DISSERTATIONES SCHOLA DOCTORALIS SCIENTIAE CIRCUMIECTALIS,
ALIMENTARIAE, BIOLOGICAE. UNIVERSITATIS HELSINKIENSIS

17/2017

MARJA RANTANEN

Light and Temperature as Developmental Signals in Woodland Strawberry and Red Raspberry

DEPARTMENT OF AGRICULTURAL SCIENCES
FACULTY OF AGRICULTURE AND FORESTRY
DOCTORAL PROGRAMME IN PLANT SCIENCES
UNIVERSITY OF HELSINKI

LIGHT AND TEMPERATURE AS DEVELOPMENTAL SIGNALS IN WOODLAND STRAWBERRY AND RED RASPBERRY

Marja Rantanen

Doctoral School in Environmental, Food and Biological Sciences (YEB)
Doctoral Programme in Plant Sciences (DPPS)

Department of Agricultural Sciences
Faculty of Agriculture and Forestry
University of Helsinki

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Agriculture and Forestry, University of Helsinki, for public examination in lecture room 2, Info Centre Korona, Viikinkaari 11, Viikki on September 15th 2017, at 12 noon.

DISSERTATIONES SCHOLA DOCTORALIS SCIENTIAE CIRCUMIECTALIS, ALIMENTARIAE, BIOLOGICAE 17/2017

Supervisors:	Assistant professor Timo Hytönen Department of Agricultural Sciences University of Helsinki, Finland
	Doc., University lecturer Pauliina Palonen Department of Agricultural Sciences University of Helsinki, Finland
	Professor Paula Elomaa Department of Agricultural Sciences University of Helsinki, Finland
Follow-up group:	Doc. Sari Tähtiharju Dr. Annakaisa Elo
Reviewers:	Professor Kurt Fagerstedt Department of Biosciences Faculty of Biological and Environmental Sciences University of Helsinki, Finland
	Professor Jorunn Elisabeth Olsen Department of Plant Sciences Norwegian University of Life Sciences, Norway
Opponent:	Associate Professor Wim van Ieperen Department of Plant Sciences Wageningen University, the Netherlands
Custos:	Professor Paula Elomaa Department of Agricultural Sciences Faculty of Agriculture and Forestry University of Helsinki, Finland

ISSN 2342-5423 (print)
ISSN 2342-5431 (Online)
ISBN 978-951-51-3635-0 (paperback)
ISBN 978-951-51-3636-7 (PDF)
Electronic publication at http://ethesis.helsinki.fi
Hansaprint 2017

Cover: Over-expression 35S:*FvFT1* in perpetual flowering woodland strawberry ‘Hawaii’ results in very early flowering

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are hereafter referred to by their Roman numerals in this text:

I **Marja Rantanen**, Takeshi Kurokura, Panpan Jiang, Katriina Mouhu and Timo Hytönen. 2015. Strawberry homologue of TERMINAL FLOWER1 integrates photoperiod and temperature signals to inhibit flowering. The Plant Journal 82: 163-173.

II **Marja Rantanen**, Takeshi Kurokura, Katriina Mouhu, Paulo Pinho, Eino Tetri, Liisa Halonen, Pauliina Palonen, Paula Elomaa and Timo Hytönen. 2014. Light quality regulates flowering in *FvFT1/FvTFL1* dependent manner in the woodland strawberry *Fragaria vesca*. Frontiers in Plant Science 5: 271.

III Pauliina Palonen, Saila Karhu, Hanna Savelainen, **Marja Rantanen** and Olavi Junttila. 2011. Growth and cropping of primocane and biennial raspberry cultivars grown under a film absorbing far-red light. Journal of Horticultural Science & Biotechnology 86: 113-119.

AUTHORSHIP STATEMENT

In (I), MR designed and carried out the photoperiod and photoperiod-temperature interaction growth experiments. RNA extraction and RT-qPCR were performed and analyzed by MR. TK and PJ designed and carried out the high-low temperature experiment, RNA extractions and RT-qPCR of which were done by PJ. MR and KM carried out the statistical analyses. Manuscript was written by MR and TH with input of all the other authors. TH supervised the study.

In (II), MR designed the experiments together with TH, TK and KM. P.Pinho, ET and LH designed and constructed LED lighting. MR designed, produced and analyzed the Fv*FT1* over-expression transgenic lines together with KM. MR carried out the experiments, extracted RNA, performed RT-qPCR analyses and analyzed the data. Manuscript was written by MR and TH with input of all the other authors. TH, P.Palonen and PE supervised the study.

In (III), the experiments were designed by MR, PP, HS and SK. The growth experiments were carried out by MR and HS. HS analyzed the berry quality. OJ performed the gibberellin analysis. Statistical analyses were run by SK, PP and MR. Manuscript was written by PP, MR and SK. All the other authors contributed manuscript preparation. PP supervised the study.

ABSTRACT

Plants adjust their development by responding to temperature, photoperiod and light quality as indicators of season and the prevailing environment. Seasonal flowering strawberries (*Fragaria sp.*) and red raspberry (*Rubus idaeus*) are temperate species that are typically induced to flower under the short days (SD) that occur in the autumn. Temperature strongly influences the SD effects. Contrary to the seasonal flowering genotypes of these species, long photoperiods advance flowering in ever-bearing genotypes.

Woodland strawberry (*Fragaria vesca*) is a model plant for the garden strawberry (*F.× ananassa*) and woody *Rosaceae* plants. The floral repressor FvTERMINAL FLOWER1 (FvTFL1) in the woodland strawberry causes seasonal flowering. Under non-inductive long day (LD) activation of *FvFLOWERING LOCUS T1 (FvFT1) >FvSUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (FvSOC1) > FvTFL1*-pathway suppresses flowering. The objective of the research summarised in this thesis was to study how temperature regulates flowering in the woodland strawberry. In addition, the effects of light quality in the woodland strawberry and the tunnel-grown red raspberry were studied at the molecular and physiological levels.

Expression studies and transgenic plant approaches showed that FvTFL1 protein integrates the photoperiod and temperature signals in the woodland strawberry. At non-inductive high temperature *FvTFL1* is highly activated to suppress flowering. Other regulators than FvSOC1 are involved in the up-regulation of the *FvTFL1* gene: especially under SD conditions. The FvSOC1 dependent photoperiodic regulation of *FvTFL1* has a major role but only at intermediate temperatures. The down-regulation of the *FvTFL1* gene allows for flower induction at cool temperatures. The photoperiod response at intermediate temperatures was found to depend on light quality. Long days (LD) and day-length extension with far-red wavelength (FR) light substantially activated the expression of *FvFT1,* which correlated negatively with flowering in the seasonal flowering woodland strawberry. Under conditions of red wavelength (R) light and SD no *FvFT1* mRNA was detected though the plants were induced to flower. In the perpetual flowering accession, the effect of light quality on *FvFT1* expression was similar, but the flowering response was opposite. In the absence of functional FvTFL1 protein, the FvFT1 was found to mediate light quality signals and function as an LD dependent floral activator.

Light quality also affected flowering in the raspberry in a cultivar dependent manner. The use of an FR absorbing photoselective film led to increased number of flowers in primocane fruiting cultivars (LD plants) and decreased number of flowers in floricane fruiting cultivars (SD plants).

This study provides evidence that FvTFL1 has a central role in the integration of temperature and photoperiod signals in regulating flowering in the woodland strawberry. The light quality signal is mediated by *FvFT1*, but the floral responses of the SD and LD flowering genotypes are opposite and thus, depend on the presence of FvTFL1. The results suggest that the modification of a combination of temperature, photoperiod and light quality is a potential approach to use in controlling vegetative and generative growth in strawberry and raspberry.

Keywords: strawberry, ambient temperature, light quality, flowering

ABBREVIATIONS

AP1, FvAP1	*APETALA 1*, strawberry homolog of *AP1*
B	blue light
cDNA	complementary DNA
CO	*CONSTANS*
COP1	*CONSTITUTIVE PHOTOMORPHOGENIC1*
EB	ever-bearing
F.vesca	*Fragaria vesca*, woodland strawberry
F. × ananassa	*Fragaria × ananassa*, garden strawberry
FKF1	*FLAVIN-BINDING, KELCH REPEAT, F-BOX1*
FR	far-red light
FLC	*FLOWERING LOCUS C*
FT, FvFT1, FaFT1/3	*FLOWERING LOCUS T*, strawberry homologs of *FT*
FUL, FvFUL1	*FRUITFUL,* strawberry homolog of *FUL*
GA	gibberellin
GI	*GIGANTEA*
H2A	histone 2A
H2A.Z	histone 2A variant
H3K27me3	histone 3 lysine 27 trimethylation
HOS1	*HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1*
HPS	high pressure sodium lamps
IAA	Auxin, indole acetic acid
LD	long day
LED	light emitting diode
LKP2	*LOV KELCH Protein 2*
LOV	light-oxygen-voltage domain
miR156	microRNA 156
Perpetual, ever-bearing	strawberry accession that is an LD plant
PEP1	*PERPETUAL FLOWERING 1*
PFT1	*PHYTOCHROME AND FLOWERING TIME1*
PHL	*PHYTOCHROME DEPENDENT LATE-FLOWERING*
PIF4	*PHYTOCHROME INTERACTING FACTOR 4*
qPCR	quantitative real time polymerase chain reaction
R	red light
SD	short day
SFL	*SEASONAL FLOWERING LOCUS*

SOC1,	*SUPPRESSOR OF OVEREXPRESSION OF*
FvSOC1, FaSOC1	*CONSTANS1,* strawberry homologs of *SOC1*
SPA1	*SUPPRESSOR OF PHYTOCHROME A 1*
TFL1,	*TERMINAL FLOWER 1*,
FvTFL1, FaTFL1	strawberry homologs of *TFL1*
VRN1–3	*VERNALIZATION* genes 1–3
ZTL	*ZEITLUPE*

1 INTRODUCTION

1.1 Environmental signals in the control of flowering

Plants use photoperiod and temperature to adjust their growth in relation to the environment to optimize their survival and reproduction. Synchronization of the flowering within a species also impels the outbreeding and genetic recombination (Thomas and Vince-Prue 1997). Temperature and light quality treatments within the horticulture photoperiod are widely utilized in the timing of germination, growth and flowering. Garner and Allard (1920) were pioneers in studying the effects of photoperiod on flowering. The physiological significance of photoperiod and temperature for the perennials, and the role of leaves in the photoperiodic signaling were reported some decades later (Wareing 1956). A few model plants, mainly the annual long day (LD) plant *Arabidopsis thaliana* (L.) Heynh and a short day (SD) plant rice (*Oryza sativa* L.) have been subjected to molecular level studies. These studies have identified genetic pathways activated by age, gibberellin, vernalization, light quality, ambient temperature or photoperiod that regulate flower induction. The genetic pathways converge in the *FLOWERING LOCUS T (FT)*, which functions as a universal floral signal in plants (Corbesier et al. 2007; Andrés and Coupland 2012; Taoka et al. 2013). Although the control of flowering in annual plants has been intensively studied, the research into the molecular basis of the physiological responses in perennial plants is still under way to increase our understanding.

1.1.1 Photoperiod and light quality as regulators of flowering

Photoperiod is a predictable and reliable signal to indicate the time of the year. Garner and Allard (1920) studied flowering in a wide selection of plants and introduced the terminology of short day (SD) and long day (LD) plants according to the respective plant's inductive photoperiod i.e. the number of hours of daylight associated with the induction of flowering. Although short and long night plants would be a more exact terminology as stated by Borthwick et al. (1952) it is the length of the dark period that is definitive for flower induction. Garner and Allard (1920) set the boundary between SD and LD plant groups simply at 12 hours. The interval of 12 hours or less was inductive for SD plants, whereas an interval of 12 hour or more for LD plants. Many SD plants were later found to flower within a photoperiod that ranged

from 12 to 14 hours, i.e. nominally outside the optimum period for flowering in SD plants (Garner 1933). The inductive day length is rarely 12 hours in either group, and therefore the critical photoperiod describes photoperiodic sensitivity better (Thomas and Vince-Prue 1997). The limits for the critical photoperiod for some plant species can be very clearly defined (Garner 1933) and flower induction may even require both SD and LD to occur in a certain order (Thomas and Vince-Prue 1997). In contrast, the flower induction of what is termed the day neutral plants is independent of the photoperiod.

The sensitivity to the floral signal is diverse among plants. In general, the length of the photoperiod is a more important factor than the intensity of light. Thus, the day extension in the end of the photoperiod or night break in the middle of the dark period with low irradiation incandescent light is sufficient to induce or repress flowering in photoperiod sensitive plants (Garner and Allard 1920). The number of inductive cycles required for the flower induction varies from one cycle in some annual plants to several weeks in perennials (Hartmann 1947; Williams 1960; Hempel et al. 1997; Heide and Sønsteby 2007). Japanese morning glory *(Pharbitis nil* (L.) Choisy) and cocklebur (*Xanthium pensylvanicum* Gand.) are two examples of very responsive SD species in which a night break of as little as five minutes may prevent flower induction (Fredericq 1964; Borthwick and Downs 1964). The LD species tend to be less responsive. A night break that lasts from 30 minutes to 2 hours induces flowering under SD conditions depending on the LD species (Lane et al. 1965; Vince 1965; Runkle et al. 1998).

Light quality as defined by the specific wavelength spectra of light that irradiates plants has an important role in the floral response. In many LD species the far-red (FR) and blue (B) wavelengths of light promote flowering, whereas red (R) has the opposite effect (Brown and Klein 1971; Martinez-Zapater and Somerville 1990; Bagnall 1995; Guo et al. 1998). At the end of a natural day, a day extension treatment with low intensity R or FR light can be as short as 15 minutes to affect the flowering (Lane et al. 1965; Vince 1965; Runkle et al. 1998). Furthermore, in many species the effect of R-FR light pulse series depends on the last light pulse given (Downs 1956; Cathey and Borthwick 1957; Olsen and Junttila 2002). Reversible R/FR light responses, however, have a limited time window (Takimoto and Hamner 1965; Thomas and Vince-Prue 1997). For example, the same treatment given at the beginning of photoperiod to *Arabidopsis,* a LD plant, and the garden

strawberry (*Fragaria ×ananassa* Duch.) a SD plant may not affect flowering (Vince-Prue and Guttridge 1973; Goto et al. 1991).

The ratio of R to FR wavelengths under natural light conditions is close to 1. Photosynthetic pigments absorb R and B wavelengths, and thus the FR wavelength light is greatly enhanced under the canopy, which decreases the R:FR ratio (Smith 1982; Sellaro et al. 2012). Plants can either tolerate or avoid shade (Smith 1982; Gommers et al. 2013). A low R:FR ratio for shade avoiding plants is a signal of insufficient irradiation that has to be eluded (Smith and Whitelam 1997). Shade avoiding plants respond to low R:FR ratios by enhanced petiole and stem elongation, hypocotyl growth, light green leaves and accelerated flowering (Halliday et al. 1994; Smith and Whitelam 1997).

1.1.2 Photoreceptors

Light activates photoreceptors that control gene expression through protein interaction, stabilization and destabilization of specific transcription factors in the plant. Phytochromes, cryptochromes and LOV-domain photoreceptors have been found to mediate light signals to regulate flower induction (Guo et al. 1998; Mockler et al. 2003; Takase et al. 2011).

Phytochromes, photoreceptors of R and FR light are dimeric chromoproteins that contain a linear tetrapyrrole phytochromobilin as a light absorbing chromophore (Quail 2002; Takano et al. 2009; Möglich et al. 2010; Strasser et al. 2010). Phytochromes exist in two conformational forms, biologically inactive P_r and active P_{fr} that respectively absorb R and FR light (Butler et al. 1959). The R light converts P_r into P_{fr} that is subsequently imported into the nucleus and interacts with putative target molecules (Smith 2000; Kircher et al. 2002; Wang and Wang 2015). When P_{fr} absorbs FR light it is converted back to the inactive P_r form. This conversion also occurs under darkness, but at a much slower rate than under FR light (Li et al. 2011).

In *Arabidopsis* five (*PhyA- PhyE*) and in rice three (*PhyA- PhyC*) genes encode phytochrome proteins (Sharrock and Quail 1989; Clack et al. 1994; Basu et al. 2000). PhyA and PhyB in *Arabidopsis* are dominant phytochromes at various stages of plant development (Reed et al. 1993; Johnson et al. 1994; Devlin et al. 1996; Smith 2000). PhyA mediates the FR signal to advance flowering (Reed et al. 1994; Devlin et al. 1996; El assal. 2003; Mockler et al. 2003), whereas PhyB has a contrasting effect on flower induction (Halliday et al. 1994; Smith and Whitelam 1997; Blázquez and

Weigel 1999). PhyC mainly affects the vegetative growth in *Arabidopsis* (Franklin et al. 2003; Monte et al. 2003) but in wheat (*Triticum aestivum* L.) and another monocot *Brachypodium distachyon* PhyC is required to activate photoperiodic flowering (Chen et al. 2014; Woods et al. 2014).

Blue wavelength light specific photoreceptors were found much later than the phytochromes despite their essential role in plant photomorphogenesis. A plant's cryptochromes and light-oxygen-voltage domain (LOV) photoreceptors such as phototropins and ZTL-proteins mediate the B-light signals (Möglich et al. 2010). Cryptochromes contain a flavin adenine dinucleotide (FAD) chromophore that absorbs B and UV-A part of the spectrum (Lin et al. 1995; Cashmore et al. 1999; Lin 2000) and mediate the blue:green ratio signals (Sellaro et al. 2010). Two genes *CRYPTOCHROME1* (*Cry1*) and *CRYPTOCHROME2* (*Cry2)* encode cryptochromes in *Arabidopsis*. They both promote flowering although the role of Cry2 is more pronounced (Guo et al. 1998; Mockler et al. 1999; El-Assal et al. 2003; Liu et al. 2008a; Liu et al. 2008b).

The proteins of the ZTL-family in *Arabidopsis,* namely: FLAVIN-BINDING, KELCH REPEAT, F-BOX1 (FKF1), LOV KELCH Protein 2 (LKP2) and ZEITLUPE (ZTL), all have the prerequisite photoreceptor characteristics (Ito et al. 2012). FKF1, LKP2 and ZTL mediate photoreceptor activity through the LOV domain that binds to the flavin mononucleotide chromophore (Christie et al. 1999; Nelson et al. 2000; Salomon, 2000; Imaizumi et al. 2003). B-light causes conformational change in FKF1 photoreceptor, but dark reversion is slower than in the phytochromes, and the excited form probably remains in that state until it becomes degraded (Ito et al. 2012). All three members of the family are involved in the regulation of flowering and the circadian clock (Somers et al. 1998; 2000; 2004; Imaizumi 2003; Takase et al. 2011).

