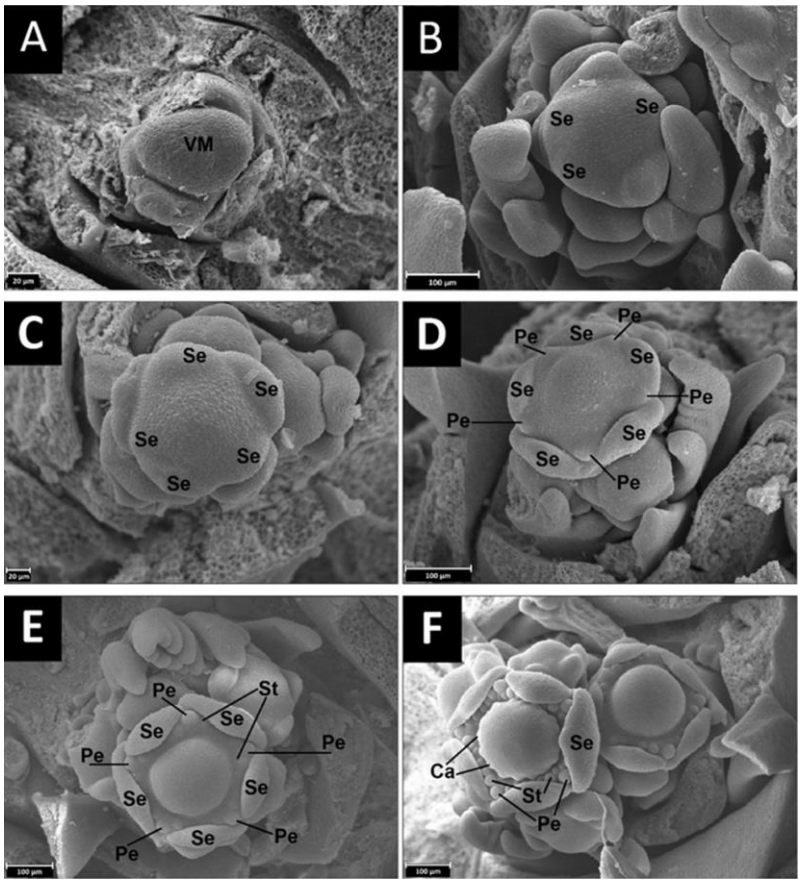

Figure 9. Floral initiation and differentiation in 'Glen Ample', Developed by Tomasz L. Woznicki et.al (2016). A. Stage 1: A vegetative shoot apex meristem. B. Stage 2: First visible sign of floral initiation, broadening of apex with indications of sepal primordial positions. C. Stage 3: Sepal primordial differentiated. D. Stage 4: Sepal primordial fully differentiated and petal primordial visible. E. Stage 5: Perianth ring complete and first stamen primordial visible. F. Stage 6: All flower parts differentiated. VM, vegetative meristem; Se, petals; St, stamens; Ca, carpels.

3.4. Scanning electron microscopy (SEM)

In late January, shoots of 'Glen Ample', 'Veten' and 'Anitra' where collected from Åsbakken and put in water for further bud development along with 'Schöneman', 'Vajolet', 'Lagorai Plus', 'Tulameen' 'Vene' and 'Balder' from cold storage at Apelsvoll. Buds from these shoots were removed and dissected, and buds that had reached a differentiation stage of 6 or more were fixated overnight in glutaraldehyde (1.25%) and para-formaldehyde in 0.05 M PIPES buffer, pH 7.2 and kept at 4 °C in 0.05 M PIPES buffer until dehydration. The buds were dehydrated in a series of ethanol solutions, one time for 10 min each in 70, 90 and 96% ethanol, and finally four times for 10 min each in 100% ethanol. Afterwards, the samples were dried in a critical point dryer (CPD 0.30, Bal-Tec, Balzers, Lichtenstein) using liquid

CO_2, and when dried, attached to a double-faced carbon tabs on stubs (Agar Scientific, Essex, UK). The apex was then sputter coated with 500 Å Pt in a SC7640 sputter coater (Quorum Technologies Ltd, Newhaven, U.K.). When coated, the apex was examined in a Zeiss EVO-50 scanning electron microscope, operated at 20-25 kV (Zeiss, Jena, Germany), and pictures were taken of all buds that were fully differentiated.

3.5. Statistical analysis

Two-way analyses of variance (ANOVA) were performed to test the effect of cultivar and temperature, and means were compared by Tukey's multiple comparison test. The calculations were performed using a MiniTab® Statistical Software Program package (Release 15; Minitab Inc., State College, PA, USA). Percentage values were always subjected to an arc sin transformation before the ANOVA.

4. Results

4.1. Controlled environment experiment

4.1.1. Growth in constant temperature

Growth curves for the seven cultivars at different constant temperatures are presented in Fig. 10. When exposed to a natural photoperiod from August 18, extension growth began to level off immediately in all cultivars at 9°C, and ceased completely within 1-2 weeks (Fig. 10). Plants exposed to 15°C grew at constant rate until September 8, when growth started to decrease. After seven weeks of the experiment, all cultivars at 15°C had ceased growth completely. The first cultivar to level off extension growth at 15°C was 'Glen Ample' after three weeks, the following week growth ceased completely. All the other cultivars except 'Schöneman' showed first sign of decreased growth one week after 'Glen Ample', with complete growth cessation within 5-8 weeks. 'Schöneman' did not show decreased growth until September 29, with complete growth cessation the following week. At 18°C, the growth started to level off in 'Glen Ample', 'Vene', 'Anitra' and 'Balder' from September 29, when the photoperiod had decreased to 12 hours (Fig. 10). The other cultivars did not show any growth retardation at 18°C during the 8-week experimental period. At 21°C, none of the cultivars showed any sign of growth cessation within the 8-week experimental period (Fig. 10). Plants grown at ambient temperature and natural photoperiod showed more or less the same trend as plants grown at 15°C. Extension growth began to level off in 'Glen Ample', 'Balder', 'Vene' and 'Anitra', on September 15, followed by 'Tulameen', 'Vene' and 'Schöneman' on September 22. All cultivars ceased growth completely the following week, by September 29.

Node increment (Fig. 11) showed the same trend as the growth increment in all cultivars, but was overall later to cease than growth. At 9°C, leaf development ceased within 2-3 weeks after shoot growth. In plants grown at 15°C, 18°C and ambient temperature, leaf development started to level off 1-2 weeks later than shoot extension.

Fig. 12 illustrates the final differences in growth between the four constant temperatures for 'Tulameen'. Table 2 shows the final shoot length, total growth increment, final number of nodes and total node increment for all seven cultivars and temperatures.

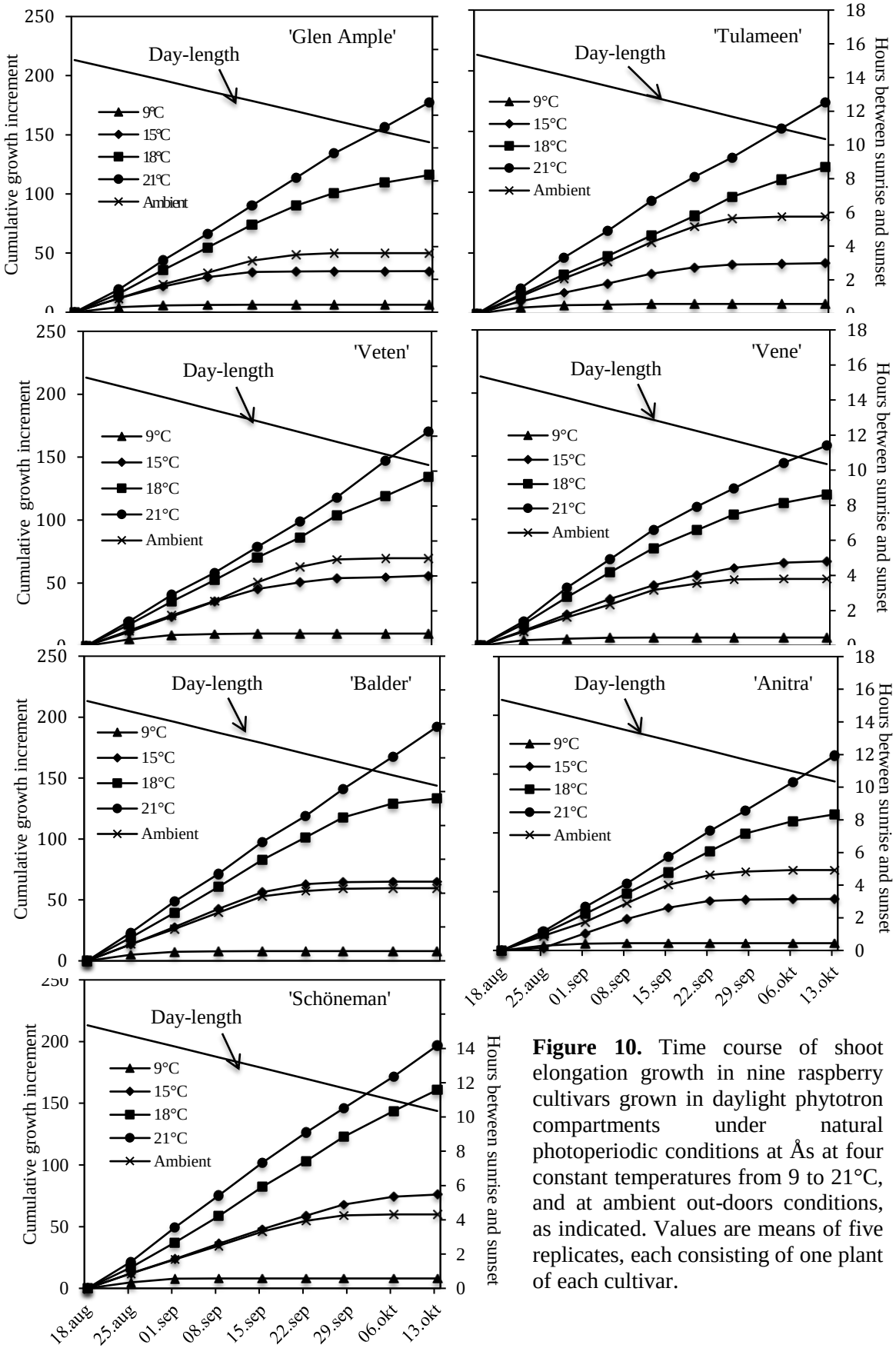

Figure 10. Time course of shoot elongation growth in nine raspberry cultivars grown in daylight phytotron compartments under natural photoperiodic conditions at Ås at four constant temperatures from 9 to 21°C, and at ambient out-doors conditions, as indicated. Values are means of five replicates, each consisting of one plant of each cultivar.

