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Investigation into the annual cycle of development in *Fragaria vesca*

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It’s not the strongest species that survives, nor the most intelligent, but the one most responsive to change.

Charles Darwin

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and full acknowledged.

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(Samantha K Bedry)

June 2017

Acknowledgements

To my family who made me believe anything is possible.

To my friends for the endless support.

To everyone who kept me smiling and laughing through it all.

To the University and the School for making this a reality.

To Prof. Nick Battey, thank you beyond words.

Abstract

The growth and development of *Fragaria vesca*, as a model plant system, was investigated to better understand the control of perenniality in plants. Experiments revealed that conditions prior to and during the resumption of vegetative growth in the spring could alter the order of emergence of runners and flowers. Under natural conditions, rapid terminal shoot apical meristem growth occurred during the spring and early summer, followed by a marked decline from June; experimental study suggested this was likely to be a response to runner development. Environment was found to regulate the fate of axillary buds: low temperature (11°C) and short days (10h) promoted branch crown development, whereas at high temperatures (>18°C) runners were initiated regardless of photoperiod. Autumn conditions induced flowering and *F. vesca* ecotypes varied in their timing of flower initiation (inferred from emergence); a possible relationship to latitude was confounded by response variation between years in one ecotype. There was also ecotypic variation in runnering and typically those that flowered earlier runnered less. Detailed experiments on one ecotype showed that spring flower emergence and vegetative growth had a more complex response to winter chilling than that reported for many tree species, raising the question of the suitability of *F. vesca* as a model perennial. Greater chill accumulation advanced runner and flower emergence during forcing; the influence of warm periods during chilling was inferred using a range of chill models. Overall, the research highlights the importance of studying plant development in the natural context. An experimental approach is proposed to allow better understanding of plant ecological development, and suggestions provided for the possible implications of predicted climate change.

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Abbreviations

Aa *TFL1*	*Arabis alpina* ortholog of *TERMINAL FLOWER1*
Aa *LFY*	*Arabis alpina* ortholog of *LEAFY*
AG	*AGAMOUS*
AGL24	*AGAMOUS-LIKE 24*
ANOVA	analysis of variance
AP1	*APETALA1*
ARK1	*ARBORKNOX1*
AS1	*ASYMMETRIC LEAVES1*
AXB	axillary bud
CENL1	*CENTRORADIALIS-LIKE1*
CETS	*CENTRORADIALIS*/*TERMINAL FLOWER 1*/*SELF-PRUNING*
CiFT	*Citrus* homolog of *FLOWERING LOCUS T*
CLAVATA	*CLV*
CsAP1	*Citrus sinensis* homolog of *APETALA1*
CsLFY	*Citrus sinensis* homolog of *LEAFY*
CsTFL1	*Citrus sinensis* homolog of *TERMINAL FLOWER1*
cm	centimetres
CO	*CONSTANS*
CU	chill units
DAM	*DORMANCY ASSOCIATED MADS-BOX*
DIF	difference between night temperature and day temperature
EVG	*EVERGROWING*
F	*F*-value
F	Finland
FaPFRU	QTL controlling both the perpetual flowering and runnering traits in *Fragaria* x *ananassa*
FaTFL1	*Fragaria* x *ananassa* homolog of *FLOWERING LOCUS T1*
FD	*FLOWERING LOCUS D*
FLC	FLOWERING LOCUS C
FRI	*FRIGIDA*
FT	*FLOWERING LOCUS T*
FUL	*FRUITFULL*
FvAP1	*Fragaria vesca* homolog of *APETALA1*
FvCO	*Fragaria vesca* homolog of *CONSTANS*
FvFUL1	*Fragaria vesca* homolog of *FRUITFULL1*

FvFT1	*Fragaria vesca* homolog of *FLOWERING LOCUS T1*
FvTFL1	*Fragaria vesca* homolog of *TERMINAL FLOWER1*
FWA	*FLOWERING WAGENINGEN*
GDD	growing degree days
GA	gibberellin
h	hours
HU	heat units
KNOX	*KNOTTED* gene family
LFY	*LEAFY*
LD	long days – the number of hours in a LD varied between experimental Chapters and is defined in the methods in each Chapter
m	metres
MdTFL1	*Malus* x *domestica* homolog of *TERMINAL FLOWER1*
MFT	*MOTHER OF FT AND TFL1*
miR	microRNA
ml	millilitres
mm	millimetres
°C	degrees Celsius
°N	degrees north
p	*p*-value
PEP	*PERPETUAL FLOWERING*
PIN	*PIN-FORMED*
PmDAM	*Prunus mume DORMANCY ASSOCIATED MADS-BOX* genes
PmSOC1	*Prunus mume* homolog of *SUPRESSOR OF OVEREXPRESSION OF CONSTANS1*
PpCYCD3s	pear *cyclin* genes
PpDAM	*Pyrus pyrifolia DORMANCY ASSOCIATED MADS-BOX* genes
PpEXPA2	pear *expansin* genes
PtMADS	*Populus* dormancy associated genes
PttCLV	*Populus* homolog of *CLAVATA*
PttHB3	*HOMEOBOX3* – *Populus* homolog of *WUSCHEL*
PttKNOX	*Populus KNOTTED*-like homeobox
PttRLK3	*Populus* homolog of *RECEPTOR-LIKE KINASE3*
PttSTM	*Populus* putative ortholog of *SHOOTMERISTEMLESS*
PttWUS	*Populus* putative ortholog of *WUSCHEL*
QTL	quantitative trait locus