1.1.3 Photoperiod and light quality in flowering –molecular aspects

The photoperiodic regulation of flowering is well known in the model plant *Arabidopsis* that is a quantitative LD plant. The regulation of flowering by light in *Arabidopsis* involves multiple factors that are dependent on both light and darkness periods, which affect the transcriptional, translational and post-translational levels. In addition to the photoperiod, the light quality regulates flowering by affecting the genes of the photoperiod pathway or specific light quality pathway (Simpson and Dean 2002; Cérdan and Chory 2003).

The external coincidence model explains photoperiodic flowering in *Arabidopsis* under LD conditions. The key components of the model are rhythmic expression of the *CONSTANS* (*CO*) gene and the stabilization of the resulting CO protein by light; especially at B and FR wavelengths (Putterill et al. 1995; Suarez-Lopez et al. 2001; Yanovsky and Kay 2002; Cérdan and Chory 2003; Valverde et al. 2004; Fig 1). *CO* mRNA expression peaks in the late afternoon, and thus under SD conditions *CO* expression occurs largely under darkness, which destabilizes the expressed CO protein (Suarez-Lopez et al. 2001; Yanovsky and Kay 2002). The CO protein therefore accumulates only under LD conditions (Suarez-Lopez et al. 2001; Valverde et al. 2004). The CO protein in the leaf phloem activates the *FLOWERING LOCUS T (FT)* the mobile flower induction signal (Samach et al. 2000; Suarez-Lopez et al. 2001; Yoo et al. 2005; Tamaki et al. 2007; Corbesier et al. 2007). The FT protein is transported to the shoot apical meristem (Corbesier et al. 2007) where it forms a protein complex with a bZIP domain transcription factor FLOWERING LOCUS D (FD) (Abe et al. 2005; Wigge et al. 2005; Andrés and Coupland 2012). In consequence, the meristem identity genes *LEAFY*, *APETALA1* and *FRUITFULL* are activated resulting in flowering (Mandel and Yanovsky 1995; Hempel et al. 1997*;* Abe et al. 2005; Wigge et al. 2005).

The transcription of the *CO* gene is regulated by several factors (Fig. 1A). CYCLING DOF FACTORs (CDFs) bind directly onto a *CO* promoter to repress transcription in the morning (Imaizumi et al. 2005; Fornara et al. 2009). The expression of the *CO* gene is enabled in the afternoon because the circadian regulated GIGANTEA (GI) and FLAVIN-BINDING, KELCH REPEAT, F-BOX1 (FKF1) proteins form a complex that degrades CDF1 (Park et al. 1999; Nelson et al. 2000; Imaizumi et al. 2003; 2005; Sawa et al. 2007; Fornara et al. 2009). B wavelengths enhance the formation of the quaternary

complex because FKF1 interacts with GI only under B or white light (Sawa et al. 2007, Song et al. 2012).

At the protein level, the duration of light (photoperiod) and light quality affect the stability of the CO protein (Fig 1B). Irradiation by R light in the morning destabilizes the CO protein through the action of phyB in (Valverde et al. 2004; Song et al. 2012). Ubiquitin ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 1 (HOS1) also destablizes the CO protein especially under R light conditions, and therefore the formation of the phyB-HOS1-CO complex has been suggested to be the major step in carrying out this destabilization (Lazaro et al. 2012; 2015) whereas the function of phyB is suppressed by PHYTOCHROME DEPENDENT LATE-FLOWERING (PHL) (Endo et al 2013). Under darkness CO is degraded by a complex formed from ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC (COP1) and SUPPRESSOR OF PHYTOCHROME A 1 (SPA1) (Laubinger et al. 2006; Jang et al. 2008; Liu et al. 2008b). In addition, phosphorylation may also expose the CO protein for degradation under darkness and R light (Sarid-Krebs et al. 2015). On the other hand, B wavelength light has an important role in the stabilization of CO that allows the accumulation of the protein. LD and specifically B-light enhance the interaction of FKF1 and CO protein to stabilize CO in the afternoon (Song et al. 2012). Furthermore, B-light activates Cry2 to suppress the formation of SPA1-COP1 complex (Zuo et al. 2011).

The responses to the photoperiodic cues in plant species that flower under SD conditions are different from those of the LD flowering *Arabidopsis* model. Many genes of the photoperiodic pathway are conserved between *Arabidopsis* and SD plant rice (Yano et al. 2000; Hayama et al. 2003). *Heading date 1 (Hd1)* and *Heading date 3a* (*Hd3a*) are orthologues of *Arabidopsis CO* and *FT*, respectively (Tamaki et al. 2007; Komiya et al. 2009). The expression of *Hd1* is low around noon but peaks at 16 hours after dawn independently of the photoperiod (Yano et al. 2000; Hayama et al. 2003). In contrast to the *CO* gene, *Hd1* expression has to coincide with darkness to activate *Hd3a* gene, which induces flowering in the shoot apical meristem (Yano et al. 2000; Hayama et al. 2003; Tamaki et al. 2007; Komiya et al. 2009; Ishikawa et al 2011; Taoka et al. 2011).

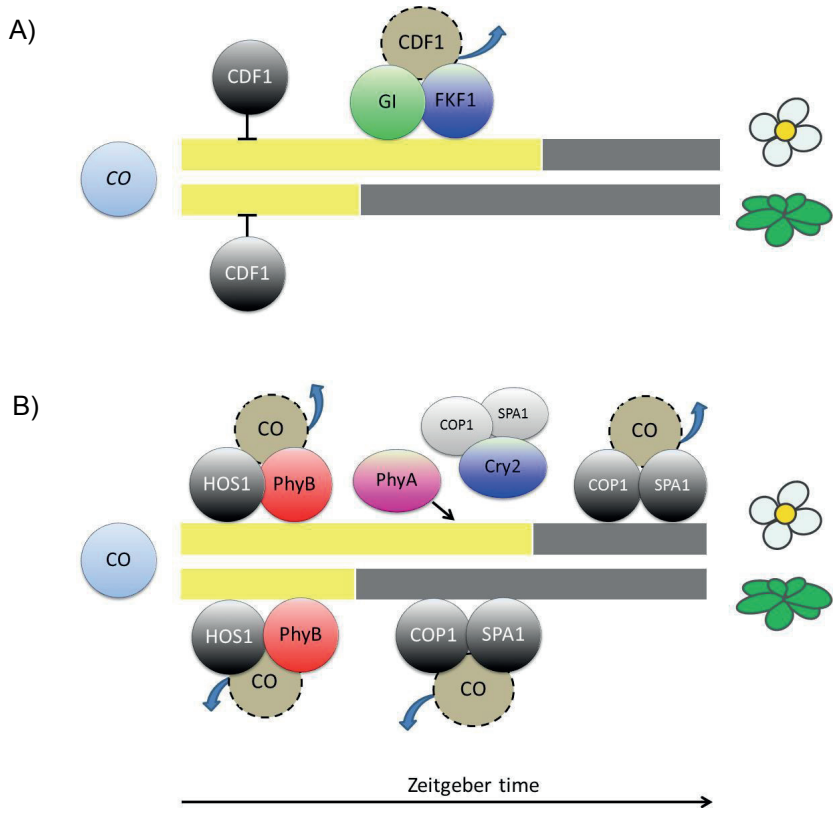

Fig 1. Regulation of *CO* transcription (A) and the subsequent regulation of CO protein (B) are the basis of the external coincidence model that explains photoperiodic flowering in *Arabidopsis*. A) CDF1 represses the transcription of *CO* in the morning. In the afternoons, GI and FKF1 proteins form a complex in LD plants that degrades CDF1 thus allowing *CO* transcription to occur.
B) In the morning HOS1 and PhyB form a complex that destabilizes the CO protein in LD plants. Another quaternary complex COP1-SPA1 degrades the CO protein under darkness. Light and especially FR and B light mediated by PhyA and Cry2, respectively, stabilize the CO protein. Thus, the accumulation of CO required for activation of FT, the moving flower inducing signal, only occurs under LD, which results in flowering. Bar=repression, arrow=stabilization, circle with dashed line=unstable protein. Modified from Andrés and Coupland (2012).

Although the photoperiod is a seasonal indicator, the light quality carries qualitative information about the local environment such as shade and cloud cover. The actions of PhyB and other stable phytochromes have been suggested to comprise a specific light quality pathway that regulates flowering (Simpson and Dean 2002). PHYTOCHROME AND FLOWERING

TIME1 (PFT1), a nuclear protein, has been proposed to mediate light quality signals downstream of phyB in order to regulate FT independently of the CO protein (Cérdan and Chory 2003; Iñigo et al. 2012). In contrast, Wollenberg et al. (2008) reported that PFT1 function under FR enriched light requires CO.

1.1.4 Temperature as a floral signal

Photoperiod as a stable seasonal indicator does not reflect the growing conditions of the respective growing season. Temperatures may vary highly between seasons. The combined effect of temperature and photoperiod therefore has a special role for the plant development and also the timing of flowering in nature (Garner 1933).

The vernalization pathway

Exposure of plants to chilling temperatures such as those in winter is a prerequisite for some plants to flower. To subject a plant artificially to such a cold exposure treatment is known as 'vernalization' (Chouard 1960). Winter annual accessions of *Arabidopsis* and related perennial species such as *Arabis alpina* L. and *Cardamine flexuosa* With. require vernalization to enable the induction of flowering. After vernalization, the epigenetic and stable down-regulation of the floral repressor *FLOWERING LOCUS C (FLC)* enables flower induction in winter annual *Arabidopsis* (Michaels and Amasino 1999). Trimethylation of lysine 27 in DNA-packaging histone protein H3 (H3K27me3) is the major silencing system that targets mainly transcription factors in *Arabidopsis* (Zhang et al. 2007). Vernalization changes H3K27me3 status in *FLC* chromatin, which is thought to recruit repressors that assist in the maintenance of a stable repression of *FLC* (Bastow et al. 2004; Zhang et al. 2007).

Vegetative and generative phases alternate in the life cycle of perennial plants, which requires the resetting of the signaling cascade (Adrian et al. 2009). Vernalization in *A. alpina* down-regulates *PERPETUAL FLOWERING 1 (PEP1),* the orthologue of *FLC* via the increased histone modification H3K27me3 in the *PEP1* locus (Wang et al. 2009b). The temporal down-regulation of *PEP1* makes *A. alpina* competent for LD induced flowering. After vernalization the level of histone modification is decreased, which allows *PEP1* transcription and which promotes vegetative development again (Wang et al. 2009b).

The FLC1 and PEP1 proteins are not the only regulators of vernalization. Generative development is prevented in the first growing season in biennial crops such as sugar beet (*Beta vulgaris* L.) and winter cereals. The flowering of the sugar beet is controlled by two antagonistic homologs of *FT*, *BvFT1* and *BvFT2* (Pin et al. 2010). Before vernalization *BvFT1* down-regulates *BvFT2* (Pin et al. 2010). The gradual down-regulation of *BvFT1* during vernalization in the winter allows the expression of *BvFT2* under the lengthening days of the following spring, which activates flowering (Pin et al. 2010). Flowering in winter wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) is controlled by three *VERNALIZATION* genes (*VRN1, VRN2, VRN3*) (Trevaskis et al. 2007; Distelfeld et al. 2009). The floral repressor gene *VRN2* maintains the vegetative phase in the autumn until vernalization induces the expression of *VRN1*, which is a meristem identity gene encoding a transcription factor that down-regulates *VRN2*. The lengthening days in the spring up-regulate *VRN3* in the absence of *VRN2*, the orthologue of *FT* that further activates the expression of *VRN1*.

The perennial lifecycle also sets a demand for sufficient vegetative growth that is achieved by a combination of age and vernalization that is dependent on regulation (Zhou et al. 2013). The phase change of vegetative juvenile into the vegetative adult plant that is competent for subsequent generative development requires the activation of several signal transduction pathways (Poethig 2003). The miR156 entity is a non-coding RNA molecule (Zhang et al. 2006) that has been observed to control the stage at which *C. flexuosa* and *A. alpina* are responsive to vernalization (Bergonzi et al. 2013). Furthermore, miR156 regulates the phase change from juvenility to the adult vegetative phase in the hybrid poplar (*Populus* × *canadensis*) (Wang et al. 2011). Expression analysis in several woody species further indicated that miR156 may have a wide role in the regulation of juvenility in woody species (Wang et al. 2011).

The ambient temperature pathway

The ambient temperature pathway refers to flowering that is accelerated at a higher temperature range; especially under SD conditions. The first research reports related to flowering found that the effects of temperature were connected to the phenomenon that the metabolic reactions are retarded at lower temperatures (Garner and Allard 1920). Ambient temperature was found to mediate the flowering pathway through two genes *Flowering time control protein FCA* and *Transducing family protein FVE* that were

originally part of the vernalization pathway (Martinez-Zapater and Somerville 1990; Koornneef et al. 1991) but which were later observed to affect the flowering at elevated temperatures through *SHORT VEGETATIVE PHASE (SVP*) (Blázquez et al. 2003; Lee et al. 2007).

Ambient temperature signals are integrated in *FT* (Balasubramanian et al. 2006). The temperature change from 16 to 23°C (Blázquez et al. 2003, Lee et al. 2007) and a further change from 22 to 27°C highly activates the *FT* mRNA expression, which causes early flowering (Kumar et al. 2012, Lee et al. 2013). The increased expression of *FT* is dependent on the interaction between two MADS-box proteins that are encoded by floral repressor genes *SVP* and *FLOWERING LOCUS M (FLM)* (Scortecci et al. 2001; 2003; Lee et al. 2007; 2013; Posé et al. 2013, Fig 2). The *FLM* gene is alternatively spliced, which results in the temperature dependent splice variant proteins FLMβ and FLMσ being expressed (Scortecci et al. 2001). At cool temperatures FLMβ is the more abundant protein, whereas the quantities of FLMσ increase along with the rise in temperatures (Lee et al. 2013; Posé et al. 2013). SVP forms complexes with both FLM splice variants with the same affinity (Lee et al. 2014) but only the SVP-FLMβ variant binds to the promoter of *FT* and thus represses the transcription specifically at cool temperatures (Lee et al. 2013). Furthermore, SVP is more vulnerable to degradation at high temperatures (Lee et al. 2013). It is probable that both the prevalence of FLM-variants and the temperature dependent stability of SVP protein results in accelerated flowering at high temperature (Capovilla et al. 2015).

The accessibility of DNA affects the gene expression. DNA often occurs as nucleosomes which are repeating subunits of chromatin where DNA coils around histones that consist of the histone octamer. The canonical histone in the nucleosome may be replaced with a histone variant, which affects the nucleosome structure and stability (Talbert and Henikoff 2010; Jarillo and Pineiro 2015). Histone modifications and specifically H2A.Z has been suggested to regulate temperature mediated by transcription (Kumar and Wigge 2010). At cool temperatures, the H2A.Z wrap DNA tightly and this nucleosome structure forms a physical barrier to the access to chromatin of the RNA polymerase. In contrast, the occupancy of H2A.Z is decreased at higher temperature that allows transcription (Kumar and Wigge 2010).

PHYTOCHROME INTERACTING FACTOR 4 (PIF4) is a putative target of regulation by the alternative histone H2A.Z (Proveniers and van Zanten 2013, Fig 2). *PIF4* affects plant architecture as a response to ambient temperature (Koini et al. 2009) and promotes early flowering in *Arabidopsis*

under SD (Kumar et al. 2012). The over-expression of *PIF4* under constitutive *35S*-promoter highly increased the expression of *FT* at 22°C. However, accelerated flowering was abolished at 12°C. Kumar et al. (2012) concluded that at cool temperature the PIF4 protein is unstable and the presence of H2A.Z decreases binding of PIF4 onto the *FT* promoter. Therefore, the eviction of H2A.Z at high temperature enables the high expression of *FT*.

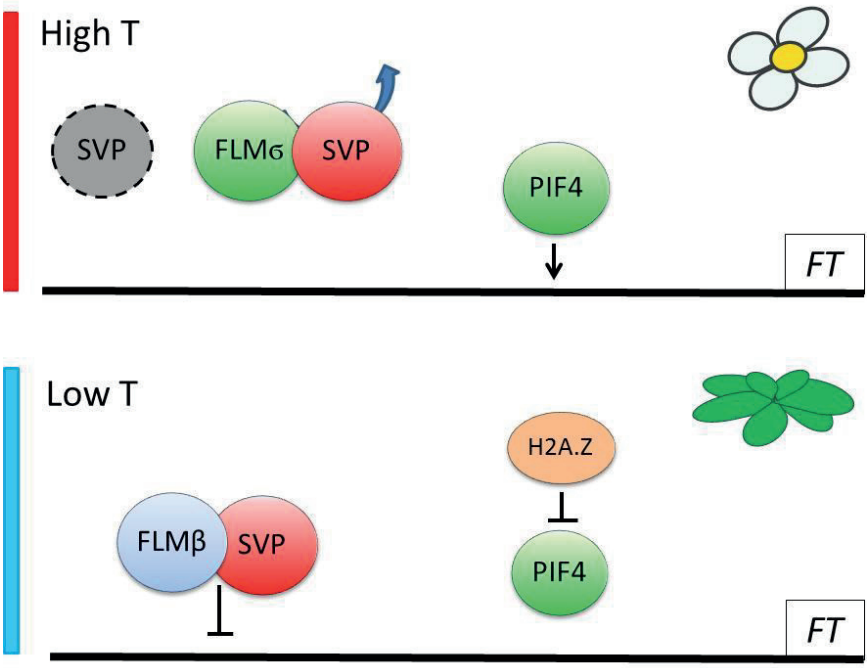

Fig 2. High temperature accelerates flowering in *Arabidopsis*. FLM is alternatively spliced; FLMβ is more abundant at low temperature and forms a complex with SVP to repress *FT* transcription. H2A.Z inhibits the binding of promoter onto *FT* chromatin. However, at high temperature H2A.Z is evicted and PIF4 enhances transcription of *FT*. At high temperature FLMσ variant forms the complex with SVP, but FLMσ-SVP complex does not bind onto *FT*. The SVP protein is unstable at high temperature. The PIF4 promotes transcription of FT at high temperature, but at low temperature H2A.Z binds to the chromatin and represses binding of PIF4 onto the *FT* promoter. The dashed circle=unstable protein, bar=repression, arrow=promotion. Modified from Andrés and Coupland (2012).