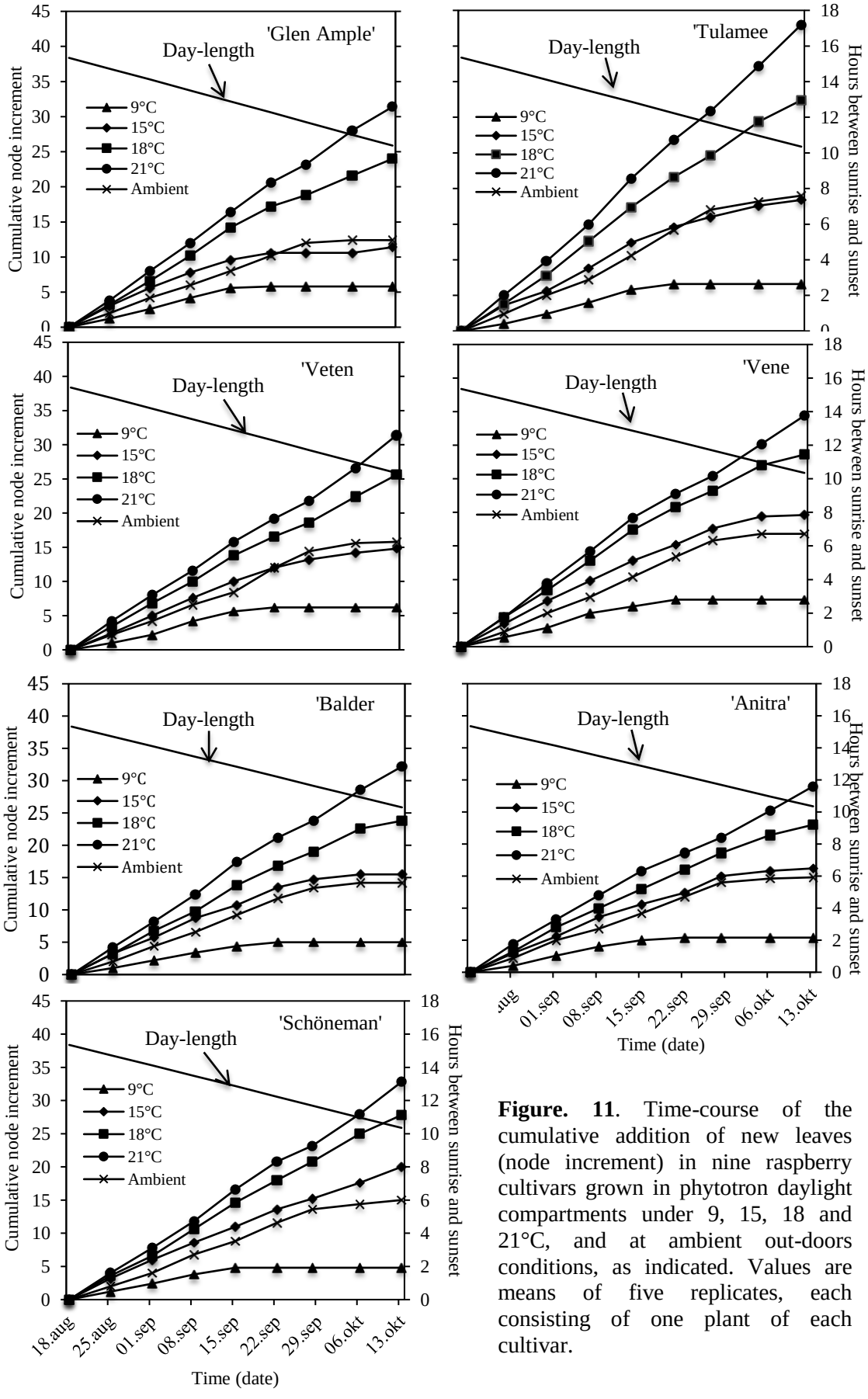

Figure. 11. Time-course of the cumulative addition of new leaves (node increment) in nine raspberry cultivars grown in phytotron daylight compartments under 9, 15, 18 and 21°C, and at ambient out-doors conditions, as indicated. Values are means of five replicates, each consisting of one plant of each cultivar.

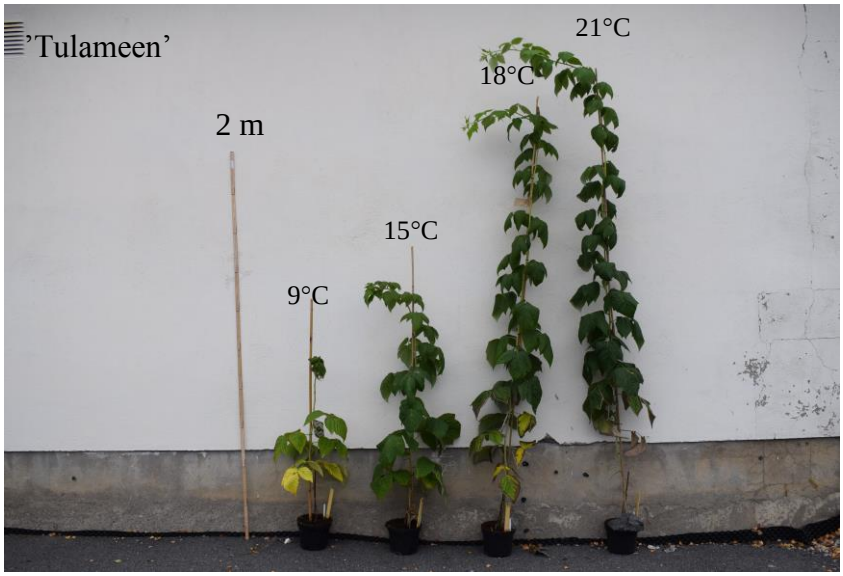

Figure. 12. Illustration of plant growth in 'Tulameen' after 8 weeks (on October 13) at constant temperatures of 9, 15, 18 and 21°C (from left to right). Photo: Anita Sønsteby

Table 2. Effects of autumn temperatures on growth and development at the end of various temperatures in seven raspberry cultivars.

Cultivar	Temperature (°C)	Shoot height (cm)	Growth increment (cm)	Total no. of nodes	Node increment
'Glen Ample'	9	92.6 tu	6.4 n	25.8 mn	5.8 n
	15	120.8 pqrst	34.8 m	31.4 kl	11.4 m
	18	204.4 ghi	122.4 f	47.8 de	28.0 ef
	21	258.4 cd	177.4 b	51.4 cde	31.4 cde
	Ambient	132.4 nopqr	50.0 kl	32.8 jkl	12.4 lm
Mean		*161.7*	*78.2*	*37.8*	*17.8*
'Tulameen'	9	86.0 u	8.0 n	26.0 mn	6.6 n
	15	127.2 opqrs	41.6 lm	37.8 ghil	18.4 ghij
	18	226.6 efgh	130.8 ef	57.4 b	36.6 b
	21	266.4 bc	173.8 b	64.4 a	43.0 a
	Ambient	161.2 jklm	80.2 g	41.0 gh	19.0 ghi
Mean		*173.5*	*86.9*	*45.3*	*24.7*
'Veten'	9	98.4 stu	9.8 n	24.4 mn	6.2 n
	15	145.8 lmnop	55.8 jk	32.6 jkl	14.8 jklm
	18	265.0 bc	149.2 d	51.4 cde	32.2 cd
	21	263.4 bc	170.2 bc	49.8 cde	31.4 cde
	Ambient	171.1 jkl	69.9 ghi	37.6 ghij	15.8 hijkl
Mean		*188.8*	*91.0*	*39.2*	*20.1*
'Vene'	9	97.0 stu	6.4 n	28.6 lm	7.0 n
	15	158.8 klmn	66.8 hij	42.2 fg	19.6 gh
	18	220.0 fgh	126.2 ef	54.8 bc	31.8 cde
	21	248.8 cdef	158.4 cd	57.0 b	34.4 bc
	Ambient	134.8 mnopq	52.8 kl	37.0 ghij	16.8 ghijk
Mean		*171.9*	*82.1*	*43.9*	*21.9*

‘Balder’	9	104.4 rstu	8.0 n	25.8 mn	5.0 n
	15	161.0 jklmn	65.0 hijk	36.0 hijk	15.5 hijklm
	18	231.6 defg	137.0 e	46.8 ef	26.0 f
	21	289.2 ab	192.0 a	52.8 bcd	32.2 cd
	Ambient	153.6 lmno	59.6 ijk	34.6 ijk	14.2 klm
Mean		*189.1*	*93.5*	*39.3*	*18.7*
‘Anitra’	9	141.8 lmnop	6.2 n	29.0 lm	5.4 n
	15	186.4 ijk	51.8 kl	37.8 ghij	16.2 ghijkl
	18	253.2 cde	120.4 f	49.0 de	27.8 ef
	21	290.6 ab	165.8 bc	48.6 de	29.0 def
	Ambient	199.2 hi	68.4 ghij	36.6 hijk	14.8 jklm
Mean		*214.2*	*82.5*	*40.2*	*18.6*
‘Schöneman’	9	107.8 qrstu	8.0 n	22.6 n	4.8 n
	15	187.4 ij	76.0 gh	39.4 ghi	20.0 g
	18	307.6 a	178.0 b	52.2 bcd	34.0 bc
	21	298.0 a	196.8 a	51.4 cde	32.8 bcd
	Ambient	164.5 jkl	60.5 ijk	34.4 ijk	15.0 ijklm
Mean		*213.1*	*103.9*	*40.0*	*21.3*
Probability levels of significance by ANOVA					
Source of variation					
Temperature (A)		< 0.001	< 0.001	< 0.001	< 0.001
Cultivar (B)		< 0.001	< 0.001	< 0.001	< 0.001
A x B		< 0.001	< 0.001	< 0.001	< 0.001

*Mean values within the same column followed by different lower-case letters indicate a significant difference ($P < 0.05$) between temperatures (n = 5).

The data are the means of five replicates, each with one plant of each cultivar in each temperature.

4.1.2. Floral development

Buds were collected weekly during the experimental period, and serial dissection of buds was examined. Almost all cultivars grown at 9°C developed fully differentiated flower buds (stage 6) by the end of the experimental period (Fig. 13). 'Veten' and 'Vene' had the most differentiated flowers, reaching development stage 6 in both cultivars. However, 'Glen Ample', 'Balder', and 'Anitra' had developmental stages close to 6, and were considered to have fully differentiated flower buds. Least developed buds had 'Tulameen' and 'Schöneman', but overall, there were no statistical significant difference between the cultivars for floral development (Table 3). The cultivars 'Glen Ample', 'Balder' and 'Anitra' had the fastest flower differentiation rate, reaching a development stage 5 after six weeks, on September 28. The other cultivars reached the same developmental stage one week later, on October 6.

Plants of 'Veten' and 'Vene grown at 15°C, showed the most differentiated flower buds of all. Both reached developmental stage 3.6 by the end of the experiment, on October 13. The difference was, however, not statistical by significance compared to the other cultivars (Table 3). The other cultivars reached a final developmental stage between 2 and 3 at 15°C. The first cultivar to respond at 15°C was 'Glen Ample' after one week, on August 25. This was one week earlier than 'Tulameen', 'Balder', 'Anitra' and 'Schöneman'. 'Veten' and 'Vene' did not respond similarly until week three, on September 8. The earliest response at ambient temperature was observed in 'Anitra', after one weeks, on August 25, followed by 'Glen Ample' and 'Balder' in week two, 'Schöneman' in week three, 'Tulameen' and 'Vene' in week six and finally 'Veten' after eight weeks at ambient temperature. Plants at ambient temperature showed in general a lower developmental stage than plants at 15°C. The largest difference was observed in 'Veten', were flower bud development at 15°C was significantly higher ($P < 0.05$) with a developmental stage 3.6 compared to 1.1 at ambient temperature. 'Vene' and 'Balder' did also reach a developmental stage that was significantly higher ($P < 0.05$) at 15°C than at ambient temperature (Fig. 13, Table 3). For the other cultivars, the difference was smaller and not statistical significant.