1.1.5 The strawberry plant and flowering

Strawberries (*Fragaria* sp.) belong to the widespread family of rose plants (*Rosaceae*). Wild strawberry species have spread to the northern hemisphere, and parts of South America (Liston et al. 2014). The garden strawberry (*Fragaria* x *ananassa*) is a hybrid of two wild, octoploid species, beach strawberry (*Fragaria chiloensis* Georgi) and the virginia strawberry (*Fragaria virginiana* Duch.), which are native to North America, and to Chile in South America (Darrow 1966; Liston et al. 2014). Strawberry has significant economic importance in the world with the production of 8.1 Million tons in 2014 (FAOSTAT Crops). In Finland strawberry is the most significant berry crop: 14 000 Tons were grown in an area of 3385 ha in 2015 (Horticultural Statistics 2017). The current economic value of strawberries motivates and maintains a need for breeding of improved varieties and also creates a demand for molecular tools (Longhi et al. 2014). The diploid woodland strawberry (*F. vesca*) and octoploid garden strawberry have similar regulation of flowering. An efficient transformation method and publication of the genome in 2010 have resulted in the woodland strawberry becoming a model plant not only for the garden strawberry but also for the whole *Rosaceae* family (Oosumi 2006; Shulaev 2011).

1.1.6 Flowering physiology of the strawberry

Strawberries are perennial rosette plants. Their internodes are short, and they have a thick stem, and a crown that is formed close to the soil (Darrow 1966). Lateral buds in the leaf axis of the crown may remain dormant, but when they are not, they form a side crown or develop into stolons that are called runners. The first two internodes in the runner elongate and this is followed by the emergence of short nodes that form a crown of a daughter plant. The growth habit of strawberries is sympodial. When the plant turns generative, the apical meristem develops into a terminal inflorescence and the vegetative growth is continued from the uppermost axillary bud (Brown and Wareing 1965). Axillary buds near the shoot apical meristem usually form a side crown that may become generative. Growth of side crowns continues independently of the main crown and follows the same pattern.

Woodland strawberry and the garden strawberry are SD plants. Growth is vegetative under long day conditions and during the high temperatures of summer (Brown and Wareing 1965). During the vegetative phase the axillary buds develop into runners. Towards late summer runner production is decreased, and the shortening days favour the formation of branch crowns

(Darrow and Waldo 1934). The shortening photoperiod and falling temperature initiate the flower induction. Floral development occurs first in the apical meristem and spreads into other meristems (Darrow and Waldo 1934; Brown and Wareing 1965). The flower initials develop as long as environmental conditions remain favourable for growth (Darrow and Waldo 1934; Jahn and Dana 1970). The longer this period is, the higher the number of flowers that will develop (Darrow and Waldo 1934). Inflorescences grow out in the following spring and after fruiting vegetative growth continues until the next generative cycle starts.

The regulation of flowering in the garden strawberry and the woodland strawberry is similar. The interaction of temperature and photoperiod is pronounced in both species and is especially apparent in the garden strawberry cultivars that are adapted to the northern climate (Darrow and Waldo 1934; Heide 1977; Sønsteby and Nes 1998; Sønsteby and Heide 2007b; Heide et al. 2013). Under SD conditions the optimal temperature for the development of the flower initials in both species is in the 15–18°C range (Heide 1977; Sønsteby and Nes 1998; Heide and Sønsteby 2007). Under conditions of cool temperatures and SD the requirement attenuates until it is lost and the flower induction occurs independently of the photoperiod. However, some garden strawberry cultivars are also obligatory SD plants at low temperature (9°C) (Heide et al. 2013). In contrast, high temperature suppresses flower induction. The woodland strawberry remains vegetative at temperatures exceeding 21°C (Heide and Sønsteby 2007), whereas induction of flowering at 24°C is still possible in the garden strawberry, though the number of flowers is drastically decreased (Heide 1977, Bradford et al. 2010).

Perpetual flowering, ever-bearing (EB) forms exist both in the woodland strawberry and the garden strawberry. Perpetual flowering woodland strawberry accessions and EB garden strawberry cultivars are quantitative LD plants at intermediate temperatures (Sønsteby and Heide 2007a; 2007b; 2008b). After the formation of a few nodes the apical meristem becomes generative while vegetative growth continues from the uppermost axillary bud. The flowering continues throughout the summer under LD conditions (Brown and Wareing 1965; Sønsteby and Heide 2007a; 2007b; 2008b). The interaction of temperature and photoperiod regulates flower induction in EB strawberries, which is similar to that of seasonal flowering accessions. The LD response becomes stronger and finally essential for flower induction along increased temperature (Sønsteby and Heide 2007b). SD combined with high temperature increases vegetative growth in both species and may

induce runner production in genotypes that do not produce runners at intermediate temperatures (Sønsteby and Heide 2007a; 2007b; 2008b).

The beach strawberry (*F. chiloensis*), one of the ancestors of the garden strawberry grows over a wide latitudinal area from Alaska to Chile. Populations of the other ancestor, the virginia strawberry are found in an area whose southern boundary is the western parts of Louisiana and Georgia and northernmost boundary in Alaska (Darrow 1966; Liston et al. 2014). Sønsteby and Heide (2008c; 2009a) studied the flowering regulation of both species using natural accessions originating from latitudinal extremes of the distribution area. All beach strawberry populations were shown to be principally SD plants at 15 and 21°C, but similarly to the woodland strawberry, they can flower independent of photoperiod at 9°C. High temperature in general inhibits flowering of the beach strawberry. The virginia strawberry is sometimes classified as a day-neutral strawberry (Serçe and Hancock 2005). However, eight of nine virginia strawberry populations have been shown to be quantitative SD plants, and the optimum temperature range for flower induction for the virginia strawberry ranges between 15 and 21°C (Sønsteby and Heide 2008c). Suppression by temperature seems to be connected to the geographical origin; flowering of low altitude populations of the virginia strawberry was not affected by temperature (Sønsteby and Heide 2008c).

1.1.7 Molecular control of flowering in strawberry

Brown and Wareing (1965) used the classical crossing method to study the inheritance of flowering habit and the formation of runners in the woodland strawberry. The generative and vegetative development correlate negatively in the seasonally flowering woodland strawberry. However, crossings of seasonal flowering accession × perpetual flowering, non-runnering accession showed that the flowering habit and the formation of runners are controlled by two genes that segregate separately. Seasonal flowering was found to be caused by a dominant allele of the single gene *SEASONAL FLOWERING LOCUS* (*SFL*) (Brown and Wareing 1965; Cekic et al. 2001; Albani et al. 2004). Quite recently *SFL* was identified as a homolog of *Arabidopsis TERMINAL FLOWER 1* (*FvTFL1)* (Koskela et al. 2012). The two base-pair mutation in *FvTFL1* in the perpetual flowering accessions results in non-functional protein and a loss of seasonal flowering (Koskela et al. 2012).

Recent studies have elucidated a molecular model for photoperiodic regulation of flowering in the strawberry (Fig. 3). The LD woodland strawberry highly up-regulates *FvFT1* (Koskela et al. 2012). On the other hand, when *FvFT1* is silenced (*FvFT1* RNAi), the expression of a homolog of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*FvSOC1)* is down-regulated (Mouhu et al. 2013). The *FvSOC1* gene in the seasonal flowering woodland strawberry is highly expressed at constant intermediate temperature (18°C) under LD, but under inductive SD the expression is decreased. Mouhu et al. (2013) demonstrated that *FvSOC1* regulates *FvTFL1*. Silencing of *FvSOC1* down-regulated *FvTFL1* and resulted in photoperiod independent flowering in the woodland strawberry. These observations suggest that under LD conditions *FvFT1*>*FvSOC1*>*FvTFL1* pathway is highly activated to repress flower induction until inductive SD gradually leads to decreasing expression of *FvTFL1* in the shoot apical meristem, and the lowered expression allows flower induction (Koskela et al. 2012; Mouhu et al. 2013). LD activates both *FvFT1* and *FvTFL1*, thus the expression of *FvFT1* correlates negatively with flower induction in the seasonal flowering woodland strawberry (Mouhu et al. 2013). In the perpetual flowering accession 'Hawaii-4' in the absence of the functional FvTFL1 protein, the *FvFT1* gene strongly correlates with flowering, whereas the FvSOC1 protein has only a weak promotive effect (Koskela et al. 2012; Mouhu et al. 2013). In both genotypes up-regulation of the meristem identity genes *FvAP1* and *FvFUL1* is detected at the time of flower induction (Koskela et al. 2012; Mouhu et al. 2009; 2013).

Recent reports by Nakano et al. (2015) and Koskela et al. (2016) have elucidated the regulation of flowering in the garden strawberry at the molecular level and both studies reported similarities to woodland strawberry. *FaFT1* is expressed in the leaves only under LD conditions (Nakajima et al. 2014; Nakano et al. 2015; Koskela et al. 2016). Interestingly, inductive conditions, SD and low temperatures, activate *FaFT3* expression in the shoot tips indicating that *FaFT3* may have an important role in the flower induction (Nakano et al. 2015). The *FaSOC1* gene is expressed in the apical meristem under LD as in woodland strawberry, but *FaSOC1* is down-regulated after two weeks of SD treatment (Koskela et al. 2016). The expression of *FaTFL1* correlates negatively with flower induction (Nakano et al. 2015; Koskela et al. 2016). Silencing of the *FaTFL1* gene resulted in continuous flowering in the obligatory SD cultivar Elsanta (Koskela et al. 2016). However, the expression pattern of *FaTFL1* varies depending on the cultivar and it may also be related to juvenility in the garden strawberry

(Koskela et al. 2016). In contrast to that found for *FaTFL1* expression, *FaFT1* and *FaSOC1* mRNA levels were similar among the different cultivars. Therefore, the *FaFT1-FaSOC1*–pathway is not likely to be the only mechanism to regulate *FaTFL1* (Koskela et al. 2016).

Taken together, the repressor FvTFL1 causes seasonal flowering in the strawberry (Koskela et al. 2012; 2016; Fig.3). The FvSOC1 protein regulates photoperiodic flowering through FvTFL1 in the woodland strawberry; under LD it up-regulates *FvTFL1* to repress flowering. *FvFT1* is up-regulated only under LD and probably activates *FvSOC1*. In the absence of FvTFL1, the *FvFT1* gene activates flowering.

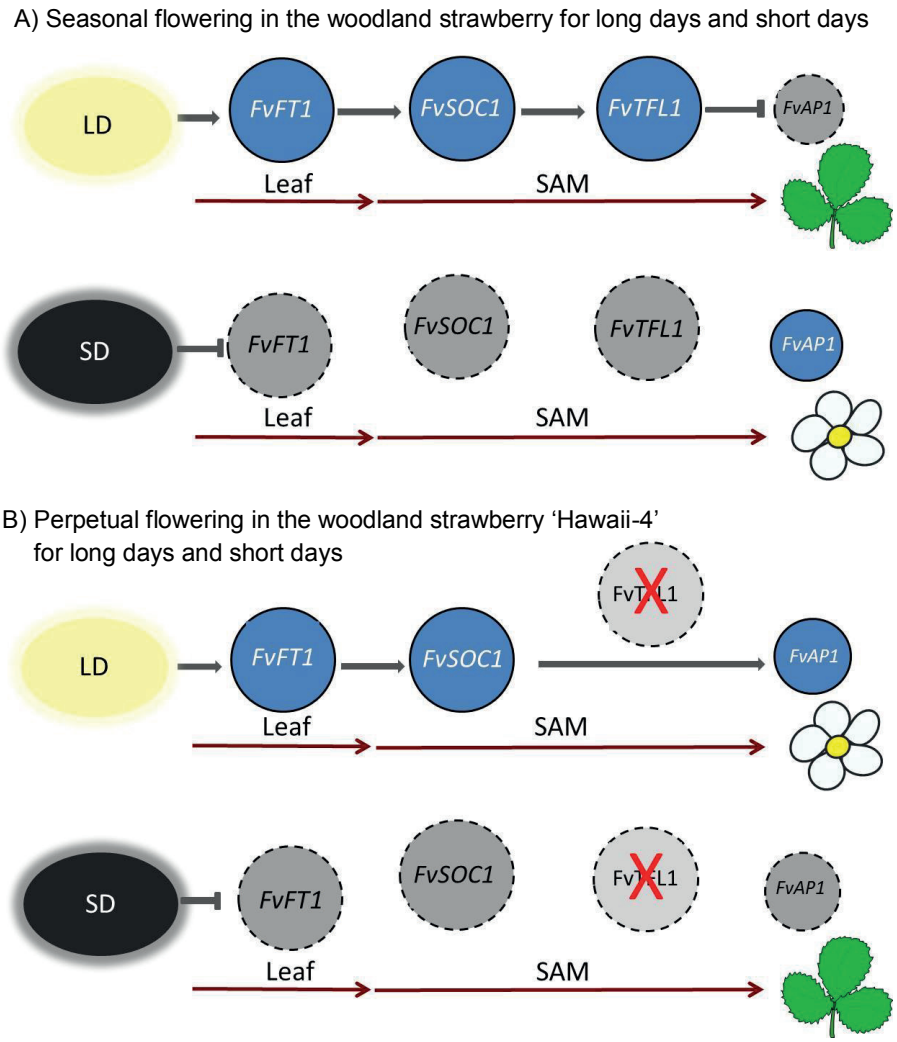

Fig 3. Model of the photoperiodic regulation of flowering in the woodland strawberry (*F. vesca)*. A) LD activates *FvFT1*>*FvSOC1*>*FvTFL1* pathway in the seasonal flowering woodland strawberry to repress flowering. Under SD conditions *FvFT1* is down-regulated, and consequently *FvSOC1* and *FvTFL1* are suppressed, which results in flowering. B) In the perpetual flowering accession 'Hawaii-4' repressor FvTFL1 is not functional, and activation of *FvFT1*>*FvSOC1* results in flowering under LD conditions. Under SD *FvFT1* is not expressed, and accordingly *FvSOC1* and flowering is suppressed. In both accessions the meristem identity gene *FvAP1* is activated at the time of flower induction. *FvFT1* is expressed in the leaf; *FvSOC1, FvTFL1* and *FvAP1* in the shoot apical meristem. Blue and grey circles with dashed line describe active or inactive gene, respectively. Arrow=activation, bar=repression. Modified from Koskela et al. (2012) and Mouhu et al. (2013).

1.2 The raspberry plant and flowering

The subgenus *Ideobatus* of the genus *Rubus* consists of over 200 species that grow wild in all temperate regions of Europe, Asia and North-America (Jennings 1988). Two subspecies can be separated under the common name of red raspberry: cultivated red raspberry *Rubus idaeus* subsp. *idaeus* and American red raspberry *R. idaeus* subsp. *strigosus*. Interfertility between these two subspecies is high and has contributed to the allelic diversity of modern cultivars (Longhi et al. 2014). The term 'raspberry' hereafter refers to cultivated red raspberry cultivars.

1.2.1 Physiology of flowering

Raspberry (*Rubus idaeus*) has a perennial root system though its shoots are biennial. New shoots emerge mainly from root buds or axillary, basal buds near to the ground, and replace old shoots that die after fruiting (Hudson 1959). In their first growing season shoots are called primocanes and floricanes in the second year. Raspberry cultivars are grouped into primocane (annual) and floricane (biennial) fruiting cultivars based on the timing of flower induction and fruiting.

The interaction between temperature and photoperiod controls growth and flowering in the *Rubus* species (Heide and Sønsteby 2011), but regulation of flower induction at the genetic level has not been demonstrated. Primocane fruiting is a quantitative character (Keep 1961). Some cultivars typically show tip flowering from the few upper most buds in the first growing season, whereas the rest of the cane behaves like a floricane fruiting cultivar (Williams 1959c). The primocane/floricane fruiting characteristic can be seen as a temporal continuum (Jennings 1988; Carew et al. 2000). According to Sønsteby and Heide (2008a; 2009b) flowering of primocane fruiting cultivars occurs before dormancy and in floricane fruiting cultivars after dormancy, which divides raspberry cultivars into two distinct groups.

The axillary buds break and grow into flowering laterals in the first growing season in primocane fruiting cultivars. Growth vigour and earliness of flowering define the cane height as growth ceases when flowering begins (Sønsteby and Heide 2012). Some buds in the lower part of the primocane may remain dormant, overwinter and flower in the following spring similar to that of the floricane fruiting cultivars.

Primocane fruiting cultivars can be classified into LD plants (Sønsteby and Heide 2009b). LD conditions advances flowering in general but the response is not linear, and in some cultivars the photoperiod does not clearly affect flower induction (Carew et al. 2003; Sønsteby and Heide 2009b; 2012). The concurrent effect of temperature and day photoperiod is pronounced. Relatively high temperatures up to 24–28°C under LD enhance flowering whereas higher temperatures reduce the number of flowers (Carew et al. 2003; Sønsteby and Heide 2009b; Sønsteby and Heide 2012). Low temperature during the active growing phase leads to a decreased number of generative nodes (Sønsteby and Heide 2009b). The chilling of root buds or young shoots at the early stage of development has been observed to accelerate flowering and raise the number of flowers (Vasilakakis et al. 1980; Takeda 1993; Carew et al. 2001; Sønsteby and Heide 2009b; 2012). An insufficient chilling of the root buds may cause erratic flowering (Vasilakakis et al. 1980; Takeda, 1993; Carew et al. 2001).

The canes of floricane fruiting raspberry cultivars elongate in the first growing season. High temperature and long photoperiod maintains vegetative growth (Sønsteby and Heide 2008a). Canes do not branch i.e. axillary buds do not break in the first growing season when the canes are not tipped, and the terminal bud remains intact (Williams 1959a). Flower initiation occurs in the autumn, and starts at a position of a few buds below the terminal bud and continues rootward along the cane (Williams 1959c; Sønsteby and Heide 2008a). The canes that have at least 10 to 15 nodes are competent for flower induction and this requires 4–6 weeks of inductive conditions to occur (Williams 1960; Sønsteby and Heide 2008a). The axillary buds enter dormancy that is broken by adequate accumulation of chilling during the winter. In the following spring, buds break and elongate into laterals that flower. The biennial canes senesce and die after fruiting.

In natural growth cycle shortening days and decreasing temperature in the autumn cease growth and induce flowering (Hudson 1959; Williams 1960; Sønsteby and Heide 2008a). Flower induction requires SD within an intermediate temperature range of 13–15°C, whereas high temperature represses flowering and cool temperature induces flowering independent of photoperiod (Williams 1960; Sønsteby and Heide 2008a). However, the interaction between the temperature and the photoperiod regulates the development of raspberry. Growth at 20°C continues even at 9 hours of photoperiod whereas at 16°C the terminal bud is formed at 14 hours of photoperiod (Williams 1959b; Sønsteby and Heide 2008a). A longer

photoperiod of 16 hours maintains vegetative growth at 16°C (Williams 1960).

1.2.2 Raspberry production in high-tunnels

Raspberry is an economically important berry crop in Finland. A majority of the raspberry growing area of 435 hectares was as open-field production in 2016 (Horticultural Statistics 2017). However, only a few cultivars can be grown commercially in the open-field in Finland due to the short growing season and the cold winters. The growing season for raspberries can be artificially extended by several weeks by growing the raspberries in polyethene covered high-tunnels that increase the temperature earlier in the spring and later in the autumn than under open-field conditions, allowing a wider cultivar selection in cold climates (Demchak 2009; Hanson et al. 2011; Yao and Rosen 2011). The response in yields in high-tunnel grown raspberries ranged between 30–50% higher than open-field production or even more than doubled the yield potential (Hanson et al. 2011; Palonen et al. 2015; 2017). A single cane may produce a yield of 1.5–3 kg (Sønsteby et al. 2009; 2013), which is much higher than the typical yield in open-field production. These factors together with high berry quality have contributed to the establishment of high-tunnel production as the principal cultivation technique in Finland during the last years.

1.2.3 Light quality affects plant appearance and the fruit quality

Raspberry canes may exceed a height of 2 m (Heiberg et al. 2008; Sønsteby et al. 2013; Palonen et al. 2015). Tall canes, however, make practical management and harvest difficult, and therefore compact plants with a high number of nodes are preferable in commercial raspberry production (Dale and Blom 2004; Hanson et al. 2011). One option for growth regulation is the modification of light environment with reflective and photoselective films, nets or mulches. Photoselective films can be used to control the growth of ornamental bedding plants as an alternative strategy to that of plant growth regulators (Rajapakse et al. 1999; Li et al. 2000; Runkle and Heins 2002; Clifford et al. 2004; Mata and Botto 2009).

The use of photoselective films has been reported to shorten the internodes of raspberries, but they may also delay flowering (van Haeringen et al. 1998; Mata and Botto 2009). Spectral modification of sunlight by using photoselective films or by augmenting daylight by LEDs does not only affect

morphology and timing of flowering in plants, but they change other growth factors such as temperature and relative humidity (Zoratti et al. 2015), temperature of the leaf surface (Nelson and Bugbee 2015) and behaviour and life cycles of pests (Vänninen et al. 2010; Johansen et al. 2011). All these factors may require changes in the whole growing system.

The quality of fruit is important in berry crops. The light intensity and light quality affect the synthesis of many secondary metabolites such as phenolic compounds including flavonoids and anthocyanins. These metabolites function in the plant's defence mechanisms and protect plants against stress, but they also contribute to the aroma and colour of the fruit (Maga and Katz 1978; Cheng and Breen 1991; Tomás-Barberán and Espín 2001; Jaakola et al. 2002). The UV and B-light frequencies of the spectrum in particular activate the flavonoid pathway genes, and biosynthesis of flavonols and anthocyanins in berry crops (Koyama et al. 2012; Kadomura-Ishikawa et al. 2013; Kondo et al. 2014; Zoratti et al. 2015). Thus, the amounts of anthocyanins and the antioxidant capacity in berries may be lowered by using any cladding material that blocks UV light (Hanson et al. 2011; Šavikin et al. 2013). However, Ordidge et al. (2010) noted that the amount of UV-irradiation is naturally low in the temperate climate and therefore, the use of a UV-blocking film may not significantly affect the amounts of phenolic compounds.

2 THE OBJECTIVES OF THE STUDY

Light and temperature are fundamental growth factors that regulate the vegetative and reproductive development of temperate berry crops. Winter hardiness and yield are closely connected to floral development in temperate regions. Therefore, it is essential to know how different environmental factors affect flower induction in production systems to develop cultivation techniques and to breed well-adapted cultivars.

Seasonal flowering in the woodland strawberry is controlled by photoperiod depending on temperature (Heide and Sønsteby 2007). This study focused on temperature, and the interactions of temperature, photoperiod, and light quality in the regulation of flowering in the woodland strawberry and also in the red raspberry. The objectives were to study the effects of temperature and light in the flowering response in the woodland strawberry at the molecular level and the effects of modified light environment at the physiological level in raspberry production.

The first paper (I) focused on the interaction of photoperiod and temperature in the regulation of flowering in the woodland strawberry. The aim of the study was to determine i) what is the critical photoperiod for flower induction in the woodland strawberry ii) what is the critical temperature for photoperiod independent flowering in the woodland strawberry and iii) what are the roles of *FvFT1*, *FvSOC1* and *FvTFL1*, the genes of the photoperiodic flowering pathway, in the regulation of flowering under varying conditions of temperature and photoperiod.

The second paper (II) describes the effects of light quality on flower induction in the woodland strawberry. The aims were to study the following questions: i) do blue-light (B), far-red-light (FR) and red-light (R) wavelengths affect flowering in the woodland strawberry and ii) do the central floral genes identified in the woodland strawberry mediate this regulation at the molecular level.

In the third paper (III) the effects of modified light quality were studied in the primocane and floricane fruiting raspberries. The study aimed to find out, how decreased FR irradiation affects cane elongation growth, flowering and fruiting in primocane and floricane raspberries.

3 MATERIAL AND METHODS

Methods used in this thesis are summarized in Table 1. Detailed information is described in publications I-III.

Table 1. Summary of methods used in this study. Co-authors had the main responsibility for the methods marked with an asterisk.

Method	Publication
cDNA synthesis	I, II
Photoperiod treatments	I, II
Flowering time measurements	I, II, III
Genetic transformation	II*
Gibberellin analysis, GC-MS	III*
LED-treatment	II
Modification of light spectrum	II, III
Quantitative PCR	I, II
RNA extraction	I, II
Spectral measurements	II, III
Temperature treatments	I

3.1 Plants studied

3.1.1 Woodland strawberry

Seasonal flowering woodland strawberry *(Fragaria vesca)* accession used in the experiments originates from Punkaharju, southeastern Finland (I, II). Perpetual flowering woodland strawberry accession 'Hawaii-4' was used in the light quality experiments (II). The transgenic over-expression and RNAi lines showing altered expression of *FvFT1*, *FvSOC1* and *FvTFL1* genes (Koskela et al. 2012; Mouhu et al. 2013) were used in temperature and light quality treatments as outlined in Table 2. Transgenic plants in the seasonal flowering and perpetual flowering 'Hawaii-4' background were propagated from runners and seeds, respectively. Non-transformed control plants were propagated in a similar manner as the transformed plants.

3.1.2 Red raspberry

The raspberry plants used in this study were obtained from commercial nurseries (Table 2). Cold stored, bare root plants of primocane fruiting cultivars 'Autumn Bliss' and 'Polka' were pruned to the soil level and were allowed to establish before transfer to the light quality treatments. Plants of floricane fruiting cultivars 'Glen Ample' and 'Tulameen' were received as small pot plants that were propagated from root cuttings. The plants were grown for one season in the experimental field of the University of Helsinki (60°13'N, 25°01'E), cold stored and pruned to the soil level before transfer to the light quality treatments.

3.2 Light and temperature treatments

3.2.1 Strawberry

Before the treatments plants were grown under non-inductive conditions. Plants of seasonal flowering SD inducible accession were grown under LD at 21°C. Seedling plants of seasonal flowering accession were grown until 5 to 6 leaves were fully opened before the plant was transferred to the treatments. Plants of perpetual flowering accession were germinated under LD and transferred to the non-inductive SD at the cotyledon stage. They were transplanted and subjected to the treatments after the plants were established at 1 or 3–4 -leaf stage (II). Light quality treatments were carried out as day-extension treatments where the main photoperiod (12 hours) was provided by high pressure sodium lamps (HPS). An LD photoperiod was achieved by extending the main photoperiod with low intensity LED light (6 hours) (II). Narrow band wavelengths of B, R or FR light or incandescent lamps were used immediately after the main photoperiod (end-of-day treatment). After six weeks in this regime the plants were transferred to LD conditions (18 h) provided by the HPS for the flowering observations.

Temperature-photoperiod treatments (I) were carried out either in the greenhouse compartment or in the growth room equipped with forced air circulation and whole spectrum LED light bars (Spectra AP67, Valoya, Helsinki, Finland). Six weeks of inductive treatment was used after which plants were transferred to the greenhouse (18 hours HPS; 150 μmol m^{-2} s^{-1}).

Strawberry has a determinate flowering habit. The flowering time was therefore recorded as the number of leaves before apical inflorescence or as days until the anthesis of the terminal inflorescence (I, II).

3.2.2 Raspberry

The plants of both primocane and floricane fruiting raspberry cultivars were subjected to the differing light quality treatments by growing them in the tunnel covered with either FR absorbing photoselective film (Solatrol, Visqueen, England) or clear film (Visqueen, England). The tunnels were located in Piikkiö, Southern Finland (60°23'N; 22°33'E). Floricane fruiting cultivars were cold stored after the light quality treatment for 10 weeks (+1°C) and then forced in the greenhouse conditions (16 h; 150 μmol m^{-2} s^{-1}; 18°C day /15°C night).

The first season elongation growth was recorded weekly during the light quality treatments. The number of raspberry flowers and the timing of the flowering were recorded in the first growing season or during the greenhouse forcing of the primocane and floricane fruiting cultivars, respectively. Yield and fruit quality were determined only for the primocane fruiting cultivars.

3.3 RNA extraction, cDNA synthesis and real-time PCR with strawberry samples

Strawberry leaves or shoot apical meristem samples were collected as three biological replicates of samples of 3–8 pooled plants for gene expression analyses. The middle leaflet of the youngest opened leaf was collected as a leaf sample. Apical meristem samples were collected by cutting approximately a 1 mm piece of the shoot apical meristem. The samples were frozen immediately in liquid nitrogen.

RNA extraction was done by using the pine tree method (Monte and Somerville 2002). One μg of total RNA was used for cDNA synthesis (Superscript III reverse transcriptase, Invitrogen) analysis. Real time PCR reactions were performed using SYBR Green Master Mix (Roche, Basel, Switzerland) and 3 μM primer mix (F+R) using LightCycler 480-instrument (Roche). The relative expression of selected genes was calculated by the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001) with stable *FvMSI1* as the normalization gene. The primer efficiencies were close to 2 for all primer pairs.

Table 2. Origin, photoperiodic flowering response type and genotypes of the plant species studied in this thesis.

Background	Origin	Photoperiodic flowering response	Genetic modification	Publi-cation
Fragaria vesca	Punkaharju, southeastern Finland	Seasonal	Wild type	I, II
			35S:FvSOC1 ox	I
			FvSOC1 RNAi	
			35S:FvTFL1ox	
			FvTFL1 RNAi	
F. vesca 'Hawaii-4'		Perpetual flowering, ever-bearing	Wild type	II
			35S:FvFT1ox	
			FvFT1 RNAi	
			35S:FvSOC1ox	
			FvSOC1 RNAi	
Rubus idaeus 'Autumn Bliss'	Gravens Plantskole, Denmark	Primocane fruiting	None	III
R. idaeus 'Polka'	Hargreaves, England, UK	Primocane fruiting		
R. idaeus 'Glen Ample'	Marja-Suomen taimituotanto, Finland	Floricane fruiting		
R. idaeus 'Tulameen'	Elite plant station Balsgård, Sweden	Floricane fruiting		

4 RESULTS AND DISCUSSION

4.1 Photoperiod and temperature in the control of flowering in the woodland strawberry (I, II)

4.1.1 FvTFL1 mediates the temperature signal

The interaction of temperature and photoperiod is pronounced in the regulation of flowering in the woodland strawberry. It is a facultative SD plant that is induced into flowering under SD at intermediate temperatures that range between 15 and 20°C (Heide and Sønsteby 2007). Photoperiodic flowering is caused by *TERMINAL FLOWER1 (FvTFL1)* that is down-regulated under inductive SD (Koskela et al. 2012; Mouhu et al. 2013). In addition to woodland strawberry, homologs of *TFL1* encode major floral repressors in many other *Rosaceae* plants (Kotoda et al. 2006; Iwata et al. 2012; Flachowsky et al. 2012).

The genes that regulate photoperiodic flowering are also putative regulators in the mediation of temperature signals. Seasonal flowering woodland strawberry (SD woodland strawberry) plants were grown under temperature series of 10; 13; 16 and 23°C to study their role. The floral response in the present study agreed with earlier observations that flowering was independent of photoperiod both at cool and high temperatures (Heide and Sønsteby 2007). All plants flowered at 10°C, whereas at 23°C plants remained vegetative (I). A majority, but not all plants were induced at 13°C under LD, which indicates that the critical temperature for the photoperiod independent flower induction is close to 13°C (I). Heide and Sønsteby (2007) observed that, depending on the woodland strawberry population, even at 9°C under continuous light some portion of the plants remained in the vegetative mode. They further found that along with a temperature rise from 15 to 18°C, critical photoperiod was shorter and the percentage of the flowering plants was decreased. Together these observations illustrate a quantitative temperature-photoperiod response in the woodland strawberry.

The gene expression study (I) revealed that the level of *FvTFL1* mRNA correlated negatively with flowering observations at all temperature-photoperiod combinations. *FvTFL1* was down-regulated at cool temperatures, and no difference between SD and LD conditions was detected

at 10°C. *FvTFL1* mRNA level increased gradually along with the rise in temperature between 13 and 23°C under LD conditions. Interestingly, the lowest expression level of *FvTFL1* was detected under SD at 16°C. It is well documented that SD together with intermediate temperatures in the 15–18°C range is optimal for flower initiation both in the woodland strawberry and the garden strawberry, which is in line with this observation (Heide and Sønsteby 2007; Heide et al. 2013). Another noteworthy observation was the high up-regulation of *FvTFL1* at 23°C. The expression study indicated that *FvTFL1* has a central role in the temperature responses of flowering. Consequently, the transgenic plants were subjected to cool and high temperatures to confirm this role. The RNA interference silencing of *FvTFL1* (RNAi) resulted in flowering at high temperature, whereas overexpression prevented flowering at cool temperatures (I), which provided strong evidence that FvTFL1 is a key factor not only in the photoperiodic flowering, but also in temperature mediated flowering.

The gradual rise of *FvTFL1* expression with an increase in temperature under LD resembles the regulation of the gene expression in the H2A.Z histone variant. Histone proteins wrap DNA in DNA replication, chaperoning and supercoiling, and the coiling forms a physical barrier to the binding of transcription factors to the promoter (Ferl and Paul 2000). Replacement of H2A with alternative histone H2A.Z makes the chromatin sensitive to temperature changes (Redon et al. 2002). The repression of transcription by H2A.Z at cool temperature is released as temperatures rise since histone variants are evicted from the chromatin (Kumar and Wigge 2010). The H2A.Z histones regulate PIF4 mediated promotion of the transcription of *FT* at high temperature in *Arabidopsis* (Kumar et al. 2012). Hypothetically, *FvTFL1* could be a target of H2A.Z regulation under LD conditions. However, the expression of *FvTFL1* under SD conditions did not show similar linear dependence on the temperature. The strong interaction of temperature and photoperiod indicates also that other mechanisms are involved. Therefore, direct or indirect regulation of *FvTFL1* by H2A.Z should be the target of a careful further study.

4.1.2 Photoperiodic control of flowering

Functioning of FvSOC1 is temperature dependent

Arabidopsis SOC1 integrates several floral pathways to activate flowering in an *FT* dependent or independent manner (Lee et al. 2000; Moon et al. 2005; Yoo et al. 2005; Wang et al. 2009a; Lee and Lee 2010). The *FvSOC1* controls vegetative and generative development up-stream of *FvTFL1* at 18°C in the woodland strawberry (Mouhu et al. 2013). Therefore, FvSOC1 is a putative candidate for the regulation of temperature mediated flowering in the woodland strawberry. The *FvSOC1* gene was clearly up-regulated under LD conditions compared with SD for all temperatures between 10 and 23°C, and thus no expression pattern correlating with flowering was observed (I). A further study showed that the functional role of FvSOC1 as a floral regulator depends on the temperature. At 16°C silencing of *FvSOC1* enabled flower induction under both photoperiods (I) similar to that found at 18°C by Mouhu et al. (2013), but at 23°C no flowering was observed in either *FvSOC1* RNAi or non-transgenic plants. In line with the phenotype, expression of *FvTFL1* was high in non-transgenic plants at 23°C. In LD the expression of *FvTFL1* increased at high temperature. In *FvSOC1* RNAi plants this photoperiodic control of *FvTFL1* had vanished, which indicates that the effect of photoperiod is not completely lost at high temperature. Therefore, in addition to the LD specific photoperiod pathway the *FvFT1-FvSOC1-FvTFL1* complex that was suggested by Mouhu et al. (2013), some as yet unknown additional activator is needed to up-regulate *FvTFL1* under SD conditions to repress flowering at high temperatures.

At cool temperatures the role of *FvSOC1* seems to be more complicated. The expression data at various temperatures (I) gave rise to the hypothesis that down-regulation of *FvTFL1* would be independent of FvSOC1 at low temperatures. However, a high expression of *FvTFL1* and suppression of flowering was found in *35S:FvSOC1* overexpression plants under conditions of LD at 11°C. In contrast, a low *FvTFL1* mRNA level was detected in the control plants that were also induced into flowering (I). The expression of *FvSOC1* in non-transgenic plants did not correlate with *FvTFL1* under conditions of LD and cool temperatures, which was possibly due to the non-functionality of the photoperiod pathway or insufficient quantities of FvSOC1 protein. The *35S*-promoter causes constitutive, abnormally high ectopic expression, which could also be a reason for the up-regulation of *FvTFL1* and the repression of flowering in *35S:FvSOC1*-plants at cool temperatures.

Critical photoperiods for flower induction correlate with the expression of *FvFT1* that activates flowering in perpetual flowering accession 'Hawaii-4'

The critical photoperiod for flower induction in SD woodland strawberry at 18°C was found to be between 14 and 16 hours (I), which is also typical for many seasonal flowering garden strawberry cultivars (Heide 1977; Konsin et al. 2001; Verheul et al. 2007). The expression of *FvFT1* correlates negatively with flowering in the woodland strawberry (Koskela et al. 2012; Mouhu et al. 2013). The *FvFT1* gene was highly up-regulated at 16 hours of photoperiod whereas the expression was hardly detected at 14 hours (I). This indicates that the FvFT1 protein has a similar role to those proteins encoded by *FT*-like genes of other species, which mediate the critical photoperiod signal to control flowering and bud set (Böhlenius et al. 2006; Hayma et al. 2007; Itoh et al. 2010; Higuchi et al. 2013). Interestingly, the highest *FvFT1* mRNA–level was found at 16°C, whereas the expression was lower at both 13 and 23°C (I). *FaFT1* expression in the garden strawberry has a similar expression pattern (Nakano et al. 2015; Koskela et al. 2016), which underlines the role of *FT1* in the photoperiodic flowering in strawberry at intermediate temperatures. However, the expression pattern of *FvFT1* at ambient temperature differs from *Arabidopsis* (Blázquez et al. 2003; Balasubramanian et al. 2006; Lee et al. 2007).

'Hawaii-4' is a perpetual flowering woodland strawberry accession that lacks functional FvTFL1, which makes it an LD plant (Koskela et al. 2012). The finding that LD activates *FvFT1* is in line with early flowering in 'Hawaii-4' (Koskela et al. 2012; Mouhu et al. 2009; 2013), thus the role of FvFT1 as a floral activator was studied in this accession. The RNAi silencing of *FvFT1* (II) delayed flowering under LD in 'Hawaii-4' plants (Koskela et al. 2012). Furthermore, the over-expression of *FvFT1* resulted in extremely early flowering after a few leaves had emerged independently of photoperiod. Taken together, FvFT1 probably mediates photoperiodic signal in both SD woodland strawberry and perpetual flowering accession, but activates flowering only in the absence of FvTFL1.