Plants grown at 18°C (Fig. 13) showed no or very little floral bud development. The only cultivars with visible flower bud formation were 'Glen Ample' and 'Vene' which reached stage 3 within 8 weeks. Tip-flowering was observed in 'Vene' at 18°C and 21°C, and in 'Anitra' at 18°C (Fig. 14). Because the collected buds were taken several nodes below the

terminal bud, this is not illustrated in the graphs in Fig. 13 or in Table 3. The first observation of open flowers in ‘Vene’ was made after five weeks at 18°C and after eight weeks at 21°C. At the end of the experiment four out of five plants at 18°C, and three out of five plants at 21°C were tip-flowering. In ‘Anitra’ the first observation of tip-flowering was made after ten weeks, on October 27, in one plant. Except for the cultivar ‘Vene’, there was no flower initiation in plants grown at 21°C.

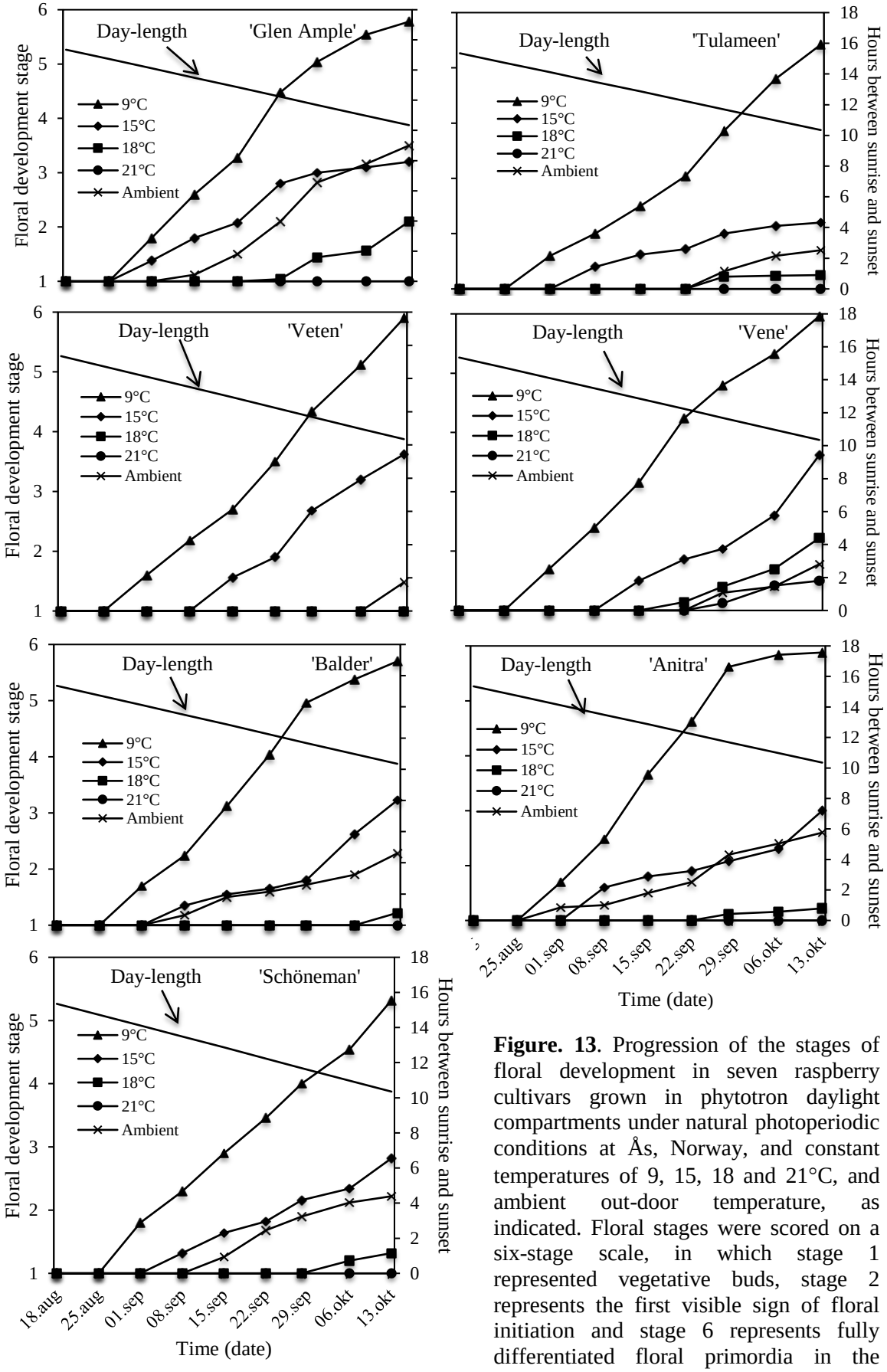

Figure. 13. Progression of the stages of floral development in seven raspberry cultivars grown in phytotron daylight compartments under natural photoperiodic conditions at Ås, Norway, and constant temperatures of 9, 15, 18 and 21°C, and ambient out-door temperature, as indicated. Floral stages were scored on a six-stage scale, in which stage 1 represented vegetative buds, stage 2 represents the first visible sign of floral initiation and stage 6 represents fully differentiated floral primordia in the primary flower.

Figure 14. Tip-flowering in the cultivar 'Vene' after 8 weeks at 18°C and natural photoperiod. Photo: Randi Hodnefjell

Table 3. Bud development of seven raspberry cultivars grown in phytotron daylight compartments under natural photoperiodic conditions at Ås, Norway, and constant temperatures of 9, 15, 18 and 21°C, and ambient out-door conditions for 8 weeks.

Cultivar	Temperature (°C)	Generative nodes (%)	Vegetative nodes (%)	Floral dev. stage (1-6)**
'Glen Ample'	9	68.3 cde	31.7 abc	5.8 a
	15	69.9 bcde	30.1 abcd	3.1 bcd
	18	74.3 abcd	25.7 bcde	2.8 cdef
	Ambient	80.3 abc	19.7 cde	3.2 bcd
Mean		*73.2*	*26.8*	*3.2*
'Tulameen'	9	67.9 cde	32.1 abc	5.4 a
	15	54.0 e	46.0 a	2.0 fghi
	18	-	-	1.5 ghij
	Ambient	59.7 de	40.3 ab	1.6 ghij
Mean		*60.5*	*39.5*	*2.3*
'Veten'	9	69.6 bcde	30.4 abcd	5.9 a
	15	77.8 abc	22.2 cde	3.6 bc
	18	-	-	1.0 j
	Ambient	83.3 abc	16.7 cde	1.1 ij
Mean		*76.9*	*23.1*	*2.5*
'Vene'	9	69.4 bcde	30.6 abcd	6.0 a
	15	74.9 abcd	25.1 bcde	3.6 bc
	18	84.0 ab	16.0 cde	3.8 b
	Ambient	75.7 abcd	24.3 bcde	1.4 hij
Mean		*76.0*	*24.0*	*3.2*

‘Balder’	9	83.4 abc	16.6 cde	5.7 a
	15	82.8 abc	17.2 cde	3.2 bcd
	18	83.8 abc	16.2 cde	2.7 cdef
	Ambient	83.4 abc	16.6 cde	1.7 ghij
Mean		*83.3*	*16.7*	*2.9*
‘Anitra’	9	83.2 abc	16.8 cde	5.9 a
	15	85.2 ab	14.8 de	3.0 bcde
	18	-	-	1.2 ij
	Ambient	89.8 a	10.2 e	2.3 defg
Mean		*86.1*	*13.9*	*2.7*
‘Schöneman’	9	77.9 abc	22.1 cde	5.3 a
	15	68.3 cde	31.7 abc	2.8 cdef
	18	-	-	1.1 ij
	Ambient	68.1 cde	31.9 abc	2.1 efgh
Mean		*71.5*	*28.5*	*2.4*

Probability levels of significance by ANOVA			
Source of variation			
Temperature (A)	0.04.	0.04.	< 0.001
Cultivar (B)	< 0.001	< 0.001	< 0.001
A x B	0.001	0.001	< 0.001

*Mean values within the same column followed by different lower-case letters indicate a significant difference ($P < 0.05$) between temperatures (n = 5).

The data are the means of five replicates, each with one plant of each cultivar for each temperature.

**The floral developmental stage (scale 1-6) of bud number 5-7 below the shoot tip recorded after 8 weeks at different temperatures.

4.1.3. Profiles of flower bud development along the entire shoot

The trends in the timing of floral initiation were confirmed by dissection of all buds along the entire shoot length of three plants of each cultivars at 9°C, 15°C and ambient outdoor condition temperatures on October 13, as well as for three cultivars at 18°C (Fig. 15-18). At 9°C (Fig. 15), plants of all cultivars had differentiated fully advanced floral buds (stage 5-6) in all buds from about node 8 and upward, including the terminal bud. Development along the shoot was similar for all cultivars, except that 'Balder', 'Anitra' and 'Schöneman' had developed floral buds a bit further down the shoot (node 5) (Fig. 15) than the others. All buds were highly advanced from the top and down to the lower part of the shoot, where there was a sharp decrease to vegetative buds.

At ambient temperature, 'Tulameen' was found to be the least developed among the seven cultivars (Fig. 16). This cultivar had a significantly ($P < 0.05$) lower number of generative buds than 'Glen Ample', 'Veten', 'Balder' and 'Anitra' (Table 3). There was no statistical significant difference among the other cultivars considering the number of generative buds (Table 3). Most of the cultivars at ambient temperature had a flower development architecture resembling the shape of a Christmas tree (Fig. 16). Very little or no development in the top of the shoot and increasing development down to the middle part of the shoot, development in the lowest part is fast decreasing or stops sharply. There are, however, some differences, 'Glen Ample' and 'Balder' were most advanced. In addition 'Schöneman' and 'Tulameen' had vegetative buds in the top of the shoot, while the other cultivars had generative buds also in the top of the shoot. Of all temperatures, the lowest number of vegetative buds near the base was found in plants from ambient temperature (Fig. 16).

Floral bud development along the shoot was more advanced along the entire shoot at 15°C, than at ambient temperature. However, the number of vegetative buds close to the base was higher in plants grown at 15°C (Fig. 17, Table 3). The cultivar 'Tulameen' was again slow to respond, and had a significantly ($P < 0.05$) lower number of generative buds than 'Balder' and 'Anitra' (Table 3). At 15°C, 'Tulameen' had only one bud at developmental stage 3, the rest of the buds were close to stage 2 or lower. Buds of 'Schöneman' had developed to stage 3, which is slightly more than 'Tulameen', but neither was significantly different from the other cultivars (Table 3). Both 'Schöneman' and 'Tulameen' in both temperatures had vegetative buds in the top of the shoot. Also 'Balder' and 'Anitra' had less advanced apical buds at 15°C and ambient temperature. 'Vene' tip-flowered at 18°C (Fig. 14

and 18) and had flowers or aggregate fruits in the top-ten buds. Development further down the shoot in 'Vene' decreased slowly, and generative activity was presents along the whole shoot, except for the lowest 8 vegetative buds. 'Balder' was vegetative in the top four buds and most other buds did not exceed stage 3 in development, the exception was one bud with a development stage of 3.7. 'Glen Ample' had vegetative buds in the top six buds of the shoot, and no buds along the shoot had exceeded a development stage 2, except one bud with a development stage 3.8.