The strawberry species have three *FT*-like genes that have different spatial expression patterns. Strawberry homologs of *FT*, *FvFT1* and *FaFT1* are expressed exclusively in the leaves whereas *FvFT2* is detected mainly in the floral buds (Koskela et al. 2012; Nakano et al. 2015). *FaFT2* does not show any particular expression pattern in the leaves or shoot tips either (Nakano et al. 2015). The *FaFT3* gene is a potential activator of flowering in the garden

strawberry since it is detected mainly in the shoot apical meristem under inductive SD in combination with flowering (Nakano et al. 2015). The examples from poplars (*Populus sp.)*, sugar beet (*Beta vulgaris*) and wild chrysanthemum (*Chrysanthemum seticuspe*) show that *FT*-like genes may respond differently to environmental cues and promote either vegetative or generative development or even have an antagonistic role in the flowering process (Pin et al. 2010; Hsu et al. 2011 Oda et al. 2012; Higuchi et al. 2013). Therefore, the functional role of strawberry FTs and their seasonal variation in relation with TFL1 should be studied further.

4.2 The role of light quality in the flowering of the woodland strawberry (II)

Daylight that irradiates a plant consists of various combinations of spectra of wavelengths that include the visible light spectrum and also wavelength spectra beyond the visible spectrum range, and any combination of these spectra comprise light quality. Daylength quality may vary a lot depending on the surrounding vegetation, season, latitude and the time of the day (Holmes and Smith 1975; 1977). In addition to photoperiod, these qualitative light signals are important for the development of the plants. The FR wavelength light often accelerates flower induction in LD plants whereas R wavelength light suppresses flowering. The same was observed in LD flowering *Rosaceae* plants such as the japanese pear (*Pyrus pyrifolia* (Burm. f.) Nakai) (Ito et al. 2014). The experiments conducted by Vince-Prue and Guttridge (1973) indicated that flower induction of the seasonally flowering garden strawberry (*Fragaria* × *ananassa*) is also sensitive to R/FR light but in contrast to that found for the LD plants, the FR day extension delayed flowering. Flowering and runner production often correlate negatively in different phases of the strawberry life cycle. Therefore, tailored light quality modification could be used as tool by nurseries, to direct growth either into the vegetative or generative growth phases to maximize plant propagation or to produce a plant capable of flowering.

4.2.1 FvFT1 mediates light quality signal

The role of light quality on flowering was studied under a short main photoperiod that was extended by narrow wavelength bands R, FR and B and by LEDs. Several transgenic lines of SD woodland strawberry and perpetual flowering 'Hawaii-4' that lacks functional FvTFL1 were used in study II in order to gain detailed knowledge on light quality responses at the molecular level. The FR wavelength light accelerated flowering in 'Hawaii-4' (II) to a similar extent to that found in many other LD plants (Eskins 1992; Cérdan and Chory 2003). The B wavelength light treatment also enhanced flowering but the terminal inflorescence developed a few leaves later than in plants under FR light treatment. Day extension by R did not promote flowering compared with the SD regime (II). As discussed earlier, the LD led to highly up-regulated *FvFT1*, whereas the expression under SD was extremely low or could not be detected at all (I, II). A natural consequence of flowering results was the hypothesis that FvFT1 could also mediate light quality signals. The FR treatment strongly activated *FvFT1* to a similar extent as that found

under LD conditions (II), whereas no expression under R wavelength light was detected as was also the case under SD. The RNA silencing (RNAi) of *FvFT1* in 'Hawaii-4' background delayed flowering under FR and B day extension treatments (II). The over-expression of *FvFT1* resulted in early flowering under all light quality treatments and also under SD alone (II).

Light quality of SD affected the flowering in the woodland strawberry in an opposite way compared with that found for 'Hawaii-4'. The FR and B wavelength day extension treatments under SD conditions inhibited flowering in the SD woodland strawberry, whereas under the R wavelength light quality treatment the plants were induced to flower. The effect of light quality on the flowering of woodland strawberry has not been reported previously. However, Vince-Prue and Guttridge (1973) found that R irradiation as an end-of-day treatment of the garden strawberry did not affect flowering, whereas FR irradiation inhibited flower induction. Thus, FR extension treatment activates the signaling that plant recognizes as a non-inductive LD. The *FvFT1* gene was up-regulated under different light quality treatments in a similar way under SD conditions in the woodland strawberry and 'Hawaii-4' (II). Although there is no functional data of the role of *FvFT1* in the SD woodland strawberry so far, the results indicate that the FvFT1 protein mediates light quality signals in both SD woodland strawberry and perpetual flowering 'Hawaii-4'. However, negative correlation between *FvFT1* mRNA level and flowering indicates that a *FvFT1*>*FvSOC1*>*FvTFL1*– pathway exists under the studied light qualities in the SD woodland strawberry.

4.2.2 FvSOC1 has B-light specific effects on light quality responses

The silencing of *FvFT1* in 'Hawaii-4' down-regulates *FvSOC1* in the apical meristem (Mouhu et al. 2013). The expression of *FvFT1* and *FvSOC1* in the present study correlated positively with each other under LD and under different light quality treatments (II). The FvFT1 protein was observed to activate flowering and to mediate light quality signals in perpetual flowering 'Hawaii-4', therefore FvSOC1 was expected to be involved in this signaling. Indeed, silencing of *FvSOC1* delayed the flowering of B treated plants (II). Unexpectedly, FR could still initiate the flowering of *FvSOC1* RNAi plants sooner and to a similar extent than in non-transgenic plants. Thus, FR may enhance flower induction independently of FvSOC1, whereas B-light accelerates flowering through the *FvFT1-FvSOC1* pathway (II), which

indicates that FR and B responses are mediated at least partly through different pathways.

In the photoperiodic flowering of *Arabidopsis* rhythmic expression of *CONSTANS (CO)* in the afternoon and accumulation of CO protein under light determine LD flowering. The B-light stabilizes CO protein that activates *FT* (Valverde et al. 2004; Sawa et al. 2007). In contrast to *Arabidopsis*, the effect of B-light on *FvFT1* expression was mild compared with FR light, which correlates with flowering (II). The effect of light quality treatments on *FvCO* and *FvFT1* rhythm was studied in LD accession 'Hawaii-4', but neither photoperiod nor light quality affected the rhythm of *FvCO* expression, whereas the effect on *FvFT1* mRNA-level was clear. The expression of *CO* peaks in *Arabidopsis* in the late afternoon and activates *FT* under LD (Suarez-Lopez et al. 2001). The *FvCO* peaked in the morning but no difference in the expression rhythm or the mRNA level was detected between treatments. This lack of difference suggests a similarity to two *CONSTANS-LIKE* (*COL1* and *COL2*) genes of *Arabidopsis,* which have no or only minor roles in the flowering (Ledger et al. 2001). According to Thomas and Vince-Prue (1997) the strong effect of B-light on flowering time is well documented for many *Cruciferae* plants but the effect is weaker in other plants. However, the present data do not exclude the possibility of post-translational regulation of FvCO. Given that the stability of the CO protein is central in the photoperiodic flowering in *Arabidopsis*, and CO has very diverse functional roles in plants (Yano et al. 2000; Liu et al. 2001; Böhlenius et al. 2006; Chia et al. 2008; Almada et al. 2009; Holefors et al. 2009; Campoli et al. 2012; Yang et al. 2014; Fu et al. 2015; Song et al. 2015), the role of B-light and FvCO in the photoperiodic flowering in the woodland strawberry warrants further study.

4.3 Physiological effects of light quality on growth and cropping of the red raspberry (III)

Vegetative growth of the red raspberry cane is vigorous, therefore the regulation of growth is a physiological necessity. FR-irradiation is known to cause increased stem elongation as a response to shading by the surrounding canopy (Morgan and Smith 1976). A decrease of FR by using tunnels covered by photoselective films for cultivating ornamental pot plants has the potential to replace treatments with plant growth regulators to yield compact plants (Li et al. 2000; Runkle and Heins 2002; Clifford et al. 2004; Mata and Botto 2009). Consequently, two primocane ('Autumn Bliss' and 'Polka') and two floricane raspberry cultivars ('Glen Ample' and 'Tulameen') were grown for one season in a high-tunnel covered either with low-FR film (photoselective film) or regular clear greenhouse film to study the effect of low-FR spectrum in the growth control of red raspberry. The development of primocane fruiting cultivars was observed during the first growing season. Long canes of floricane fruiting cultivars were then cold stored to break dormancy (vernalized) and forced in the greenhouse conditions under a regular lighting regime.

4.3.1 The effect of low-FR on growth and flowering is cultivar dependent

Low-FR film had a minor effect on the elongation growth of primocane fruiting cultivars. Until mid-August of that same year no difference in the cane height of the cultivars was found between clear and low-FR film treatments (III). The internode length of primocane cultivars was also unaffected. However, the growth of the 'Polka' cultivar ceased a few weeks earlier under the low-FR film compared with the clear film treatment, and this resulted in an 8% lower number of nodes and a shorter cane at the end of the season. Vegetative growth was not affected in the other primocane fruiting cultivar 'Autumn Bliss'.

The low-FR irradiation treatment advanced the flowering in both primocane fruiting cultivars and increased the number of flowers by 9% in cultivar 'Polka' and by 19% in cultivar 'Autumn Bliss' compared with the clear film treatment (III). The percentage of generative nodes in the 'Polka' cultivar was also increased. Primocane fruiting raspberries are LD plants (Sønsteby and Heide 2009b; 2012). An FR-light day extension regime promotes flowering in many LD plants and the blocking-off of the FR irradiation by a

photoselective film has been observed to delay flowering of LD bedding plants when day length was close to the critical inductive photoperiod (Cerny et al. 2003). However, the results in the present study (III) indicate that primocane fruiting raspberries do not have a similar response to a low-FR environment.

Canes of the floricane fruiting cultivars 'Glen Ample' and 'Tulameen' were grown under a photoselective film in the first growing season when the flower induction occurs. Wild raspberry is a shade intolerant species (Ricard and Messier 1996) and using an end of day-FR treatment has been observed to increase cane height of the floricane fruiting raspberry (Dale and Blom 2004), which gives a reason to expect shortened cane growth under a low-FR light regime. However, low-FR affected the vegetative growth only in the 'Tulameen' cultivar by shortening the internode length whereas the vegetative growth of 'Glen Ample' was unaffected (III). Light quality did not affect the termination of elongation growth either, which occurred at the end of September for 'Glen Ample' and four weeks later for 'Tulameen'. It has been reported that the photoperiod regulates the termination of growth in *Populus*, and FR irradiation activates the strong expression of *FT* (Böhlenius et al. 2006). The same group of authors also found that, in addition to flowering, strong *FT* expression controls growth cessation and bud set (Böhlenius et al. 2006). However, critical temperature determines the timing of growth cessation in many *Rosaceae* plants including floricane raspberry cultivars (Heide and Prestrud 2005; Sønsteby and Heide 2008a), which could explain why a low-FR treatment did not affect the termination of growth. The plants were cold stored to break the dormancy and forced into 'the next spring' in the greenhouse to see the effect of modified light environment on the yield potential. No difference in the timing of flowering was found, but the number of flowers was decreased in 'Glen Ample' and 'Tulameen' by 15 and 10%, respectively, in the plants grown under the low-FR treatment (III).

The effect of low-FR on vegetative growth was cultivar dependent. However, in regard to generative development primocane/floricane fruiting habit clustered the cultivars. Low-FR led to increased flowering in primocane and decreased flowering in floricane fruiting cultivars. Low R/FR ratio or end-of-day FR irradiation lead to increased gibberellin (GA) and auxin (IAA) levels in the LD plant *Arabidopsis*, and also in the SD plant poinsettia (*Euphorbia pulcherrima*). This increase results in genotype dependent lengthening of internodes (Pierik et al. 2009; Kurepin et al. 2012; Islam et al. 2014). The

effect of exogenous GA treatment on floricane fruiting raspberry depends on the timing of the treatment (Redalen 1981). The application of GA during flowering has been observed to increase slightly the number of berries, whereas treatment during the flower induction probably correlates negatively with flowering (Måge 1986; Ghora et al. 2000; Palonen and Mouhu 2009; Palonen et al. 2013). The preliminary analysis in the present study III detected several biologically active gibberellins in floricane fruiting cultivars, but no difference was found between low-FR and clear film treatments. The relationship between light and GA is complex. Furthermore, sensitivity and spatial distribution of GA may be influenced by light quality (Lopéz-Juez et al. 1995; Olsen et al. 1997; Olsen and Junttila 2002). Therefore, an understanding of the effects of light environment on GA metabolism in raspberry requires further study.

4.3.2 Light quality did not affect external or internal fruit quality

Sensory parameters such as appearance, taste and texture are decisive qualities for the acceptability of the vegetable or fruit (Abbott 1999). Light quality parameters were investigated in primocane fruiting cultivars to evaluate the effects of modified light environments on the external and internal berry quality. Both clear UVI/EVA and the low-FR film decrease the level of UV that insect pollinators such as the bumble bee and the honey bee use for navigation (Peitsch et al. 1992). Improper pollination and large temperature variation contribute to the occurrence of crumbly berries that are formed when single drupelets are not properly developed (Graham et al. 2015). No such disorders were observed in this present study, however. Overall, fruit quality of primocane fruiting cultivars was good, and the proportion of the unmarketable yield was low and independent of the lighting regime. Furthermore, fruit colourization was equal under both treatments. Thus, these data indicated that modified light spectra did not affect external berry quality.

The low-FR treatment had only minor effects on the internal quality parameters of fruits in the present study. Soluble solids content (SSC, °Brix) was decreased in cultivar ‘Polka’ and SSC to the titratable acids ratio in both cultivars. The use of the low-FR film decreased the PAR irradiation by 10% and could be a reason for the lowered sugar content reported in an earlier study (Watson et al. 2002). Light quality affects the amount of phenolic compounds such as the anthocyanins (Kadomura-Ishikawa et al. 2013; Kondo et al. 2014; Zoratti et al. 2014; 2015). However, no difference was

found between the amounts of total anthocyanins produced, but the contents of ellagic acid, the most abundant phenolic compound in red raspberry (Häkkinen et al. 1999), was increased under low-FR in the cultivar 'Autumn Bliss'.

5 CONCLUSIONS

Light and temperature regulate flower induction in temperate climate berry crops. The woodland strawberry (*F. vesca*) serves as a model plant to study how flowering is controlled at the molecular level. The results of this study suggest a model (Fig. 4) describing the activation of the *FvFT1-FvSOC1-FvTFL1*-pathway by temperature and light signals that inhibit flower induction in the seasonal flowering woodland strawberry. The integrator of these signals is the repressor gene *FvTFL1*. The perpetual flowering accession 'Hawaii-4' flowering response depends on the activation of *FvFT1* in the absence of the functional FvTFL1 protein. Moreover, *FvFT1* mediates photoperiod signal independent of the flowering response.

The woodland strawberry is an SD plant. During the summer months a long photoperiod activates the *FvSOC1-FvTFL1*-pathway to suppress flowering. Towards autumn and also under intermediate temperatures in the 13–20°C range, the critical photoperiod for flower induction is between 14 and 16 hours. The *FvFT1* putatively mediates the LD signal to up-regulate *FvSOC1-FvTFL1,* which suppresses flower induction. However, *FvFT1* and *FvSOC1* mRNA levels at a photoperiod of 14 hours are low, which results in the down-regulation of *FvTFL1* and thus allows the induction of flowering.

Outside the intermediate temperature range, the regulation of the FvTFL1 protein is not determined by the photoperiod. The *FvTFL1* gene is highly activated at high temperatures both under SD and LD, but in addition to the *FvSOC1* and especially under SD conditions an as yet unidentified factor up-stream of *FvTFL1* is involved in the repression of flowering. At cool temperatures flower induction occurs both under short and long photoperiods. *FvSOC1* maintains a photoperiodic expression pattern, which indicates that the FvSOC1 protein as such does not regulate *FvTFL1* expression at cool temperatures. The FvSOC1 protein has a major role in the photoperiodic regulation of flowering at intermediate temperatures, and thus its role at cool temperatures requires further study.

In the absence of functional FvTFL1 in the perpetual flowering 'Hawaii-4' LD advances the flower induction. A LD regime vigorously activates *FvFT1* whose protein is a strong activator of flowering in the leaves. The expression of *FvFT1* mediates the photoperiod and light quality signals. The FR and B wavelength day extensions advance flowering through the *FvFT1* gene, and *FvSOC1* is specifically needed in the B wavelength light mediated response. Under R-light conditions *FvFT1* is not expressed and flowering does not

occur. Light quality signals in the seasonal flowering woodland strawberry have similar effect on *FvFT1* expression but the floral response is the opposite.

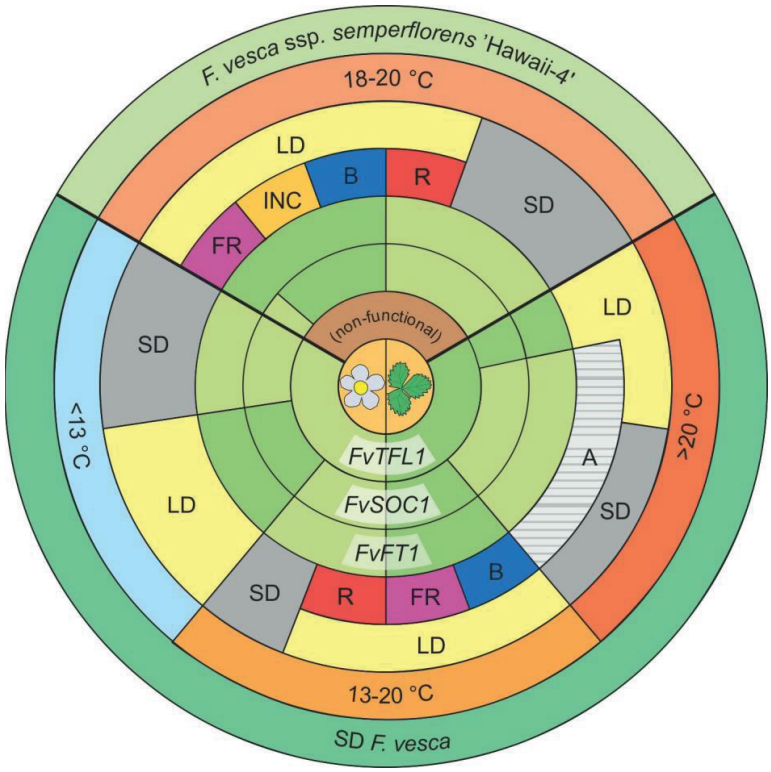

Fig. 4. A diagram showing the regulation of flowering in the SD plant woodland strawberry (*Fragaria vesca)* under different environmental conditions of photoperiod, light quality and temperature. The model is read from the uppermost sector towards center, and the floral response: activation of *FvFT1-FvSOC1-FvTFL1*-pathway controls flowering. *F. vesca*=seasonal flowering woodland strawberry, SD=short day, LD=long day, B=blue, FR= far-red, R=red, and INC=incandescent light. A=an unidentified activator. Activated and inactivated gene is represented by dark green and light-green colour, respectively. In the perpetual flowering woodland strawberry (*F. vesca ssp. semperflorens* 'Hawaii-4') the FvTFL1 protein is non-functional which reverses light quality and photoperiod effects on flower induction.

The physiological regulation of flowering by photoperiod and temperature in the woodland strawberry and the red raspberry are similar. Light quality through decreased FR irradiation affected the growth and flowering of the raspberry. Low-FR light led to increased number of flowers in primocane fruiting cultivars, whereas the number of flowers decreased in the biennial floricane fruiting cultivars. Thus, the results of light quality in this study indicate different regulation characteristics than those found in the seasonal and perpetual flowering woodland strawberry. Genome of the black raspberry (*Rubus occidentalis* L.), is a close relative that of the red raspberry, and it was published recently (Van Buren et al. 2016), which may help in the further elucidation of the regulation of flowering in the raspberry in the near future.

Temperature has a great influence on flower induction in many temperate perennial berry crops. Technical advances have made the control of light and temperature conditions possible in the propagation of high value crop plants and in sheltered production. Molecular studies combined with an understanding of the physiological changes are extremely useful in developing more precise horticultural practices in a changing environment.

ACKNOWLEDGEMENTS

This PhD thesis was conducted at the Department of Agricultural Sciences, University of Helsinki and funded by the Academy of Finland, the Jenny and Antti Wihuri Foundation, the Aino and August Johannes Tiura Foundation and the Ministry of Agriculture and Forestry of Finland. University of Helsinki and Natural Resources Institute Finland are acknowledged for the finalization grants of this thesis.

During these years of research for this thesis, I have had the pleasure to meet and work with many talented skillful persons. I want to express my warmest thanks to everyone who contributed to this PhD by providing critical comments, giving advice or and engaging in informal discussions. Even small things like smiles and laughs have been of great value.

I have had a great opportunity to be a member of Strawberry research group. I want to thank Professor Paula Elomaa, Assistant professor Timo Hytönen and Docent, University lecturer Pauliina Palonen for their excellent supervision. I am grateful not only for the introduction into the world of plant science, but also for the realistic and positive attitude on research presented to me. Combining full-time work with the writing process posed difficulties, therefore, I am especially appreciative of their encouragement.

I want to acknowledge my pre-examiners Professor Kurt Fagerstedt and Professor Jorunn E. Olsen for their critical reading and valuable comments. Follow-up group members Docent Sari Tähtiharju and Dr. Annakaisa Elo are thanked for their advice and discussions. The coordinator of the Doctoral Programme in Plant Sciences Dr. Karen Sims-Huopaniemi is thanked for student friendly attitude and for organizing occasions to meet plant scientists and plant science students.

I want to thank my Strawberry group fellows. Dr. Katriina Mouhu did not only help in many practical things in the lab and greenhouse, but also advised me on statistical questions and figures. The discussions in and between work have been of great value as well. Former and current members of the Strawberry group Dr. Takeshi Kurokura, Dr. Elli Koskela and Dr. Panpan Jiang are thanked as well for their contribution to this work. Professor Olavi Junttila and M.Sc. Hanna Savelainen are acknowledged for their participation to the raspberry work.

Greenhouse technicians, especially Matti Salovaara and Daniel Richterich are thanked for their great help performing the experiments. I have learned

many things about the technical side of the experimental set-up. All professors and colleagues at the Department of Agricultural Sciences are acknowledged for creating a good and inspiring work atmosphere. Marja Huovila, Eija Takala, Anu Rokkanen and Marjo Kilpinen are thanked for their valuable advice in the lab and lively coffee time discussions.

I acknowledge my present working colleagues Research professor Saila Karhu, Dr. Tuuli Haikonen, M.Sc. Jaana Laamanen, and all other members in the Horticulture team of the Natural Resources Institute Finland both in Laukaa and Piikkiö.

In my opinion fresh air and outdoor life is the best source of new ideas for research. I am grateful to Mari, Anne, Leena, Tuija and Arja thanks for their company during the unforgettable miles in adventure races, along endless forest paths and on trail runs. Each journey provided new perspectives for me to see that the issues are just things to be solved. Special thanks belong to my dear friend Anniina, as well.

For their support, I want to thank my mother Helena, and my brothers: Kimmo, Kari and Kalle. It is good to know where your roots are.

REFERENCES

Abbott JA (1999) Quality measurement of fruits and vegetables. Postharvest Biol Tech 15: 207-225.

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K and Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309: 1052-1056.

Adrian J, Torti S and Turck F (2009) From decision to commitment: The molecular memory of flowering. Mol Plant 2: 628-642.

Albani MC, Battey NH and Wilkinson MJ (2004) The development of ISSR-derived SCAR markers around the *SEASONAL FLOWERING LOCUS* (*SFL*) in *Fragaria vesca*. Theor Appl Genet 109: 571-579.

Almada R, Cabrera N, Casaretto JA, Ruiz-Lara S and Villanueva EG (2009) *VvCO* and *VvCOL1*, two *CONSTANS* homologous genes, are regulated during flower induction and dormancy in grapevine buds. Plant Cell Rep 28: 1193-1203.

Andrés F and Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nature rev 13: 627-639.

Bagnall DK, King RW, Whitelam GC, Boylan MT, Wagner D and Quail P (1995) Flowering responses to altered expression of phytochrome in mutants and transgenic lines of Arabidopsis thaliana (L.) Heynh. Plant Physiol 108: 1495-1503.

Balasubramanian S, Sureshkumar S, Lempe J and Weigel D (2006) Potent induction of *Arabidopsis thaliana* flowering by elevated growth temperature. PLoS Genet 2: 980-989.

Basu D, Dehesh K, Schneider-Poetsch H-J, Harrington SE, McCouch SR and Quail PH (2000) Rice PHYC gene: structure, expression, map position and evolution. Plant Mol Biol 44: 27–42.

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA and Dean C (2004) Vernalization requires epigenetic silencing of *FLC* by histone methylation. Nature 427: 164-167.

Bergonzi S, Albani MC, Ver Loren van Themaat E, Nordström KJV, Wang R, Schneeberger K, Moerland PD and Coupland G (2013) Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*. Science 340: 1094-1097.

Blázquez MA and Weigel D (1999) Independent regulation of flowering by phytochrome B and gibberellins in Arabidopsis. Plant Physiol 120: 1025-1032.

Blázquez MA, Ahn JH and Weigel D (2003) A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. Nature Genet 33: 168-171.

Borthwick HA and Downs RJ (1964) Roles of active phytochrome in control of flowering of Xanthium pensylvanicum. Bot Gaz 125: 227-231.

Borthwick HA, Hendricks SB and Parker MW (1952) The reaction controlling floral initiation. PNAS 38: 929-934.

Bradford E, Hancock JF and Warner RM (2010) Interactions of temperature and photoperiod determine expression of repeat flowering in strawberry. J Am Soc Hort Sci 135:102-107.

Brown T and Wareing PF (1965) The genetical control of the everbearing habit and three other characters in varieties of Fragaria vesca. Euphytica 14: 97-112.

Brown JAM and Klein WH (1971) Photomorphogenesis in *Arabidopsis thaliana* (L.) Heynh. Threshold intensities and blue- far-red synergism in floral induction. Plant Physiol 47: 393-399.

Butler WL, Norris KH, Siegelman HW and Hendricks SB (1959) Detection, assay, and preliminary purification of the pigment controlling photoresponsive development of plants. PNAS 45: 1703-1708.

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH and Nilsson O (2006) *CO/FT* regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312: 1040-1043.

Campoli C, Drosse B, Searle I, Coupland G and von Korff M (2012) Functional characterization of *HvCO1*, the barley (*Hordeum vulgare*) flowering time ortholog of *CONSTANS*. Plant J 69: 868-880.

Capovilla G, Schmid M and Posé D (2015) Control of flowering by ambient temperature. J Exp Bot 66: 59–69.

Carew JG, Gillespie T, White J, Wainwright H, Brennan R and Battey NH 2000. Techniques for manipulation of the annual growth cycle in raspberry. J Hort Sci Biotech 75: 504-509.

Carew JG, Mahmood K, Darby J, Hadley P and Battey NH (2001) The effects of low temperatures on the vegetative growth and flowering of the primocane fruiting raspberry 'Autumn Bliss'. J Hort Sci Biotech 76: 264-270.

Carew JG, Mahmood K, Darby J, Hadley P and Battey NH (2003) The effect of temperature, photosynthetic photon flux density, and photoperiod on the vegetative growth and flowering of 'Autumn Bliss' raspberry. J Hort Sci Biotech 128: 291-296.

Cashmore AR, Jarillo JA, Wu Y-J and Liu D (1999) Cryptochromes; Blue light receptors for plants and animals. Science 284: 760-765.

Cathey HM and Borthwick HA (1957) Photoreversibility of floral initiation in crysanthemum. Bot Gaz 119: 71-76.

Cekic C, Battey NH and Wilkinson MJ (2001) The potential of ISSR-PCR primer-pair combinations for genetic linkage analysis using the seasonal flowering locus in *Fragaria* as a model. Theor Appl Genet 103: 540-546.

Cérdan PD and Chory J (2003) Regulation of flowering time by light quality. Nature 423: 881-885

Cerny TA, Faust JE, Layne DR and Rajapakse NC (2003) Influence of photoselective films and growing season on stem growth and flowering of six plant species. J Am Soc Hort Sci 128: 486-491.

Chen A, Li C, Hu W, Lau MY, Lin H, Rockwell NC, Martin SS, Jernstedt JA, Lagarias JC, Dubcovsky J (2014) PHYTOCHROME C plays a major role in the

acceleration of wheat flowering under long-day photoperiod PNAS 111: 10037-10044.

Cheng G and Breen P (1991) Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J Am Soc Hort Sci 116: 865-869.

Chia TYP, Müller A, Jung C and Mutasa-Göttgens ES (2008) Sugar beet contains a large *CONSTANS-LIKE* gene family including a *CO* homologue that is independent of the early-bolting (*B*) gene locus. J Exp Bot 59: 2735-2748.

Chouard P (1960) Vernalization and its relations to dormancy. Ann Rev Plant Phys 11: 191-238

Christie J, Salomon M, Nozue K, Wada M and Briggs W (1999) LOV (light, oxygen, or voltage) domains of the blue light photoreceptor phototropin (nph1): binding sites for the chromophore flavin mononucleotide. PNAS 96: 8779-8783.

Clack T, Mathews S and Sharrock RA (1994) The phytochrome apoprotein family in *Arabidopsis* is encoded by five genes: the sequences and expression of *PHYD* and *PHYE*. Plant Mol Biol 25: 413-427.

Clifford SC, Runkle ES, Langton FA, Mead A, Foster SA, Pearson S, Heins RD (2004) Height control of poinsettia using photoselective filters. HortSci 39: 383-387.

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. Science 316: 1030-1033

Dale A and Blom TJ (2004) Far-red light alters primocane morphology of red raspberry. HortSci 39: 973-974.

Darrow GM (1966) The strawberry. History, breeding and physiology. Holt, Rinehart and Winston, New York, USA.

Darrow GM and Waldo GF (1934) Responses of strawberry varieties and species to duration of the daily light period. Technical bulletin 453: 1-32.

Demchak K (2009) Small fruit production in high tunnels. HortTech 19: 44-49.

Devlin PF, Halliday K, Harberd NP and Whitelam GC (1996) The rosette habit of Arabidopsis thaliana is dependent upon phytochrome action: novel phytochromes control internode elongation and flowering time. Plant J 10: 1127-1134.

Distelfeld A, Li C and Dubcovsky J (2009) Regulation of flowering in temperate cereals. Curr Op Plant Biol 12: 178-184.

Downs RJ (1956) Photoreversibility of flower initiation. Plant Phys 83: 279-284.

El-Assal SE-D, Alonso-Blanco C, Peeters AJM, Wagemaker C, Weller JL, and Koornneef M (2003). The role of Cryptochrome 2 in flowering in Arabidopsis. Plant Physiol 133: 1504–1516.

Endo M, Tanigawa Y, Murakami T, Araki T and Nagatani A (2013) PHYTOCHROME-DEPEDENT LATE-FLOWERING accelerates flowering

through physical interactions with phytochrome B and CONSTANS. PNAS 110: 18017-18022.

Eskins K (1992) Light-quality effects on *Arabidopsis* development. Red, blue and far-red regulation of flowering and morphology. Physiol Plantarum 86: 439-444.

FAOSTAT Crops. [web publication]. Food and Agriculture Organization of the United Nations. [Referred 9.1.2017]. Access method: http://www.fao.org/faostat/en/#data/QC

Ferl R and Paul A-L (2000) Genome organization and expression. In: Buchanan BB, Gruissem G and Jones RL (Eds.) Biochemistry& Molecular Biology. Am Soc Plant Phys, Maryland. pp 312-357.

Flachowsky H, Szankowski I, Waidmann S, Peil A, Tränkner C and Hanke M-H (2012) The *MdTFL1* gene of apple (*Malus*× *domestica* Borkh.) reduces vegetative growth and generation time. Tree Physiol 32: 1288-1301.

Fornara F, Panigrahi KCS, Gissot L, Sauerbrunn N, Rühl M, Jarillo JA and Coupland G (2009) *Arabidopsis* DOF transcription factors act redundantly to reduce *CONSTANS* expression and are essential for a photoperiodic flowering response. Develop Cell 17: 75-86.

Franklin KA, Davis SJ, Stoddarta WM, Vierstra RD and Whitelam GC (2003) Mutant analyses define multiple roles for phytochrome C in Arabidopsis photomorphogenesis. Plant Cell 15: 1981-1989.

Fredericq H (1964) Conditions determining effects of far-red and red irradiations on flowering response of *Pharbitis nil*. Plant Physiol 39: 812-816.

Fu J, Yang L and Dai S (2015) Identification and characterization of the *CONSTANS*-like gene family in the short-day plant *Chrysanthemum lavandulifolium*. Mol Genet Genomics 290: 1039-1054.

Garner WW (1933) Comparative responses of long-day and short-day plants to relative length of day and night. Plant Physiol 8: 347-356.

Garner WW and Allard HA (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. J Agr Res XVIII: 553-606.

Ghora Y, Vasilakakis M and Stavroulakis G (2000) Effect of growth retardants (cyclocel, daminozide and paclobtrazol) on growth and development of red raspberries, cv. 'Autumn Bliss' cultivated under plastic greenhouse conditions in Chania-Crete, Greece. Acta Hort 513: 453–458.

Gommers CMM, Visser EJW, St Onge KR, Voesenek L and Pierik R (2013) Shade tolerance: when growing tall is not an option. Trends Plant Sci 18: 65-71.

Goto N, Kumagai T and Koornneef M (1991) Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. Physiol Plantarum 83: 209-215.

Graham J, Smith K, McCallum S, Hedley PE, Cullen DW, Dolan A, Milne L, McNicol JW and Hackett CA (2015) Towards an understanding of the control of 'crumbly' fruit in red raspberry. SpringerPlus 4: 223.

Guo H, Yang H, Mockler TC, Lin C (1998) Regulation of flowering time by *Arabidopsis* photoreceptors. Science 279: 1360-1363

van Haeringen CJ, West JS, Davis FJ, Gilbert A, Hadley P, Pearson S, Wheldon AE and Henbest RGC (1998) The development of solid spectral filters for the regulation of plant growth. Photochem Photobiol 67: 407-413.

Hall IV and Ludwig RA (1961) The effects of photoperiod, temperature, and light intensity on the growth of the lowbush blueberry (Vaccinium angustifolium Ait.). Canad J Bot 39:1733-1739.

Halliday KJ, Koornneef M and Whitelam GC (1994) Phytochrome B and at least one other phytochrome mediate the accelerated flowering response of Arabidopsis thaliana L. to low red/far-red ratio. Plant Physiol 104: 1311-1315.

Hanson E, von Weihe MV, Schilder AC, Chanon AM and Scheerens JC (2011) High tunnel and open field prodcution of floricane- and primocane-fruiting raspberry cultivars. HortTech. 21: 412-418.

Hartmann HT (1947) Some effects of temperature and photoperiod on flower formation and runner production in the strawberry. Plant Physiol 22: 407–420.

Hayama R, Yokoi S, Tamaki S, Yano M and Shimamoto K. (2003) Adaptation of photoperiodic control produces short-day flowering in rice. Nature 422: 719-722.

Heiberg N, Lunde R, Nes A and Hageberg B (2008) Long cane production of red raspberry plants and effect of cold storage. Acta Hort 777: 225-230.

Heide OM (1977) Photoperiod and temperature interactions in growth and flowering of strawberry. Physiol Plant 40: 21-26.

Heide OM and Prestrud AK (2005) Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol 25: 109–114.

Heide OM and Sønsteby A (2007) Interactions of temperature and photoperiod in the control of flowering of latitudinal and altitudinal populations of wild strawberry (*Fragaria vesca*). Physiol Plant 130: 280-289.

Heide OM and Sønsteby A (2011) Physiology of flowering and dormancy regulation in annual- and biennial-fruiting red raspberry (*Rubus idaeus* L.) –a review. J Hort Sci Biotech 86: 433-442.

Hempel FD, Weigel D, Mandel MA, Ditta G, Zambryski PC, Feldman LJ and Yanofsky MF (1997) Floral determination and expression of floral regulatory genes in *Arabidopsis*. Development 124: 3845-3853.

Higuchi Y, Narumi T, Oda A, Nakano Y, Sumimoto K, Fukai S and Hisamatsu T (2013) The gated induction system of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemums. PNAS 110: 17137-17142.

Holefors A, Opseth L, Ree Rosnes AK, Ripel L, Snipen L, Fossdal CG, Olsen JE (2009) Identification of *PaCOL1* and *PaCOL2*, two *CONSTANS*-like genes

showing decreased transcript levels preceding short day induced growth cessation in Norway spruce. Plant Physiol Biochem 47: 105-115.

Holmes MG and Smith H (1975) The function of phytochrome in plants growing in the natural environment. Nature 254: 512-514.

Holmes MG and Smith H (1977) The function of phytochrome in the natural environment -I. Characterization of daylight for studies in photomorphogenesis and photoperiodism. Photochemistry and Photobiology 25: 533-538.