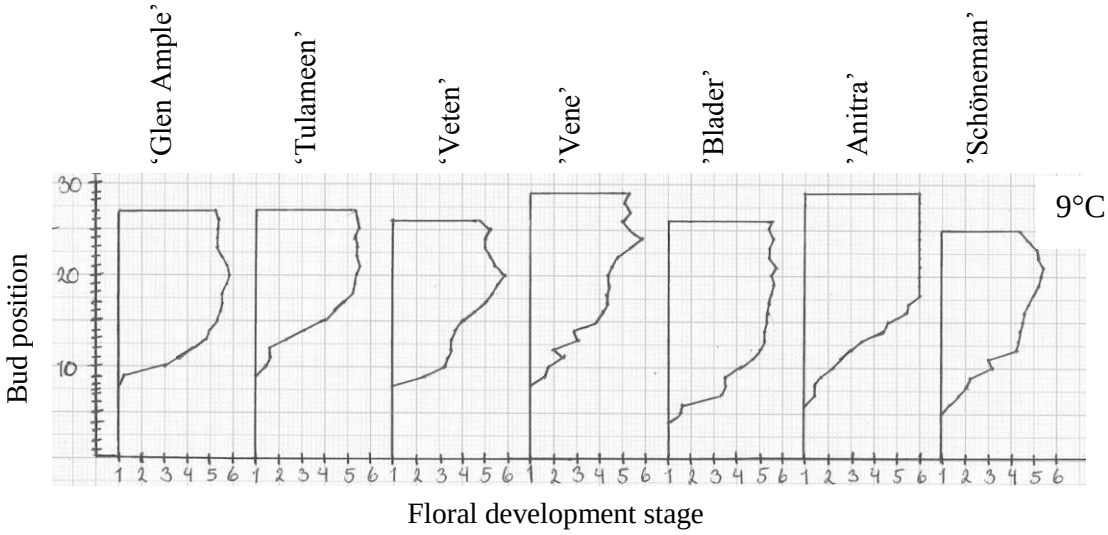

Figure 15. Profiles of the stages of flower development of buds at each node along the entire shoot length of seven raspberry cultivars grown at a constant 9°C under natural photoperiodic conditions at Ås, Norway. All buds were sampled on October 13. Bud positions were counted from the base of the shoot. All values are the means of three plants of each cultivar.

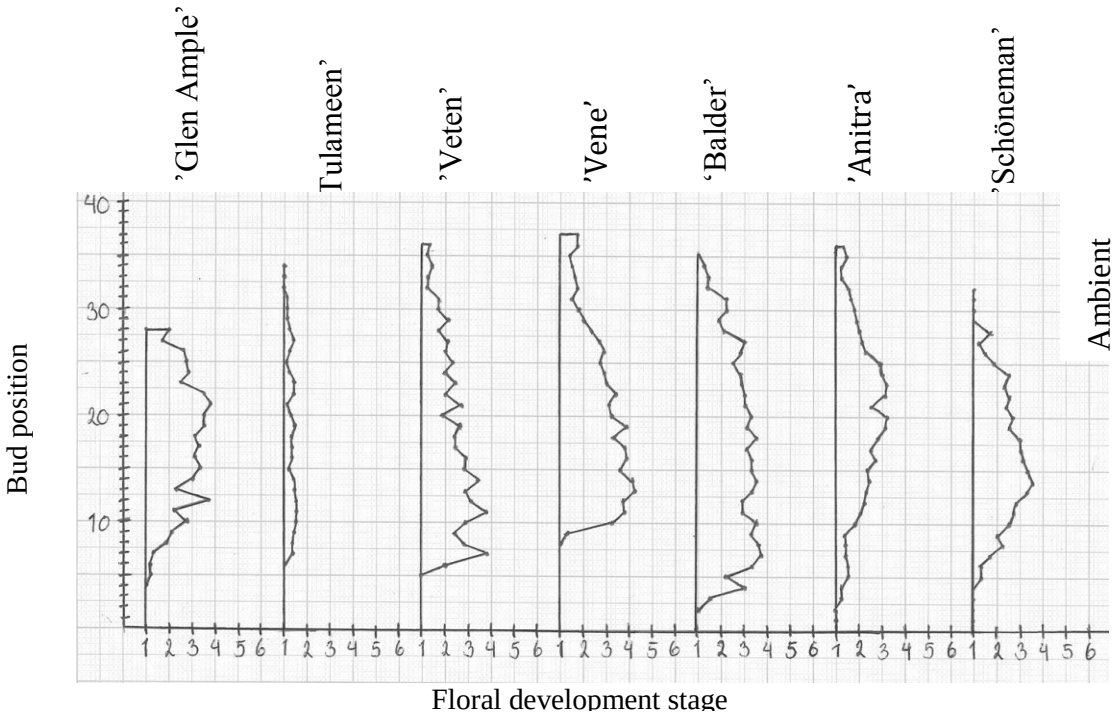

Figure 16. Profiles of the stages of flower development of buds at each node along the entire shoot length of seven raspberry cultivars grown at ambient out-door conditions under natural photoperiodic conditions at Apelsvoll Norway. All buds were sampled on October 13. Bud positions were counted from the base of the shoot. All values are the means of three plants of each cultivar.

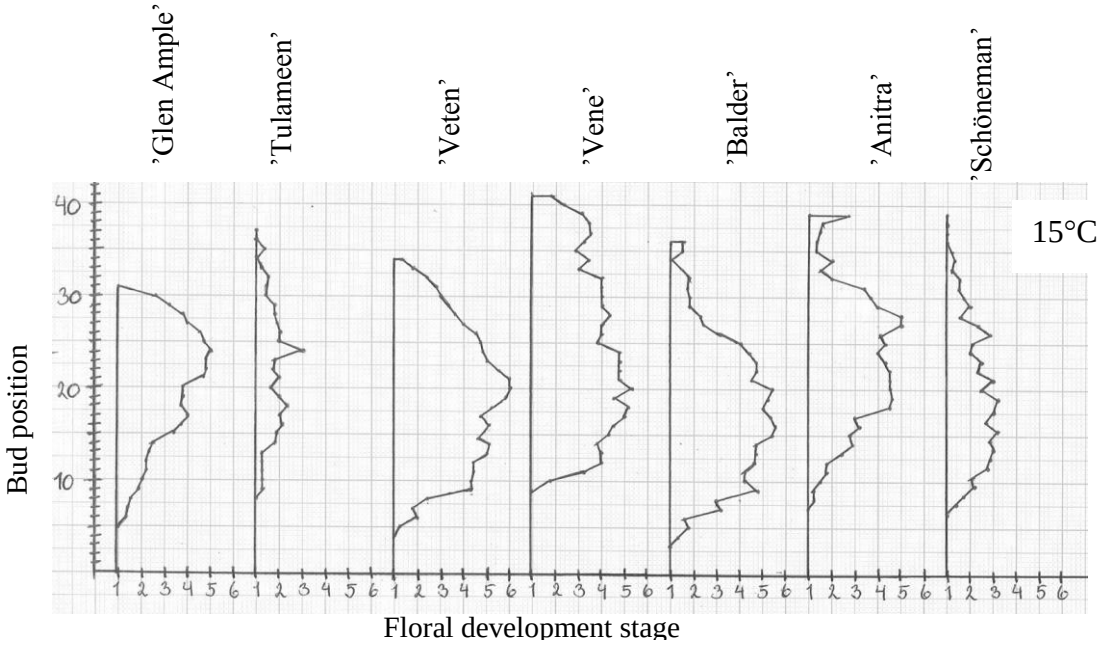

Figure 17. Profiles of the stages of flower development of buds at each node along the entire shoot length of seven raspberry cultivars grown at a constant 15°C under natural photoperiodic conditions at Ås, Norway. All buds were sampled on October 13. Bud positions were counted from the base of the shoot. All values are the means of three plants of each cultivar.

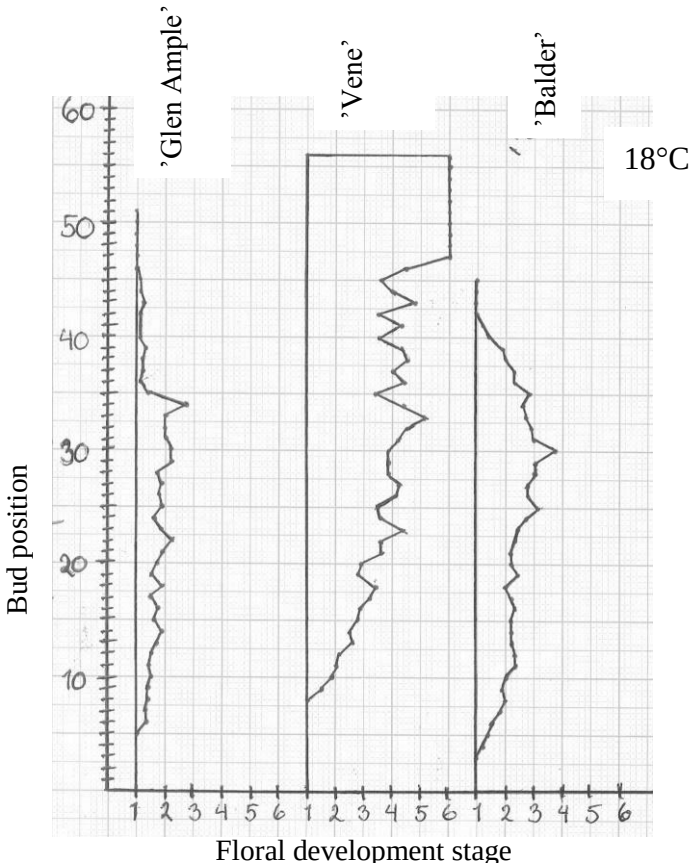

Figure 18. Profiles of the stages of flower development of buds at each node along the entire shoot length of seven raspberry cultivars grown at a constant 18°C under natural photoperiodic conditions at Ås, Norway. All buds were sampled on October 13. Bud positions were counted from the base of the shoot. All values are the means of three plants of each cultivar.

4.2. Growth cessation and flower initiation in two Italian cultivars

4.2.1. Growth in constant temperature

Growth curves for the Italian cultivars ‘Vajolet’ and ‘Lagorai Plus’ at different constant temperatures are presented in Fig. 19. Final shoot height, growth increment and total number of nodes at different constant temperatures is presented in Table 4. Equal to the other cultivars grown at 9°C, extension of shoot growth began to level off immediately, and showed complete growth cessation within 1-2 weeks. At 15°C, both cultivars ceased growth completely within three weeks of the experiment. In plants grown at 18°C, only ‘Lagorai Plus’ ceased growth completely during the experimental period of 8 weeks, on October 6. ‘Vajolet’, only showed a slight decrease in growth in the two final weeks of the experiment. At 21°C, both cultivars kept a constant growth rate. Leaf development (Fig 20) showed the same trends as shoot growth, but with a two weeks delay.

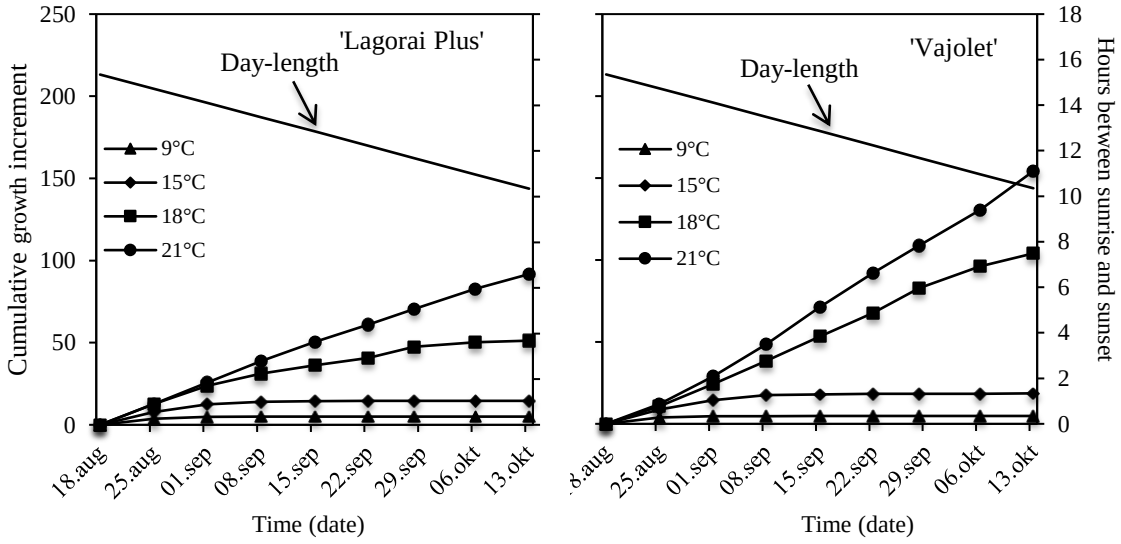

Figure 19. Time course of shoot elongation growth in two Italian raspberry cultivars grown in daylight phytotron compartments under natural photoperiodic conditions at Ås at four constant temperatures from 9 to 21°C, and at ambient out-doors conditions, as indicated. Values are means of five replicates, each consisting of one plant of each cultivar.