Horticultural Statistics [web publication]. Helsinki: Natural Resources Institute Finland [referred: 9.1.2017]. Access method: http://stat.luke.fi/en/horticultural-statistics

Hsu C-Y, Adams JP, Kim H, No K, Ma C, Strauss SH, Drnevich J, Vandervelde L, Ellis JD, Rice BM, Wickett N, Gunter LE, Tuskan GA, Brunner AM, Page GP, Barakat A, Carlson JE, de Pamphilis CW, Luthe DS and Yuceer C (2011) *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. PNAS 108: 10756-10761.

Hudson JP (1959) Effects of environment on *Rubus idaeus* L. I. Morphology and development of the raspberry plant. J Hort Sci 34: 163–169.

Häkkinen S, Heinonen M, Kärenlampi S, Mykkänen H, Ruuskanen J and Törrönen R (1999) Screening of seleceted flavonoids and phenolic acids in 19 berries. Food Res Int 32: 345-353.

Imaizumi T, Tran HG, Swartz TE, Briggs WR and Kay SA (2003) FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. Nature 426: 302–306.

Imaizumi T, Schultz TF, Harmon FG, Ho LA, and Kay SA (2005). FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*. Science 309: 293–297.

Iñigo S, Alvarez MJ, Strasser B, Califano A and Cerdán PD (2012) PFT1, the MED25 subunit of the plant Mediator complex, promotes flowering through CONSTANS dependent and independent mechanisms in Arabidopsis. Plant J 69: 601-612.

Ishikawa R, Aoki M, Kurotani K, Yokoi S, Shinomura T, Takano M and Shimamoto K (2011) Phytochrome B regulates *Heading date 1* (*Hd1*)-mediated expression of rice florigen *Hd3a* and critical day length in rice. Mol Gen Genetics 285: 461-470.

Islam MA, Tarkowska D, Clarke JL, Blystad D-R, Gislerod HR, Torre S and Olsen JE (2014) Impact of end-of-day red and far-red light on plant morphology. Sci Hort 174: 77-86.

Ito H and Saito T (1962) Studies on the flower formation in the strawberry plants I. Effects of temperature and photoperiod on the flower formation. Tohoku J Agr Res 13:191–203.

Ito S, Song YH and Imaizumi T (2012) LOV-domain-containing F-box proteins: Light-dependent protein degradation modules in *Arabidopsis*. Mol Plant 5: 573-582.

Ito A, Saito T, Nishijima T and Moriguchi T (2014) Effect of extending the photoperiod with low-intensity red or far-red light on the timing of shoot elongation and flower-bud formation of 1-year-old Japanese pear (*Pyrus pyrifolia*). Tree Physiol 34: 534-546.

Itoh H, Nonoue Y, Yano M and Izawa T (2010) A pair of floral regulators sets critical day length for *Hd3a* florigen expression in rice. Nature Genetics 42: 635-638.

Iwata H, Gaston A, Remay A, Thouroude T, Jeauffre J, Kawamura K, Oyant L, Araki T, Denoyes B and Foucher F (2012) The *TFL1* homologue *KSN* is a regulator of continuous flowering in rose and strawberry. Plant J 69: 116–125.

Jaakola L, Määttä K, Pirttilä A-M, Törrönen R, Kärenlampi S, and Hohtola A. (2002) Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. Plant Physiol 130: 729-739.

Jennings D (1988) Raspberries and blackberries: Their breeding, diseases and growth. Academic Press, London.

Johnson E, Bradley M, Harberd N and Whitelam G (1994) Photoresponses of light – grown *phyA* mutants of *Arabidopsis*. Plant Physiol 105: 141-149.

Jahn O and Dana M (1970) Crown and inflorescence development in the strawberry, Fragaria ananassa. Amer J Bot 57: 605-612.

Jang S, Marchal V, Panigrahi K, Wenkel S, Soppe W, Deng, X-W, Valverde F and Coupland G. (2008). *Arabidopsis* COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. EMBO J 27: 1277–1288.

Jarillo J and Piñeiro M (2015) H2A.Z mediates different aspects of chromatin function and modulates flowering responses in Arabidopsis. Plant J 83: 96-109.

Johansen N, Vänninen I, Pinto D, Nissinen, A and Shipp L (2011) In the light of new greenhouse technologies: 2. Direct effects of artificial lighting on arthopods and integrated pest management in greenhouse crops. Ann Appl Biol 159: 1-27.

Kadomura-Ishikawa Y, Miyawaki K, Noji S and Takahashi A (2013) *Photoropin2* is involved in blue light-induced anthocyanin accumulation in *Fragaria* × *ananassa* fruits. J Plant Res 126: 847-857.

Keep E (1961) Autumn fruiting in raspberries. J Hort Sci 36: 174-185.

Kircher S, Gil P, Kozma-Bognár L, Fejes E, Speth V, Husselstein-Muller T, Bauer D, Ádám E, Schäfer E and Nagy F (2002) Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D, and E is regulated differentially by light and exhibits a diurnal rhythm. Plant Cell 14: 1541-1555.

Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC and Franklin KA (2009) High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. Curr Biol 19: 408-413.

Komiya R, Yokoi S and Shimamoto K (2009) A gene network for long-day flowering activates *RFT1* encoding a mobile flowering signal in rice. Development 136: 3443-3450.

Kondo S, Tomiyama H, Rodyoung A, Okawa K, Ohara H, Sugaya S, Terahara N and Hirai N (2014) Abscisic acid metabolism and anthocyanin synthesis in grape skin are affected by light emitting diode (LED) irradiation at night. J Plant Physiol 171: 823–829.

Konsin M, Voipio I and Palonen P (2001) Influence of photoperiod and duration of short-day treament on vegetative grwoth and flowering of strawberry (*Fragaria* × *ananassa* Duch). J Hort Sci Biotech 76: 77-82.

Koornneef M, Hanhart CJ and van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol Gen Genet 229: 57-66.

Koskela EA, Mouhu K, Albani MC, Kurokura T, Rantanen M, Sargent DJ, Battey NH, Coupland G, Elomaa P and Hytonen T (2012) Mutation in *TERMINAL FLOWER1* reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. Plant Physiol 159: 1043-1054.

Koskela EA, Sønsteby A, Flachowsky H, Heide OM, Hanke M-V, Elomaa P and Hytönen T (2016) *TERMINAL FLOWER1* is a breeding target for a novel everbearing trait and tailored flowering responses in cultivated strawberry (*Fragaria* × *ananassa* Duch.). Plant Biotechnol J 14:1852-1861

Koyama K, Ikeda H, Poudel PR and Goto-Yamamoto N (2012) Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. Phytochem 78: 54–64.

Kumar SV and Wigge PA (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in Arabidopsis. Cell 140: 136-147.

Kumar SV, Lucyshyn D, Jaeger KE, Alós E, Alvey E, Harberd NP and Wigge PA (2012) Transcription factor PIF4 controls the thermosensory activation of flowering. Nature 484: 242-246.

Kurepin LA, Walton LJ, Hayward A, Emery RJN, Pharis RP and Reid DM. (2012) Interactions between plant hormones and light quality signaling in regulating the shoot growth of Arabidopsis (I) thaliana seedlings. Botany 90: 237-246.

Lane HC, Cathey HM and Evans LT (1965) The dependence of flowering in several long-day plants on the spectral composition of light extending the photoperiod. Amer J Bot 52: 1006-1014.

Laubinger S, Marchal V, Gentilhomme J, Wenkel S, Adrian J, Jang S, Kulajta C, Braun H, Coupland G and Hoecker U (2006) *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. Development 133: 3213-3222

Lazaro A, Valverde F, Piñeiro M and Jarillo JA (2012) The Arabidopsis E3 ubiquitin ligase HOS1 negatively regulates CONSTANS abundance in the photoperiodic control of flowering. Plant Cell 24: 982-999.

Lazaro A, Mouriz A, Piñeiro M and Jarillo JA (2015) Red light-mediated degradation of CONSTANS by the E3 ubiquitin ligase HOS1 regulates photoperiodic flowering in Arabidopsis. Plant Cell 27: 2437-2454.

Ledger S, Strayer C, Ashton F, Kay SA and Putterill J (2001) Analysis of the function of two circadian-regulated *CONSTANS-LIKE* genes. Plant J 26: 15-22

Lee H, Suh S-S, Park E, Cho E, Ahn JH, Kim S-G, Lee JS, Kwon YM and Lee I (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Genes Dev 14: 2366-2376.

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS and Ahn JH (2007) Role of *SVP* in the control of flowering time by ambient temperature in *Arabidopsis*. Genes Dev 2: 397-402.

Lee J and Lee I (2010) Regulation and function of SOC1, a flowering pathway integrator J Exp Bot 61: 2247-2254.

Lee JOM, Par H and Lee I (2008), SOC1 translocated to the nucleus by interaction with AGL24 directly regulates *LEAFY*. Plant J 55: 832–843.

Lee JH, Ryu H-S, Chung KS, Posé D, Kim S, Schmid M and Ahn JH (2013) Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. Science 342: 628-632.

Lee JH, Chung KS and Ahn JH (2014) Post-translational regulation of SHORT VEGETATIVE PHASE as a major mechanism for thermoregulation of flowering. Plant Signal Behav 9: e28193

Li S, Rajapakse NC, Young RE and Oi R (2000) Growth responses of chrysanthemum and bell pepper transplants to photoselective plastic films. Sci Hort 84: 215-225.

Li J, Gang L, Wang H and Deng XW (2011) Phytochrome signaling mechanisms. The Arabidopsis Book 2011:e0148. The American Society of Plant Biologists.

Lin C, Robertson DE, Ahmad M, Raibekas AA, Jorns MS, Dutton PL and Cashmore AR (1995) Association of flavin adenine dinucleotide with the *Arabidopsis* blue light receptor CRY1. Science 269: 968-970.

Liston A, Cronn R and Ashman T-L (2014) *Fragaria*: A genus with deep historical roots and ripe for evolutionary and ecological insights. Am J Bot 101: 1686-1699.

Liu J, Yu J, McIntosh L, Kende H and Zeevart AD (2001) Isolation of a *CONSTANS* ortholog from *Pharbitis nil* and its role in flowering. Plant Physiol 125: 1821-1830.

Liu H, Yu X, Li K, Klejnot J, Yang H, Lisiero D and Lin C (2008a) Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in *Arabidopsis*. Science 322: 1535-1539.

Liu L-J, Zhang Y-C, Li Q-H, Sang Y, Mao J, Lian H-L, Wang L and Yang H-Q (2008b) COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in *Arabidopsis*. Plant Cell 20: 292-306

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Methods 25: 402–408.

Longhi S, Giongo L, Buti M, Surbanovksi N, Viola R, Velasco R, Ward JA and Sargent DJ (2014) Molecular genetics and genomics of the Rosoideae: state of the art and future perspectives. Horticulture Res 1: 1.

Lopéz-Juez E, Kobayashi M, Sakurai A, Kamiya Y and Kendrick RE (1995) Phytochrome and hypocotyl growth. Plant Physiol 107: 131-140.

Maga JA and Katz I (1978) Simple phenol and phenolic compounds in food flavor. CRC Critical Rev Food Sci Nutr 10: 323-372.

Måge F (1986) Growth responses and physiological studies in red raspberry plants treated with growth regulators. Acta Hort 183: 291-296.

Mandel MA and Yanofsky MF (1995) The Arabidopsis *AGL8* MADS box gene is expressed in inflorescence meristems and is negatively regulated by *APETALA1*. Plant Cell 7: 1763-1771.

Martinez-Zapater JM and Somerville CR (1990) Effect of light quality and vernalization on late-flowering mutants of *Arabidopsis thaliana*. Plant Physiol 92: 770-776

Mata DA and Botto JF (2009) Manipulation of light environment to produce high-quality poinsettia plants. HortSci 44: 702-706.

Michaels SD and Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11: 949-956

Mockler T, Guo H, Yang H, Duong H, and Lin C (1999) Antagonistic actions of *Arabidopsis* cryptochromes and phytochrome B in the regulation of floral induction. Development 126: 2073-2082.

Mockler T, Yang HY, Yu XH, Parikh D, Cheng YC, Dolan S, and Lin CT (2003) Regulation of photoperiodic flowering by Arabidopsis photoreceptors. PNAS 100: 2140-2145

Monte E, Alonso JM, Ecker JR, Zhang Y, Li X, Young J, Austin-Philips S and Quail PH (2003). Isolation and characterization of *phyC* mutants in Arabidopsis reveals complex crosstalk between phytochrome signaling pathways. Plant Cell 15: 1962–1980.

Monte D and Somerville S (2002) Pine tree method for isolation of plant RNA. In: Bowtel D and Sambrook J (eds.) DNA microarrays: A molecular Cloning Manual. New York, NY: Cold Springer Harbour Laboratory Press. 124-126.

Moon J, Lee H, Kim M and Lee I (2005) Analysis of flowering pathway integrators in Arabidopsis. Plant Cell Physiol 46: 292-299.

Mouhu K, Hytönen T, Folta K, Rantanen M, Paulin L, Auvinen P and Elomaa P (2009) Identification of flowering genes in strawberry, a perennial SD plant. BMC Plant Biol 9:122.

Mouhu K, Kurokura T, Koskela EA, Albert VA, Elomaa P and Hytönen T (2013) The *Fragaria vesca* homolog of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 represses flowering and promotes vegetative growth. Plant Cell 25: 3296-3310

Möglich A, Yang X, Ayers RA and Moffat K (2010) Structure and function of plant photoreceptors. Ann Rev Plant Biol 61: 21-47.

Nakajima R, Otagaki S, Yamada K, Shiratake K and Matsumoto S (2014) Molecular cloning and expression analyses of *FaFT*, *FaTFL*, and *FaAP1* genes in cultivated strawberry: their correlation to flower bud formation. Biol Plant 58: 641-648.

Nakano Y, Higuchi Y, Yoshida Y and Hisamatsu T (2015) Environmental responses of the *FT/ TFL1* gene family and their involvement in flower induction in *Fragaria* × *ananassa*. J Plant Physiol 177: 60-66.

Nelson DC, Lasswell J, Rogg LE, Cohen MA and Bartel B (2000) *FKF1*, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. Cell 101: 331-340.

Nelson JA and Bugbee B (2015) Analysis of environmental effects on leaf temperature under sunlight, high pressure sodium and light emitting diodes. PLoS ONE 10: e0138930.

Oda A, Narum, T, L, T, Kando T, Higuchi Y, Sumimoto K, Fukai S and Hisamatsu T (2012) *CsFTL3,* a chrysanthemum *FLOWERING LOCUS T-LIKE* gene, is a key regulator of photoperiodic flowering in chrysanthemums. J Exp Bot 63: 1461-1477.

Olsen JE and Junttila O (2002) Far red end-of-day treatment restores wild type-like plant length in hybrid aspen overexpressing phytochrome A. Physiol Plantarum 115: 448-457.

Olsen JE, Junttila O, Nilsen J, Eriksson ME, Martinussen I, Olsson O, Sandberg G and Moritz T (1997) Ectopic expression of oat phytochrome aspen changes critical daylength for growth and prevents cold acclimatization. Plant J 12: 1339-1350.

Oosumi T, Gruszewski HA, Blischak LA, Baxter AJ, Wadl PA, Shuman JL, Veilleux RE and Shulaev V (2006). High-efficiency transformation of the diploid strawberry (*Fragaria vesca*) for functional genomics. Planta 223: 1219-1230.

Ordidge M, Garcia-Macias P, Battey NH, Gordon MH, Hadley P, John P, Lovegrove JA, Vysini E and Wagstaffe A (2010) Phenolic contents of lettuce, strawberry, raspberry, and blueberry crops cultivated under plastic films varying in ultraviolet transparency. Food Chem 119: 1224–1227.

Palonen P and Mouhu K. (2009) Vegetative growth and flowering of primocane raspberry 'Ariadne' as affected by prohexadione-calcium. HortSci 44: 529-531.

Palonen P, Pehkonen E and Rantanen M (2013) Growth control of 'Glen Ample' and 'Tulameen' raspberry cultivars with single and repeated ProCa applications. Europ J Hort Sci 78:22–29.

Palonen P, Pohjola M and Karhu S (2015) Cropping potential of raspberry long-cane plants is affected by their growing conditions and duration of cold storage. J Hort Sci Biotech 90:738-746.

Palonen P, Pinomaa A and Tommila T (2017) The influence of high tunnel on yield and berry quality in three floricane raspberry cultivars. Sci Hort 214: 180-186.

Park DH, Somers DE, Kim YS, Choy YH, Lim HK, Soh MS, Kim HJ, Kay SA and Nam HG (1999) Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. Science 3: 1579-1582.

Peitsch D, Fietz A, Hertel H, deSouza J, Ventura D-F and Menzel R (1992) The spectral systems of hymenopteran insects and their receptor-based colour vision. J Comp Physiol A 170:23-40

Pin PA, Benlloch R, Bonnet D, Wremerth-Weich E, KraftT, Gielen, JJL and Nilsson O (2010) An antagonistic pair of *FT* homologs mediates the control of flowering time in sugar beet. Science 330: 1397-1400.

Pierik R, Keuskamp DH, Sasidharan R, Djakovic-Petrovic T, de Wit M and Voesenek L (2009) Light quality controls shoot elongation through regulation of multiple hormones. Plant Signal Behav 4: 755-756.

Poethig RS (2003) Phase change and the regulation of developmental timing in plants. Science 301: 334-336.

Posé D, Verhage L, Ott F, Yant L, Mathieu J, Angement GC, Immink RGH and Schmid M (2013) Temperature –dependent regulation of flowering by antagonistic FLM-variants. Nature 503: 414–417.

Proveniers MCG and van Zanten M (2013) High temperature acclimation through PIF4 signaling. Trends Plant Sci 18: 59-64.

Putterill J, Robson F, Lee K, Simon R and Coupland G (1995) The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80: 847-857.

Rajapakse NC, Young RE, McMahon MJ and Oi R (1999). Plant height control by photoselective filters: Current status and future prospects. HortTech. 9: 618–624.

Redalen G (1981) Influence of GA_3, number of canes and cane height on fruit set and drupelet set in raspberries. Meldinger fra Norges Landburkshøgskole 60, nr 106.

Redon C, Pilch D, ROgakou E, Sedelnikova O, Newrock K and Bonner W (2002) Histone H2A variants H2AX and H2AZ. Curr Op Gen Develop 12: 162-169.

Reed JW, Nagpal P, Poole DS, Furuya M and Chory J (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell 5: 147-157.

Reed JW, Nagatani A, Elich TD, Fagan M and Chory J (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol 104: 1139-1149.

Ricard J-P and Messier C (1996) Abundance, growth and allometry of red raspberry (*Rubus idaeus* L.) along a natural light gradient in a northern hardwood forest. Forest Ecol Manag 81: 153-160.