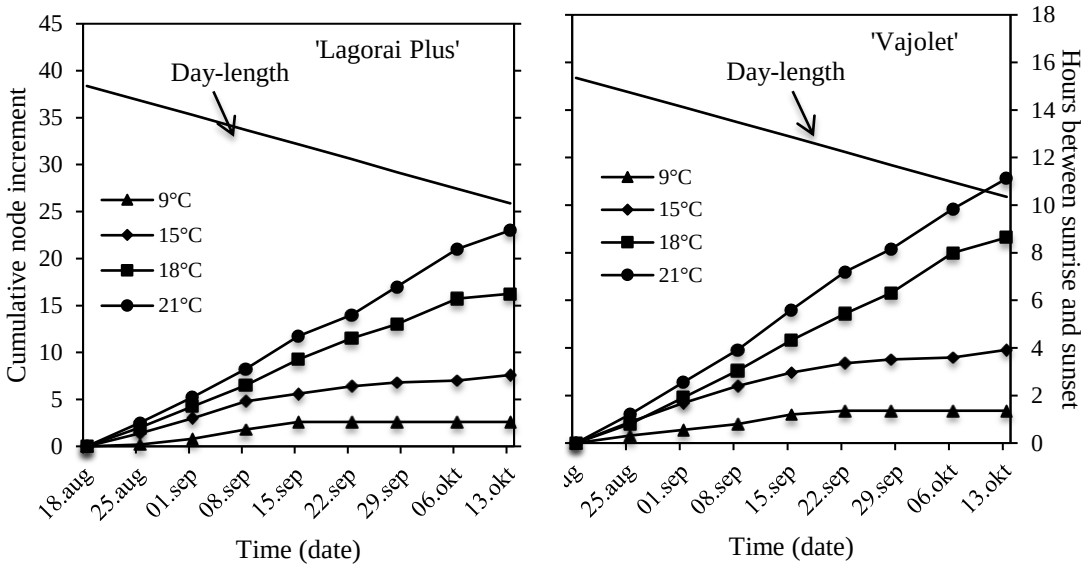

Figure. 20. Time-course of the cumulative addition of new leaves (node increment) in two Italian raspberry cultivars grown in phytotron daylight compartments under 9, 15, 18 and 21°C, and at ambient out-doors conditions, as indicated. Values are means of five replicates, each consisting of one plant of each cultivar.

4.2.2 Floral development

Whole shoots were also collected and examined for 'Vajolet' and 'Lagorai Plus', and profiles of flower development along the entire shoot are presented in Fig. 21. In Table 4, percentages of generative and vegetative buds along the entire shoot are presented together with final floral developmental stage. Plants grown at 9°C showed the same trend as the other cultivars, with at a flower developmental stage between 4 and 6. The similarities with the other cultivars are obvious in plants grown at 15°C and ambient temperature, but they were in general less differentiated. A big difference between ambient temperature and 15°C were observed in 'Vajolet' (Fig. 21), were ambient temperature showed a developmental stage of no more than 2.6 in one bud, while several buds at 15°C showed a stage 4.5.

Table 4. Growth and development of two Italian raspberry cultivars grown at controlled temperature and ambient photoperiod at Ås, Norway for 8 weeks

Cultivar	Temperature (°C)	Shoot height (cm)	Growth incre-ment (cm)	Total no. of nodes	Node incre-ment	Generative nodes (%)	Vegetative nodes (%)	Floral dev. stage (1-6)**
'Vajolet'	9	54.0	4.8 n	14.4 o	3.4 n	84.5 ab	15.5 de	-
	15	69.0	18.6 m	19.8 n	9.8 m	79.4 abc	20.6 cde	3.9 b
	18	168.0	112.0 f	37.2 ghij	25.4 f	-	-	1.5 ghij
	21	201.4	154.2 cd	38.0 ghi	27.8 ef	-	-	1.5 ghij
	Ambient	-	-	-	-	63.5 cde	36.5 abc	-
Mean		*123.1*	*72.4*	*27.4*	*16.6*	*75.8*	*24.2*	*2.3*
'Lagorai Plus'	9	40.2	5.0 n	12.6 o	2.6 n	58.5 de	41.5 ab	-
	15	55.8	15.0 n	16.8 o	7.6 n	57.6 de	42.4 ab	3.5 bc
	18	92.5	52.3 kl	26.5 mn	17.0 ghij	-	-	2.3 defg
	21	125.5	91.8 g	31.8 kl	23.0	-	-	1.1 ij
	Ambient	-	-	-	-	-	-	-
Mean		*75.1*	*37.6*	*21.1*	*11.7*	*58.0*	*42.0*	*2.4*
Probability levels of significance by ANOVA								
Source of variation								
Temperature (A)		< 0.001	< 0.001	< 0.001	< 0.001	n.s.	n.s.	< 0.001
Cultivar (B)		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
A x B		< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.001	< 0.001

*Mean values within the same column followed by different lower-case letters indicate a significant difference ($P < 0.05$) between cultivars (n = 5).

The data are the means of five replicates (growth, node and floral development) and three replicates (generative and vegetative buds), each with one plant of each cultivar for each temperature.

**The floral developmental stage (scale 1-6) of bud number 6-8 below the shoot tip recorded after 8 weeks of temperature n.s.- not significant.

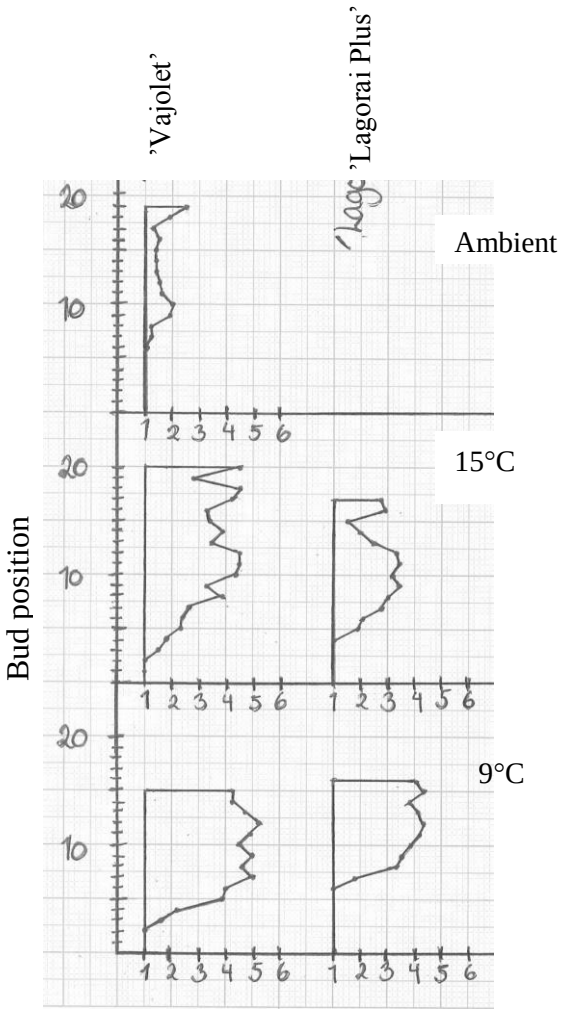

Figure 21. Profiles of the stages of flower development of buds at each node along the entire shoot length of two Italian raspberry cultivars grown at a constant 9°C, 15°C and ambient out-door conditions under natural photoperiodic conditions at Ås and Apelsvoll, Norway. All buds were sampled on October 13. Bud positions were counted from the base of the shoot. All values are the means of three plants of each cultivar.

4.3 Field experiment

4.3.1. Shoot growth

The field experiment consisted of seven cultivars grown under natural out-door conditions. Growth measurements and bud collecting was done in the same way as for the controlled environment experiment.

All cultivars in the field experiment showed a decrease in shoot growth when photoperiod was approximately 15 hours, between August 17 and August 24 (Fig. 5 and 22). After August 24, temperature was decreasing (Fig. 5) and so was shoot growth (Fig. 22). The cultivars 'Cascade Delight', 'Glen Ample', 'Ninni' and 'Malling Juno' ceased growth first, after five weeks, on September 7 (Fig. 22). The next cultivar to cease shoot growth was RU90 the following week, on September 14. The latest cultivars to cease growth completely were 'Veten' and 'Anitra', three weeks after the first. 'Anitra' even showed a slight growth increment in the last week of the experiment.

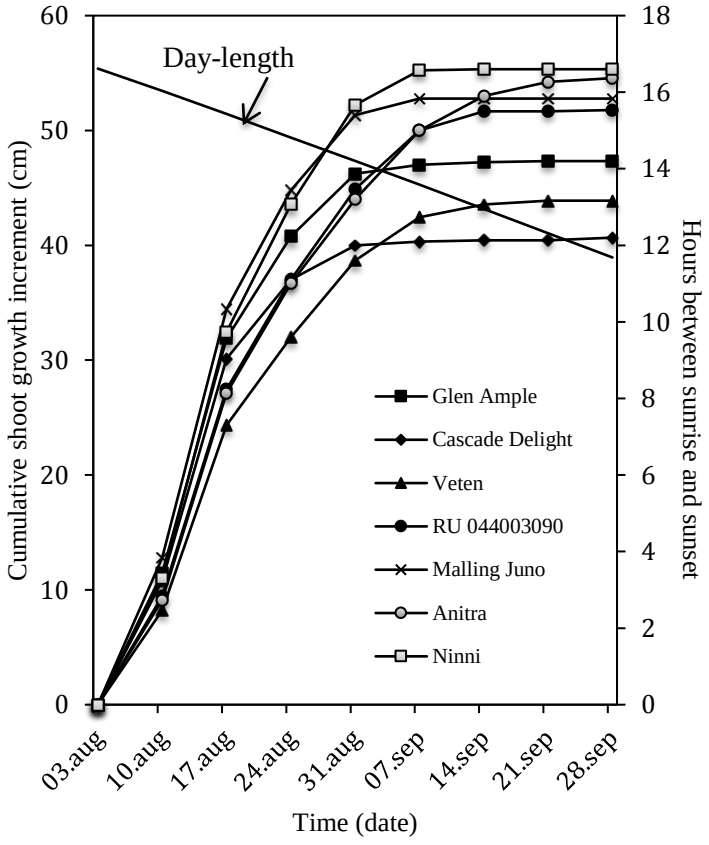

Figure 22. Time course of shoot elongation growth in seven raspberry cultivars grown in out-door conditions at Ås, Norway. Values are means of nine replicates, each consisting of one plant of each cultivar.