Runkle ES, Heins RD, Cameron AC and Carlson WH (1998) Flowering of herbaceous perennials under various night interruption and cyclic lighting treatments. HortSci 33: 672-677.

Runkle ES and Heins RD (2002) Stem extension and subsequent flowering of seedlings grown under a film creating a far-red defcient environment. Sci Hort 96: 257-265.

Salomon M, Christie JM, Knieb E, Lempert U, and Briggs WR (2000) Photochemical and mutational analysis of the FMN-binding domains of the plant blue receptor, phototropin. Biochemistry 39: 9401-9410.

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF and Coupland G (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science 288: 1613-1616.

Šavikin K, Mikulič-Petkovšek M, Djordjević B, Zdunić G, Janković T, Djurović D and Veberič R (2013) Influence of shading net on polyphenol profile and radical scavenging activity in different varieties of black currant berries. Sci Hort 160: 20-28.

Sawa M, Nusinow DA, Kay SA and Imaizumi T (2007) FKF1 and GIGANTEA complex formation is required for daylength measurement in *Arabidopsis*. Science 318: 261–265.

Sawa M and Kay SA (2011) GIGANTEA directly activates *Flowering Locus T* in *Arabidopsis thaliana*. PNAS 108: 11698-11703.

Scortecci KC, Michaels SD and Amasino RM (2001), Identification of a MADS-box gene, *FLOWERING LOCUS M*, that represses flowering. Plant J 26: 229–236.

Scortecci KC, Michaels SD and Amasino RM (2003) Genetic interactions between *FLM* and other flowering time genes in *Arabidopsis thaliana*. Plant Mol Biol 52: 915-922.

Sellaro R, Crepy M, Trupkin SA, Karayekov E, Buchovsky AS, Rossi C and Casal JJ (2010) Cryptochrome as a sensor of the blue/green ratio of natural radiation in Arabidopsis. Plant Physiol 154: 401-409.

Sellaro R, Pacín M and Casal JJ (2012) Diurnal dependence of growth responses to shade in *Arabidopsis*: Role of hormone, clock, and light signaling. Mol Plant 5: 619-628.

Serçe S and Hancock JF (2005) The temperature and photoperiod regulation of flowering and runnering in the strawberries, *Fragaria chiloensis*, *F.virginiana*, and *F.× ananassa*. Sci Hort 103: 167-177.

Sharrock RA and Quail PH (1989) Novel phytochrome sequences in *Arabidopsis thaliana*: structure,evolution, and differential expression of a plant regulatory photoreceptor family. Genes Develop 3: 1745-1757.

Shulaev V, Sargent DJ, Crowhurst RN, Mockler TC, Folkerts O, Delcher AL, Jaiswal P, Mockaitis K, Liston A, Mane SP, Burns P, Davis TM, Slovin JP, Bassil N,

Hellens RP, Evans C, Harkins T, Kodira C, Desany B, Crasta OR, Jensen RV, Allan AC, Michael TP, Setubal JC, Celton J, Rees DJG, Williams KP, Holt SH, Rojas JJR, Chatterjee M, Liu B, Silva H, Meisel L, Adato A, Filichkin SA, Troggio M, Viola R, Ashman T, Wang H, Dharmawardhana P, Elser J, Raja R, Priest HD, Bryant DW, Jr., Fox SE, Givan SA, Wilhelm LJ, Naithani S, Christoffels A, Salama DY, Carter J, Girona EL, Zdepski A, Wang W, Kerstetter RA, Schwab W, Korban SS, Davik J, Monfort A, Denoyes-Rothan B, Arus P, Mittler R, Flinn B, Aharoni A, Bennetzen JL, Salzberg SL, Dickerman AW, Velasco R, Borodovsky M, Veilleux RE, Folta KM (2011) The genome of woodland strawberry (*Fragaria vesca*). Nat Genet 43: 109-116.

Simpson GG and Dean C (2002) Arabidopsis, the Rosetta stone of flowering time? Science 296: 285-289.

Smith H (1982) Light quality, photoreception, and plant strategy. Annual Rev Plant Physiol 33: 481-518.

Smith H (2000) Phytochromes and light signal perception by plants –an emerging synthesis. Nature 407: 585-591.

Smith H and Whitelam GC (1997) The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. Plant, Cell Env 20: 840-844

Somers DE, Devlin PF and Kay SA (1998) Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. Science 282: 1488-1490.

Somers DE, Schulz TF, Milnamow M and Kay SA (2000) *ZEITLUPE* encodes a novel clock associated PAS protein from *Arabidopsis*. Cell 101: 319-329.

Somers DE, Kim WY and Geng R (2004) The F-box protein ZEITLUPE confers dosage-dependent control on the circadian clock, photomorphogenesis, and flowering time. Plant Cell 16: 769–782.

Song YH, Smith RW, To BJ, Millar AJ and Imaizumi T (2012) FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. Science 336: 1045-1049.

Song YH, Shim JS, Kinmonth-Schulz HA and Imaizumi T (2015) Photoperiodic flowering: Time measurement mechanisms in leaves. Annu Rev Plant Biol 66:441-464.

Sønsteby A and Nes A (1998) Short days and temperature effects on growth and flowering in strawberry (*Fragaria* × *ananassa* Duch.). J Hort Sci Biotech 73: 730-736.

Sønsteby A and Heide OM (2007a) Quantitative long-day flowering response in the perpetual-flowering F1 strawberry cultivar Elan. J Hort Sci Biotech 82: 266-274.

Sønsteby A and Heide OM (2007b) Long-day control of flowering in everbearing strawberries. J Hort Sc Biotech 82: 875-884.

Sønsteby A and Heide OM (2008a) Environmental control of growth and flowering of *Rubus idaeus* L. cv. Glen Ample. Sci Hort 117: 249-256.

Sønsteby A and Heide OM (2008b) Long-day rather than autonomous control of flowering in the diploid everbearing strawberry *Fragaria vesca* ssp. *semperflorens*. J Hort Sci Biotech 83: 360–366.

Sønsteby A and Heide OM (2008c) Flowering physiology of populations of *Fragaria virginiana*. J Hort Sci Biotech 83: 641-647.

Sønsteby A and Heide OM (2009a) Environmental control of flowering and runnering in three contrasting populations of *Fragaria chiloensis*. J Hort Sci Biotech 84: 267-272.

Sønsteby A and Heide OM (2009b) Effects of photoperiod and temperature on growth and flowering in the annual (primocane) fruiting raspberry (*Rubus idaeus* L.) cultivar 'Polka'. J Hort Sci Biotech 84: 439-446.

Sønsteby A and Heide OM (2012) Effects of photoperiod and temperature on growth, flowering, and fruit yield in annual-fruiting red raspberry cultivars (*Rubus idaeus* L.). Europ J Hort Sci 77: 97-108.

Sønsteby A and Heide OM (2014) Cold tolerance and chilling requirements for breaking of bud dormancy in plants and severed shoots of raspberry (*Rubus idaeus* L.). J Hort Sci Biotechnol 89: 631-638

Sønsteby A, Myrheim U, Heiberg N and Heide OM (2009) Production of high yielding red raspberry long canes in a Northern climate. Sci Hort 121: 289-297.

Sønsteby A, Stavang JA and Heide OM (2013) Production of high-yielding raspberry long canes: The way to 3 kg of fruit per cane. J Hort Sci Biotech 88: 591-599.

Sønsteby A, Stavang JA and Heide OM (2016) Functional growth analysis of 'Sonata' strawberry plants grown under controlled temperature and daylength conditions. Sci Hort 211: 26-33.

Strasser B, Sánchez-Lamas M, Yanovsky MJ, Casal JJ and Cerdán PD (2010) *Arabidopsis thaliana* life without phytochromes. PNAS 107: 4776-4781.

Strasser B, Alvarez MJ, Califano A and Cerdán PD (2009) A complementary role for ELF3 and TFL1 in the regulation of flowering time by ambient temperature. Plant J 58: 629–640.

Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2001) *CONSTANS* mediates between the circadian clock and the control of flowering in Arabidopsis. Nature 410: 1116-1120.

Takano M, Inagaki N, Xie X, Kiyota S, Baba-Kasai A, Tanabata T, Shinomura T (2009) Phytochromes are the sole photoreceptors for perceiving red/far-red light in rice. PNAS 106: 14705-14710.

Takase T, Nishiyama Y, Tanihigashi H, Ogura Y, Miyazaki Y, Yamada Y and Kiyosue T (2011) *LOV KELCH PROTEIN2* and *ZEITLUPE* repress Arabidopsis photoperiodic flowering under non-inductive conditions, dependent on *FLAVIN-BINDING KELCH REPEAT F-BOX1*. Plant J 67: 608–621.

Takeda F (1993) Chilling affects flowering of primocane-fruiting 'Heritage' red raspberry. Acta Hort 352: 247-252.

Takimoto A and Hamner K (1965) Effect of far-red light and its interaction with red light in the photoperiodic response of Pharbitis nil. Plant Physiol 40: 859-864.

Talbert PB and Henikoff S Histone variants –ancient wrap artists of the epigenome. Nature rev 11: 264-275.

Tamaki S, Matsuo S, Wong HL, Yokoi S and Shimamoto K (2007) Hd3a protein is a mobile flowering signal in rice. Science 316: 1033-1036.

Taoka K, Ohki I, Tsuji H, Furuita K, Hayashi K, Yanase T, Yamaguchi M, Nakashima C, Purwestri YA, Tamaki S, Ogaki Y, Shimada C, Nakagawa A, Kojima C and Shimamoto K (2011) 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature 476, 332-335.

Taoka K, Ohki I, Tsuji H, Kojima C and Shimamoto K (2013) Structure and function of florigen and the receptor complex. Trends Plant Sci 18: 287-294.

Tomás-Barberán FA and Espín JC (2001) Phenolic compounds and related enzymes as determines of quality in fruits and vegetables. J Sci Food Agricult 81: 853-876.

Thomas B and Vince-Prue D (1997) Photoperiodism in plants. 2nd Ed. Academic Press, San Diego.

Trevaskis B, Hemming MN, Dennis ES and Peacock WJ (2007) The molecular basis of vernalization-induced flowering in cereals. Trends Plant Sci 12: 352-357.

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A and Coupland G (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science 303: 1003-1006.

Van Buren R, Bryant D, Bushakra JM, Vining KJ, Edger PP, Rowley ER, Priest HD, Michael TP, Lyons E, Filichkin SA, Dossett M, Finn CE, Bassil NV and Mockler TC (2016) The genome of black raspberry (*Rubus occidentalis*). Plant J 87: 535–547.

Vasilakakis MD, McCown BH and Dana MN (1980) Low temperature and flowering of primocane-fruiting red raspberries. HortSci 15: 750-751.

Verheul MJ, Sønsteby A and Grimstad SO (2007) Influences of day and night temperatures on flowering of *Fragaria* × *ananassa* Duch.,cvs. Korona and Elsanta, at different photoperiods. Sci Hort 112: 200-206.

Vince D (1965) The promoting effect of far-red light on flowering in the long day plant *Lolium temulentum*. Plant Physiol 18: 474-482.

Vince-Prue D and Guttridge CG (1973) Floral initiation in strawberry -Spectral evidence for regulation of flowering by long-day inhibition. Planta 110: 165-172

Vänninen I, Pinto DM, Nissinen AI, Johansen NS and Shipp L (2010) In the light of new greenhouse technologies: 1. Plant-mediated effects of artificial lighting on anthropods and tritrophic interactions. Ann Appl Biol 157: 393-414.

Wang J-W, Czech B and Weigel D (2009a) miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. Cell 138: 738-749.

Wang R, Farrona S, Vincent C, Joecker A, Schoof H, Turck F, Alonso-Blanco C, Coupland G and Albani MC (2009b) *PEP1* regulates perennial flowering in *Arabis alpina*. Nature 459: 423-427.

Wang J-W, Park MY, Wang L-J, Koo Y, Chen X-Y, Weigel D and Poethig RS (2011) MiRNA control of vegetative phase change in trees. PLoS Genet 7(2): e1002012.

Wang H and Wang H (2015) Phytochrome signaling: Time to tighten up the loose ends. Mol Plant 8: 540-551.

Wareing PF (1956) Photoperiodism in woody plants. Ann Rev Plant Pysiol 7: 191-214.

Wareing PF (1959) Prolems of juvenility and flowering in trees. Bot J 56: 282-289.

Watson R, Wright CJ, McBurney T, Taylor AJ and Linforth RST (2002) Influence of harvest date and light integral on the development of strawberry flavour compunds. J Exp Bot 53: 2121-2129.

Wigge PA, Kim MC, Jaeger KE, Schmid, M, Lohmann JU and Weigel D (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science 309: 1056-1059.

Williams IH (1959a) Effects of environment on *Rubus idaeus* L. II. Field observations on the variety Malling Promise. J Hort Sci 34: 170-175.

Williams IH Effects of environment on *Rubus idaeus* L. III. Growth and dormancy of young shoots. J Hort Sci 34: 210—217.

Williams IH (1959c) Effects of environment on *Rubus idaeus* L. IV. Flower initiation and development of the inflorescence. J Hort Sci 34: 219-228.

Williams IH (1960) Effects of environment on *Rubus idaeus* L. V.Dormancy and flowering of the mature shoot. J Hort Sci 35: 214-220.

Williams IH and Hudson JP (1956) Effect of environment upon the growth and development of raspberry canes. Nature 177: 798-799.

Wollenberg AC, Strasser B, Cerdán PD and Amasino RM (2008) Acceleration of flowering during shade avoidance in Arabidopiss alters the balance between FLOWERING LOCUS C-mediated repression and photoperiodic induction of flowering. Plant Physiol 148: 1681-1694.

Woods DP, Ream TS, Minevich G, Hobert O and Amasino RM (2014) PHYTOCHROME C is an essential light receptor for photoperiodic flowering in the temperate grass, *Brachypodium distachyon*. Genetics 198: 397-408.

Wu F, Price BW, Haider W, Seufferheld G, Nelson R & Hanzawa Y (2014) Functional and evolutionary characterization of the *CONSTANS* gene family in short-day photoperiodic flowering in soybean. PLoS ONE 9(1): e85754.

Zhang B, Pan X, Cobb GP and Anderson TA (2006) Plant microRNA: A small regulatory molecule with big impact. Develop Biol 289: 3–16.

Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J and Jacobsen SE (2007) Whole-genome analysis of histone H3 lysine 27 trimethylation in *Arabidopsis*. PLoS Biol 5: e129.

Zhou CM, Zhang T-Q, Wang X, Yu S, Lian H, Tang H, Feng Z-Y, Zozomova-Lihová J and Wang J-W (2013) Molecular basis of age-dependent vernalization in *Cardamine flexuosa*. Science 340: 1097-1100.

Zoratti L, Sarala M, Carvalho E, Karppinen K, Martens S, Giongo L, Häggman H and Jaakola L (2014) Monochromatic light increases anthocyanin content during fruit development in bilberry. BMC Plant Biol 14:377.

Zoratti L, Jaakola L, Häggman H, Giongo L (2015) Modification of sunlight radiation through colored photoselective nets affects anthocyanin profile in *Vaccinium* spp. berries. PLoS ONE 10(8): e0135935.

Zuo Z, Liu H, Liu B, Liu X, and Lin C (2011) Blue light-dependent interaction of CRY2 with SPA1 regulates COP1 activity and floral initiation in Arabidopsis. Current Biol 21: 841-847.

Yanovsky MJ and Kay SA (2002) Molecular basis of seasonal time measurement in Arabidopsis. Nature 419: 308-312.

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y and Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12: 2473-2483.

Yang S, Weers BD, Morishige DT and Mullet JE (2014) *CONSTANS* is a photoperiod regulated activator of flowering in sorghum. BMC Plant Biol 14: 148.

Yao S and Rosen CJ (2011) Primocane-fruiting raspberry production in high tunnels in a cold region of the Upper Midwestern United States. HortTech 21: 429-434

Yoo SK, Chung KS, Kim J, Lee JH, Hong SM, Yoo S, Yoo SY, Lee JS and Ahn JH (2005) *CONSTANS* activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* through *FLOWERING LOCUS T* to promote flowering in Arabidopsis. Plant Physiol 139: 770-778.

Recent Publications in this Series

24/2016 Anu Riikonen
Some Ecosystem Service Aspects of Young Street Tree Plantings
25/2016 Jing Cheng
The Development of Intestinal Microbiota in Childhood and Host-Microbe Interactions in Pediatric Celiac Diseases
26/2016 Tatiana Demina
Molecular Characterization of New Archaeal Viruses from High Salinity Environments
27/2016 Julija Svirskaitė
Life in Extreme Environments: Physiological Changes in Host Cells during the Infection of *Halophilic archaeal* Viruses
1/2017 Pauliina Kokko
Towards more Profitable and Sustainable Milk and Beef Production System
2/2017 Juha Kontturi
Type III Polyketide Synthases from *Gerbera hybrida*
3/2017 Pauliina Kärpänoja
Antimicrobial Resistance in the Major Respiratory Tract Pathogens - Methods and Epidemiology
4/2017 Clara Isabel Lizarazo Torres
Diversifying Boreal — Nemoral Cropping Systems for Sustainable Protein Production
5/2017 Festus Anasonye
Treatment of Organic Aromatic Contaminants in Soil with Fungi and Biochar
6/2017 Essi Päivärinta
Cloudberry (*Rubus chamaemorus*) and Its Components as Chemopreventive Constituents in *Apc*^{Min} Mice and Human Colon Adenocarcinoma Cells
7/2017 Tuija Kantala
Presence of Hepatitis E Virus (HEV) and Markers for HEV Infection in Production Swine, Human Patients with Unexplained Hepatitis, and Veterinarians in Finland
8/2017 Mantas Survila
Immune Responses to Pathogen Infection in Arabidopsis
9/2017 Jessica M. Coyne
Genetics of Longitudinal Growth Data
10/2017 Xing Wan
Leuconostoc bacteriocins and Their Application in Genome Editing
11/2017 Inka Reijonen
Chemical Bioavailability of Chromium and Vanadium Species in Soil: Risk Assessment of the Use of Steel Industry Slags as Liming Materials
12/2017 Senja Laakso
Practice Approach to Experimental Governance. Experiences from the Intersection of Everyday Life and Local Experimentation
13/2017 Paulina Deptula
A Multifaceted Study of Propionibacterium Freudenreichii, the Food-Grade Producer of Active Vitamin B12
14/2017 Taneli Tirkkonen
Porcine mycobacteriosis Caused by *Mycobacterium avium* subspecies *hominissuis*
15/2017 Pär Davidsson
Oligogalacturonide Signalling in Plant Innate Immunity
16/2017 Kean-Jin Lim
Scots Pine (*Pinus sylvestris L.)* Heartwood Formation and Wounding Stress: A View from the Transcriptome

Helsinki 2017 ISSN 2342-5423 ISBN 978-951-51-3635-0