4.3.2. Leaf development

Similar to the controlled environment experiment, leaf development (Fig. 23) ceased shortly after shoot growth cessation (Fig. 22), and with the same pattern as for shoot growth. 'Cascade Delight' ceased leaf development first, on September 14, 'Glen Ample', RU 90 and 'Ninni' the following week. 'Veten', 'Malling Juno' and 'Anitra' had not ceased leaf development at the end of the experiment. However, development in these cultivars was minimal.

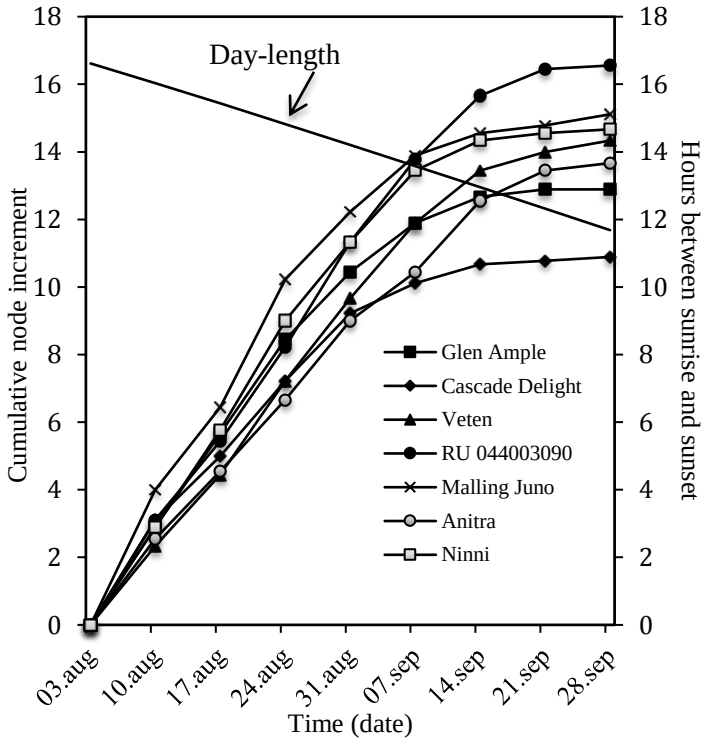

Figure 23. Time course of the cumulative addition of new leaves (node increment) in seven raspberry cultivars grown in out-door conditions at Ås, Norway. Values are means of nine replicates, each consisting of one plant of each cultivar.

4.3.3. Floral development

No bud among the collected buds from the cultivars grown in the field reached a development stage of more than 4.5 during the experiment (Fig. 23). 'Anitra' started, however, to flower five weeks into the experiment. The first observation of flowering was done on September 7. This is not presented in the results because the collected buds were taken below the flowering buds, or on shoots that was not flowering. The earliest sign of flower initiation was in 'Glen Ample' and 'Malling Juno' the first week of the experiment, on August 10. However, the development of floral buds in 'Glen Ample' did not reach a developmental stage of more than 3, while 'Malling Juno reached a stage 4.5. The other cultivars started flower initiation two weeks later. The latest cultivar to initiate flower primordia was RU 90, four weeks after 'Glen Ample'. The fastest rate of flower initiation was in 'Malling Juno', followed by 'Cascade Delight' and 'Veten' (Fig. 23).

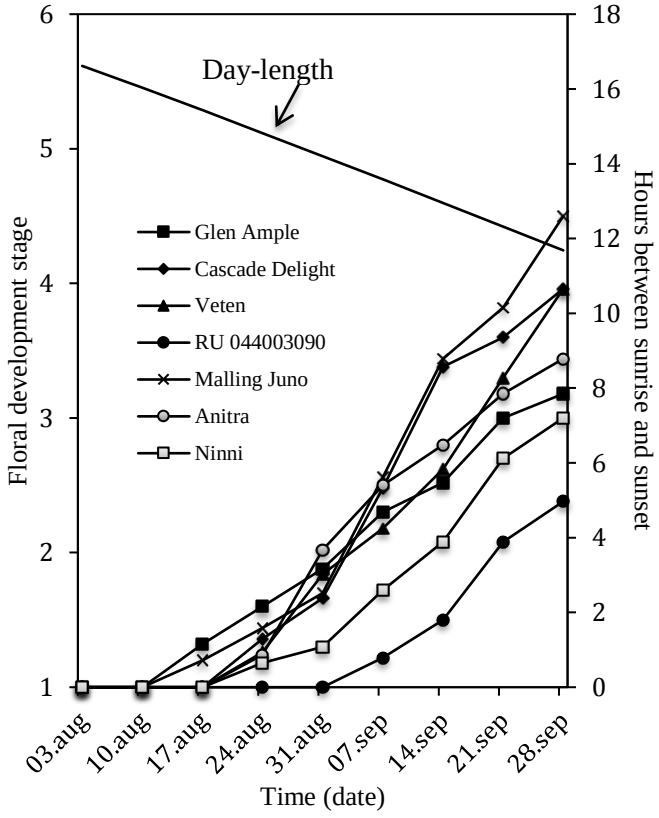

Figure 23. Progression of the stages of floral development in seven raspberry cultivars grown in natural out-door conditions at Ås, Norway. Floral stages were scored on a six-stage scale, in which stage 1 represented vegetative buds, stage 2 represented the first visible sign of floral initiation and stage 6 represented fully differentiated floral primordia in the primary flower. Values are means of nine replicates, each consisting of one plant of each cultivar

4.4. Scanning electron microscope (SEM)

Because of few canes with fully differentiated flowers, not all cultivars were available for SEM examination. Another consequence of few buds available was that not all were at the same developmental stage, making it difficult to compare flower morphology of the different cultivars. However, some buds had reached the same development stage. The flowers in Fig. 24 are more or less at the same floral development stage, stage 6 with a fully differentiated flower.

‘Glen Ample’ seems to have a more open flower, while ‘Anitra’ and ‘Veten’ displays a more closed flower. ‘Veten’ had started developing thricomes on the petals, while ‘Glen Ample’ seems to have differentiated further without developing thricomes. ‘Glen Ample’ also seems to have more stamens than the other cultivars.

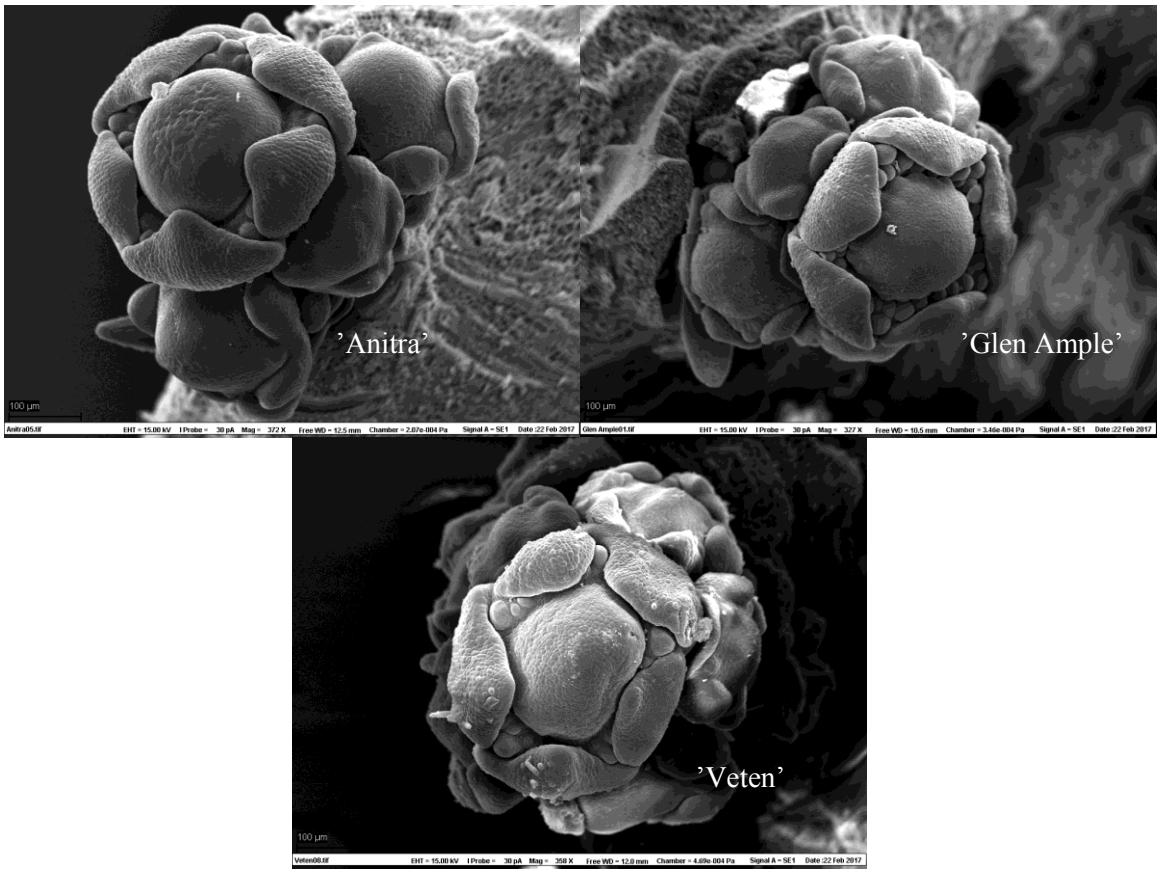

Figure 24. SEM imaging of fully differentiated flowers (stage 6) of the cultivars 'Anitra', 'Glen Ample' and 'Veten'.

Based on the development of trichomes on the buds, the flower buds in Fig. 25 are more or less on the same floral development stage, except ‘Balder’ and ‘Anitra’ which had developed slightly further than the other cultivars. By comparing the morphology of the

flower in the different cultivars, ‘Vajolet’ seems to have a more closed flower, while ‘Glen Ample’ seems to have the most open flower. ‘Balder’ also displace a more closed flower, but since ‘Balder’ had developed further than ‘Vajolet’, it is difficult to state a conclusion. ‘Veten’ seems to have bigger sepals than the rest.

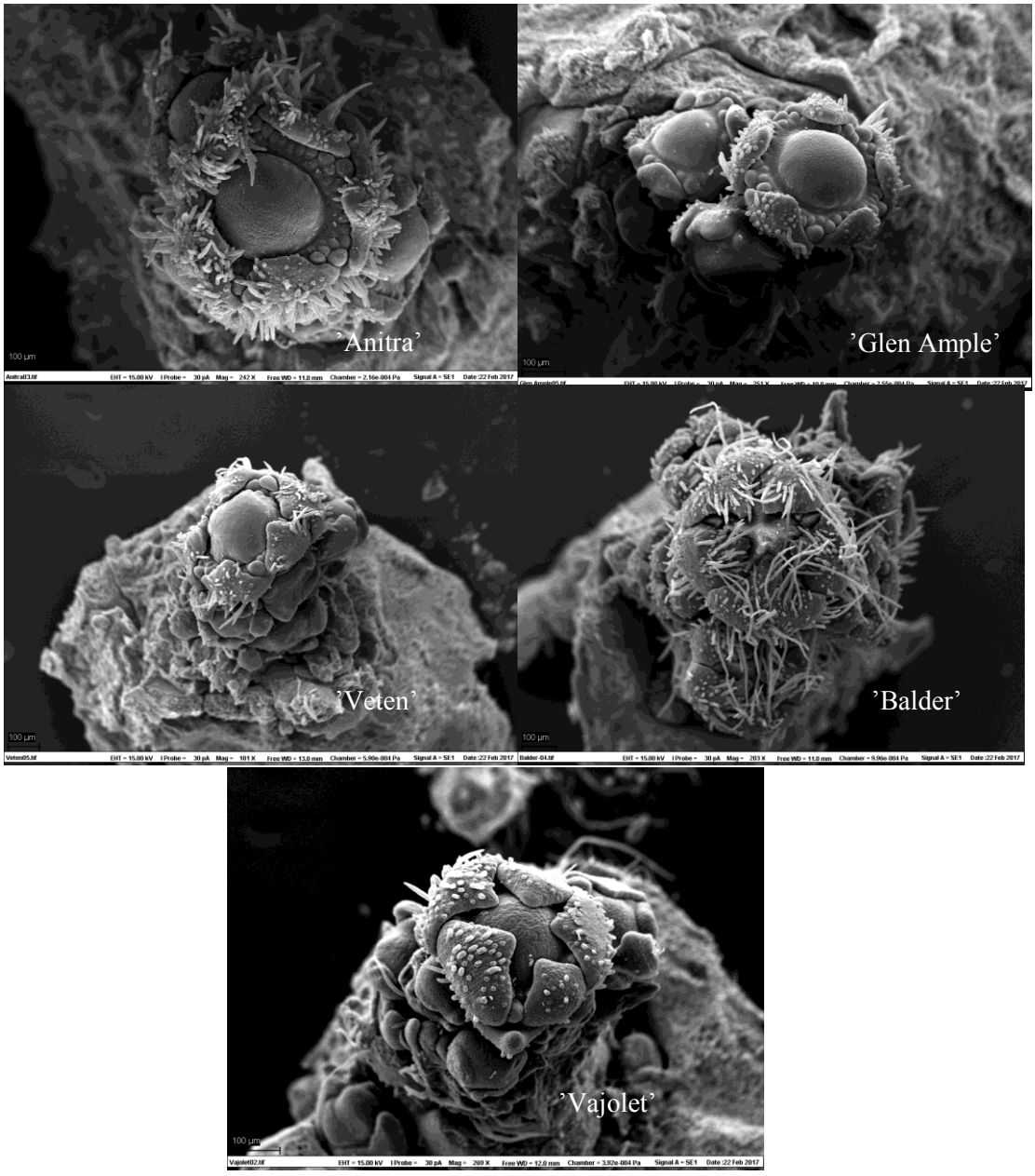

Figure 25. SEM- images of flowers differentiated to stage 6 and above of the cultivars ‘Anitra’, ‘Glen Ample’, ‘Veten’, ‘Balder’ and ‘Vajolet’.

5. Discussion

The results presented in this thesis illustrate the behaviour of raspberry cultivars during the phase of growth cessation and the onset of dormancy in two experiments conducted under controlled and ambient out-door conditions. Special attention was given to the architecture of the shoot and timing of flower initiation. Flower morphology of a few cultivars was studied as well.

5.1. Controlled environment experiment

5.1.1. Shoot growth and leaf development

The presented results show that all cultivars were highly dependent on temperature and photoperiod for growth cessation and floral initiation as previously shown for 'Malling Promise' (Williams, 1959a, b, c, 1960) and 'Glen Ample' (Sønsteby & Heide, 2008). Also, the results concur with earlier findings, that there is a temperature limit at 18°C for growth cessation (Williams, 1959b; Sønsteby & Heide, 2008). All cultivars in this experiment showed a growth pattern equal to biennial-fruiting cultivars. At a temperature of 9°C, shoot growth of all cultivars ceased after one to two weeks, while leaf development ceased after four weeks. When shoot extension started to level off, the photoperiod was still long (>15 hours), proving that at low temperatures, raspberry plants are insensitive to photoperiod (Williams, 1959b; Sønsteby & Heide, 2008) At 15°C and ambient temperature, the earliest stop in shoot growth was shown after five weeks, and leaf development after six weeks. Photoperiod was at this point less than 15 hours, as also shown by Williams (1959b), to be the critical photoperiod to be a signal for growth cessation. Plants in ambient temperature showed more or less the same response as for plants at 15°C. This response was as expected, since the average daily mean out-door temperature was approximately 15°C throughout the majority of the experimental period. All cultivars grown at 18°C, started to decrease shoot growth and leaf development at the end of the experimental period, but they never stopped completely. At 18°C, photoperiod is still a signal the plants respond to, however, at such high temperature the balance between temperature and photoperiod are different from 15°C. At 15°C, the critical photoperiod is 15 hours (Williams, 1960; Sønsteby & Heide, 2008), at 18°C, the photoperiod was under 12 hours when the plants started to respond. This indicates that the photoperiod must be shorter at higher temperatures than when the temperature is approximately 15°C. This confirms that the biennial-fruiting raspberry is a facultative short day plant (Williams, 1960; Sønsteby & Heide, 2008). Plants at 21°C did never show any sign

of decreased vegetative growth, which is in accordance with what Williams (1959b), showed for ‘Malling Promise’ and Sønsteby and Heide (2008) for ‘Glen Ample’. When temperatures increase to 21°C, the signal becomes so strong that photoperiod have no effect on vegetative growth.

The results also show differences between cultivars grown at the same temperature. All plants of all cultivars grown at 9°C showed the same response for all cultivars, with a complete growth cessation after two weeks. At 15°C and ambient temperature, there was a two weeks difference, in growth cessation between the cultivars. ‘Balder’, ‘Glen Ample’ and ‘Anitra’ ceased growth completely after five weeks at 15°C and ambient temperature. ‘Veten’, ‘Vene’ and ‘Tulameen’ ceased shoot growth the following week, and the last cultivar, ‘Schöneman’, ceased growth after another week. The cultivars to cease growth after five weeks are, however, cultivars very well adapted to a Nordic climate, and especially ‘Balder’ is breed for cold winters. The plants bred to survive cold winters require a long period of decreasing temperature and shorter photoperiod in order to gain hardiness to survive winter. The earlier a cultivar enters dormancy, the stronger the winter hardiness is (Säkö & Hiirsalmi, 1980). To get the long period they need, the plants must respond earlier to the environmental signals and use photoperiod as a very strong signal. ‘Tulameen’ and ‘Schöneman’, however, are bred for mild winter climate and were thereby responding later. This type of response is making these plants less likely to accumulate enough winter hardiness and they may therefore suffer severe winter damage in locations with cold winters. The difference between cultivars was also visible in plants grown at 18°C. Shoot growth stopped later at 18°C than at 15°C and ambient temperature, but the pattern was the same: ‘Glen Ample’ responded first and started to level off shoot extension in week six of the experiment. This indicate that cultivars adapted to Nordic conditions use decreased photoperiod as a stronger signal when temperatures are between 15°C and 18°C than cultivars originating further south in Europe.

Equal for all cultivars and temperatures, leaf development ceased later than shoot growth as shown by Williams (1959b). A delayed stop in leaf development is due to the formation of the leaf rosette when entering dormancy. While elongation of the internodes has stopped, already initiated leaves will continue to unfold and thus result in constrained internodes and the characteristic rosette at the top of the shoot (Hudson, 1959).

5.1.2. Floral development in selected buds

The result of this study concurs with results reported by Williams (1959a, b, c, 1960) and Sønsteby and Heide (2008) that showed that decreasing temperature and photoperiod are crucial signals for raspberry plants to initiate flowers. All cultivars initiated flowers at 9°C, 15°C and ambient temperature when the photoperiod was shorter than 15 hours. However, buds at 9°C had almost fully differentiated flowers in all cultivars while buds at 15°C and ambient temperature had not reached a development stage of more than 4 in any of the cultivars tested. This proves that raspberry plants are insensitive to photoperiod when temperatures are below 15°C, and photoperiod dependent when temperatures are at 15°C (Sønsteby & Heide, 2008). The exact temperature when the plants become insensitive to photoperiod has not been discovered yet, and need further research. Sønsteby and Heide (2008) also found that either dormancy or flower initiation could take place at temperatures above 18°C regardless of the photoperiod in the biennial-fruiting cultivar 'Glen Ample'. However, there were some flower development in plants grown at 18°C in this study, but since the highest developmental stage was no more than 2.2; it can be discussed if this was a result of too detailed scoring. At the same time, there were also decreased shoot growth in five out of nine cultivars at 18°C. Since the results here do not concur with that found in 'Glen Ample' by Sønsteby ad Heide (2008), further research is needed.

The difference between cultivars is also visible in flower development. All cultivars grown at 9°C initiated flower buds, but only 'Glen Ample', 'Veten', 'Vene', 'Balder' and 'Anitra' had fully differentiated floral organs (stage 6). 'Glen Ample', 'Balder' and 'Anitra' had not reached a development stage of 6 at the end of the experiment, but with a development stage of 5.8, 5.7 and 5.9, respectively, they were considered fully differentiated. Equal to shoot growth and leaf development, flower development in 'Schöneman' and 'Tulameen' was at a lower development stage at the end of the experiment (stage 5.3 and 5.4, respectively). The difference was, however, not statistically significantly.

This response can also, in some degree, be observed at temperatures of 15°C. While 'Schöneman' and 'Tulameen' responded at the same time as the other cultivars, they did not differentiate flower organs at the same rate, ending the floral development at a lower developmental stage at the end of the experimental period (stage 2.8 and 2.2, respectively). This indicates that cultivars adapted to Nordic conditions will respond to decreasing temperatures with a faster flower differentiation rate. Cultivars adapted to a warmer climate will on the other hand, need a longer differentiation period before entering dormancy.

‘Glen Ample’, which is a very well known to be good adapted cultivar to the Nordic climate started flower initiation a week before any other cultivar. ‘Glen Ample’ have a lower temperature optimum to stop shoot growth and initiate flower primordia than the other cultivars, giving it more time to differentiate flowers and long laterals in buds along the majority of the shoot before entering dormancy. A longer period of time during this period will also increase the winter hardiness as mentioned above. Cultivars bred for a southern climate will respond too late in a Nordic climate, which will most likely result in a winter damaged plants.

Ambient out-door temperature and 15°C showed a big variation in some cultivars and smaller variation in others. ‘Veten’ showed the highest variation, were flower differentiation at 15°C were more advanced than at ambient temperature. This difference was also visible in the cultivars ‘Vene’ and ‘Balder’. This is most likely due to the influence of abiotic factors under ambient temperature condition, such as wind and fluctuating day/night temperature (Privé et al., 1993). The variation can also be a result of the method used in this study. The buds dissected throughout the experiment were collected from the top of the plant, but the shoot profiles show more floral development in buds on lower positions. It is also possible that the flower initiation signal in these cultivars moves differently along the shoot than reported previously for ‘Glen Ample’ and ‘Malling Promise’ (Williams, 1959c; Sønsteby & Heide, 2008; Woznicki et al., 2016)

Plants grown at 18°C showed no or very little flower development. Highest differentiation was observed in ‘Glen Ample’ and ‘Vene’. However, since they did not develop floral buds further than stage 2, it is most likely that this is the result of too detailed scoring. The response in 18°C should be equal to that reported by Sønsteby and Heide (2008), who found no flower initiation in ‘Glen Ample’ grown at 18°C with short or long photoperiod. The same applies for plants grown at 21°C were ‘Vene’ showed some flower development. This is very likely a result of too detailed scoring. ‘Vene’ did, however, tip-flower at 18°C in four out of five plants and three out of five plants at 21°C, in addition to one out of five plants of ‘Anitra’ at 18°C, but because the buds collected were taken several nodes below the terminal bud, this is not visible in the presented graphs. Tip-flowering is a plastic trait in some annual- and biennial-fruiting cultivars, and require favourable conditions, such as high temperature over a long period of time (Sønsteby & Heide, 2009). At 18°C and 21°C these requirements were met, making ‘Vene’ and ‘Anitra’ able to tip-flower. In Norway, this is very unusual, because temperature rarely stay above 18°C for a longer period

in late summer. However, last year tip-flowering was observed in ‘Vene’ under out-door conditions (Røen, 2017b, pers comm.). With a changing climate in both Scandinavia and Europe, warmer and longer seasons are a very likely scenario, making tip-flowering more probable in cultivars with this plastic trait.

5.1.3. Floral development along the entire shoot

The illustrations of floral development along entire shoot of all cultivars show the plant architecture at the end of the experiment. They all showed the same pattern as for growth cessation and flower initiation. Plants grown at 9°C had the most differentiated buds and ambient temperature showed less development compared to 15°C. Plants grown at 18°C showed, however, a slightly different pattern than expected. ‘Vene’ tip-flowered and had an expected development, but ‘Glen Ample’ and ‘Balder’ was expected to have no flower initiation at all as proved in ‘Glen Ample’ by Sønsteby and Heide (2008). ‘Glen Ample’ showed very little development in buds along the entire shoot and this might be a consequence of too detailed scoring of developmental stage. ‘Balder’ had, however, developed further and this is hardly caused by the scoring evaluation. Sønsteby and Heide (2008) did not find any flower initiation in ‘Glen Ample’ grown at 18°C with short or long photoperiod. They examined axillary bud number five, as indicate by Williams (1959b) to be one of the first buds to initiate flower primordia. As shown in Fig. 18, both ‘Glen Ample’ and ‘Balder’ have more or less vegetative buds between axillary bud number five and ten, while buds in lower positions are further differentiated. To understand better what happens in the plants at warmer temperatures, more research on raspberry grown at temperatures between 15°C and 21°C with different photoperiods is recommended.

Equal to their response in growth cessation and flower initiation, ‘Tulameen and ‘Schöneman’ showed a slower response at 15°C and ambient temperature. This is especially visible in ‘Tulameen’ grown at ambient temperature. If the level of differentiation is real or a result of too detailed scoring is hard to determine. At 15°C, ‘Tulameen’ had differentiated slightly further, but was still not above stage 2 in a majority of the buds. Nevertheless, since some buds were above a stage 2, and the development is visible in the collected buds, there must have been some activity. In ‘Schöneman’, the difference in response is less visible compared to ‘Tulameen’. At ambient temperature, ‘Anitra’ actually showed less differentiation than ‘Schöneman’, while the difference at 15°C was the opposite. All other

cultivars had differentiated more at 15°C compared to ambient temperature. Indicating that other factors besides temperature and photoperiod have affected flower differentiation at ambient temperature. 'Schöneman', however, showed more or less the same differentiation at 15°C and ambient temperature, indicating that 'Schöneman' is not easily influenced by other factors.

5.1.4. Italian cultivars

The Italian cultivars 'Vajolet' and 'Lagorai Plus' were very small at the beginning of the experiment, and are therefore kept separately in the results and discussion parts. Both 'Vajolet' and 'Lagorai Plus' are reported to be annual-fruiting cultivars (CommunityPlantVarietyOffice, 2012, 2016), but in this experiment their behaviour concerning growth cessation indicates that they are biennial-fruiting cultivars. However, both cultivars initiated flowers and showed differentiated flower buds at a stage of approximately 5 and 4.5, respectively, at the end of the experimental period in plants grown at 9°C. At this point 'Vajolet' and 'Lagorai Plus' had 12 and 14 nodes respectively, which is below the limit of 15 leaves of generative plants, as reported for the biennial cultivar 'Glen Ample' (Sønsteby & Heide, 2008). This indicates that both 'Vajolet' and 'Lagorai Plus' are annual-fruiting cultivars. Both cultivars would most likely have burst flowers and set fruit in all temperatures with time, if they had grown to have enough nodes as described by Faunt & Hall (2013). The number of nodes a biennial-fruiting cultivar must develop before being able to sense the environmental signals and enter the generative phase has only been studied in 'Malling Promise' (Williams, 1960) and 'Glen Ample' (Sønsteby & Heide, 2008). In contrast, Sønsteby and Heide (2008) found that the annual-fruiting cultivars 'Polka' responded to inductive conditions as early as the 5-leaf stage, and did not appear to have a juvenile phase. In other biennial-fruiting cultivars, this number of nodes separating a vegetative and generative phase can be different, and further research is recommended.

5.2. Field experiment

The presented results from the field experiment prove equal to the controlled environment experiment that growth cessation and flower initiation are jointly controlled by the interaction of short photoperiod and low temperature (Williams, 1959a, b, c, 1960; Sønsteby & Heide, 2009). Shoot growth started to level off in all cultivars when the photoperiod was approximately 15 hours, while complete growth cessation followed three

weeks later. Leaf development stopped later than shoot growth as shown by Williams (1959b). The first cultivars to cease growth completely were 'Glen Ample', 'Cascade Delight', 'Malling Juno' and 'Ninni', on September 7, followed by 'Veten' and RU90, on September 21. 'Anitra' did not cease growth during the experimental period, but the activity was minimal in the last week of registration and it was expected to stop the following week. It was also expected that 'Glen Ample' and 'Ninni' would cease growth early, because they are bred in Norway, and therefore well adapted to Nordic climate conditions. 'Cascade Delight' and 'Malling Juno' however, originates from the USA and the UK, respectively, and was expected to respond later. Their earliness shows that these are cultivars with high adaptability to different environmental conditions.

'Malling Juno' and 'Cascade Delight' are also among the cultivars to show earliest flower initiation and the fastest rate of flower differentiation, reaching a floral development stage of 4.5 and 3.9, respectively, by the end of the experiment. Further indicating that these cultivars might have a high adaptability to different environmental conditions. 'Glen Ample', 'Veten', 'Ninni' and 'Anitra' did also initiate flowers early, as expected, but only 'Veten' reached a developmental stage of approximately 4. RU90 was the latest to initiate flowers, three weeks after the first ones, and did consequently, not reach a development stage further than 2.3. Based on the results of this study, RU90 seems to be a late selection that might not be suitable for locations vulnerable for freezing temperature in early autumn. Considering that RU90 is only a selection and not yet a cultivar, further testing is recommended.

When comparing the cultivars that were included in both experiments, it is shown that plants in the field experiment ceased vegetative growth and initiated flowers earlier than plants in the controlled environment experiment. This can indicate that fluctuating temperatures gives a stronger signal in these cultivars, than constant low temperature. In addition, plants established in soil will be more sensitive to abiotic stress than plants in pots (one shoot in one pot, and always access to water and nutrients). Abiotic stress includes drought, flood, lack of nutrients, competition for assimilates, pests, diseases, etcetera. It is well known that plants subjected to stress are more sensitive to inductive signal, and will therefore, initiate growth cessation and floral initiation earlier than unstressed plants. The biggest difference was observed in 'Glen Ample', which ceased growth two weeks earlier in the field experiment. 'Veten' was one week earlier in the field experiment, but 'Anitra' showed no difference between field and controlled environment. The temperature throughout the experiment (Fig. 5) showed an average temperature of approximately 15°C until mid-

September when temperatures started to decrease. At the same time the photoperiod was approximately 13 hours (September 16). This might indicate that 'Anitra' have a critical photoperiod that is shorter than earlier shown for 'Glen Ample' (Sønsteby & Heide, 2008). It can also indicate that 'Anitra' needs longer time at an optimal temperature/photoperiod before initiating growth cessation. According to Säkö et al. (1980), it can also mean that 'Anitra' enters dormancy late, and will, therefore, show weaker winter hardiness than cultivars such as 'Balder' and 'Glen Ample' that enters dormancy early.

'Anitra' did also flower in the field experiment, but because the buds collected were below the flowering buds, or on non-flowering shoots, this is not presented in the figures. The reason why 'Anitra' flowered can be due to the plastic trait that made it susceptible to tip-flowering at long periods of high temperatures. The temperatures were unusually high in the autumn, and 'Anitra' did also tip-flower at 18°C in the phytotron experiment. However, 'Anitra' did not tip-flower before 10 weeks at 18°C in the controlled environment, making tip-flowering in out-door temperature less credible. Another possibility to explain why 'Anitra' tip-flowered in the field is that the flowering shoots could have originated from replacement buds. 'Anitra' was planted later (August) than the other cultivars in the field experiment, (June) in 2015. Replacement buds (dormant buds at the base of the annual shoots) may have been kept when removing shoots in the planting year, and sprouted and chosen when thinning the shoots in spring 2016. The late flowering might be due to late node development and floral differentiation in autumn before entering dormancy, and therefore, the floral differentiation process needed to be fulfilled in spring before flowering.

5.3. Scanning electron microscopy (SEM)

Because only few cultivars were available for scanning electron microscopy, and also that the cultivars available varied much in their developmental stages, it was difficult to make any conclusion about the flower morphology. However, 'Vajolet' differed from the other cultivars. It seemed to have more closed flowers than the other cultivars. 'Glen Ample', on the other hand, seems to have the most open flower. To study the difference in flower morphology in raspberry, more research is needed.

6. Conclusion

The results of this study with a wide range of raspberry cultivars, confirm that in biennial-fruiting raspberry, growth cessation and floral initiation are jointly controlled by the interaction of low temperature and short-day conditions. The process coincided in time in both natural and controlled environments. Cultivars well adapted to Nordic conditions will initiate growth cessation and flower initiation earlier than cultivars adapted to a warmer climate. By responding earlier to decreasing photoperiod at temperatures >15°C and being insensitive to photoperiod at temperatures <12°C, cultivars suited for Nordic climate, 'Glen Ample', 'Veten', 'Vene', 'Balder', 'Ninni' and 'Anitra' are able to initiate growth cessation and flower initiation earlier than cultivars originating further south. The plants with this adaptability will also develop a stronger cold hardiness, and better survive freezing temperatures. These cultivars also show a faster rate of flower differentiation than cultivars adapted to warmer climates, meaning that their optimal temperature for flower differentiation is lower. Because of more fluctuating temperatures during autumn in Nordic locations, a faster rate of differentiation is beneficial in order to avoid damaged caused by freezing temperatures.

In controlled environments, the plants grew continuously and remained vegetative at 21°C, but at 18°, they ceased growing and initiated floral primordia at the end of the experimental period. This result is not consistent with earlier research. Except for 'Vene', who has been reported to tip-flower before, only vegetative buds were expected to occur in plants at 18°C. However, dissection of all buds on entire shoots showed differentiated buds at a stage between 4 and 5 in a majority of the buds in both 'Vene' and 'Balder'. To get better knowledge of the critical temperature for floral induction in the 15-20°C range, more research is needed. 'Vene' tip-flowered at 18°C and 21°C, in addition to one of five plants in 'Anitra' at 18°C. This is caused by a plastic trait that makes these cultivars susceptible to flower in the top buds after longer periods of high temperatures. In a changing climate were warmer temperatures are predicted; tip-flowering is expected to happen more frequently in the future.

The two Italian cultivars showed growth behaviour equal to biennial-fruiting cultivars, but because the plants responded to inductive conditions, at the 12- and 14-leaf stage, this is not a common behaviour known for biennial cultivars and this may prove that they actually are annual cultivars. Both cultivars would most likely have flowered and set fruits in an extended period of time.

In the field experiment ‘Cascade Delight’ and ‘Malling Juno’ showed a promising behaviour for growing under Nordic climate. Further testing of these cultivars for production in a Nordic climate is highly recommendable.

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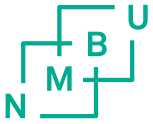

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