R	*RUNNERING LOCUS*
RoKSN	rose *TERMINAL FLOWER1* homologue
SAM	shoot apical meristem
SD	short days – the number of hours in a SD varied between experimental Chapters and is defined in the methods in each Chapter
sec	seconds
SEP	*SEPALLATA*
SFL	*SEASONAL FLOWERING LOCUS*
SFT	*SINGLE FLOWER TRUSS*
SOC1	*SUPRESSOR OF OVEREXPRESSION OF CONSTANS1*
SPL	*SQUAMOSA BINDING FACTOR-LIKE*
STM	*SHOOTMERISTEMLESS*
SVP	SHORT VEGETATIVE PHASE
TFL1	*TERMINAL FLOWER1*
UK	United Kingdom
µmol	micromoles
µm	micrometres
VvFT	*Vitis vinifera* ortholog of *FLOWERING LOCUS T*
W	watt
WUS	*WUSCHEL*
ZLL/*PNH*	*ZWILLE*/*PINHEAD*

Chapter 1 – Introduction

Plants play hugely important roles in natural ecosystems and food production. Understanding the regulation of their annual cycles and how they are influenced by external factors is therefore of great theoretical and practical importance (Tan and Swain, 2006). Plants are organisms with relatively simple body plants (compared to animals), with modular growth from meristems. Their form is shaped by the environment rather than being laid down in a complex way during embryogenesis (Leyser and Day, 2009). So the environment regulates development both in space and time. In fact time and space are in some ways equivalent in plant development because form and structure arise in space as a response to events in time.

Critical questions about how perennials regulate their annual cycles remain unaddressed, a particular problem being the pile-up of detailed molecular knowledge while understanding of the complex morphological changes occurring during the year and their regulation by the environment remains patchy. The mission of the work in this thesis was therefore to focus explicitly on developmental changes as expressed in the external morphology of *Fragaria vesca* L. over the course of the year. This would provide detailed knowledge of these changes and their regulation in a model perennial species where understanding of environmental regulation has a prior basis in experimentation. Key findings were the pronounced correlative effect of runners on growth from the terminal meristem which appeared to restrict its period of activity during the natural cycle; the complexity of the response to winter chilling and spring warmth, as the vegetative meristem within the existing (floral) structure asserts itself, giving rise to runners at a variable time in relation to the expression of flowers; and pronounced variation in flowering and runnering responses of different ecotypes to a single environment.

In this Introduction basic developmental regulation, the role of environment, the annual cycle in perennials; and the potential and known effects of climate change are reviewed. The particular focus is on rosaceous perennials, the aim being to provide essential background to the work discussed in the thesis. More detailed overviews are provided in the Introductions to the Results chapters (Chapter 3-6).

1.1 Basic developmental regulation in plants: annuals and perennials

Understanding of developmental regulation in plants has focused on processes observed in both annuals and perennials and has been substantially developed through the use of model species.

One fundamental process regulating plant development is the control of growth at the shoot apical meristem (SAM), where all aerial organs (leaves, flowers and stems) are initiated (Murray *et al*., 2012); *Arabidopsis* is a model annual species for exploration of this subject. The following summary highlights the role of key genes in *Arabidopsis* during vegetative and reproductive development and compares these roles with those in perennials. This is important background for the research described in this thesis on developmental responses in *F. vesca*: although the work has been conducted at a morphological level, many of the questions raised will need ultimately to be addressed at the molecular level.

SHOOTMERISTEMLESS (*STM*) is a member of the *KNOTTED* (*KNOX*) gene family and plays a vital role in the formation of the SAM in *Arabidopsis* embryos; low *STM* activity compromises SAM maintenance (Leyser and Day, 2009). *WUSCHEL* (*WUS*) and *ZWILLE*/*PINHEAD* (*ZLL*/*PNH*) are also necessary for initiation and/or functioning of the SAM (Mordhorst *et al*., 2002). *STM* is expressed throughout the SAM, but not in lateral organ primordia, and the action of *STM* at the SAM is to inhibit differentiation, thereby maintaining indeterminate cell fate (Dodsworth, 2009; Scofield *et al.,* 2014). *STM* interacts with related *KNOX* genes including *ASYMMETRIC LEAVES1* (*AS1*) (Scofield *et al.,* 2014). *AS1* plays a role in promoting leaf identity (Machida *et al*., 2015). Analysis of the interaction between *AS1* and *KNOX* genes has distinguished stem cells from founder cells. At the SAM, *STM* negatively regulates *AS1*, and therefore maintains the undifferentiated state (Scofield *et al.,* 2014). In differentiating (founder) cells, *STM* and related *KNOX* genes are downregulated and *AS1* expression allows leaf initiation to occur (Frerichs *et al*., 2016).

Populus is widely used as a model for perennials (Wullschleger *et al*., 2012). It has been shown that an orthologue of *STM* (*PttSTM*, *P. tremula*, Schrader *et al*., 2004; *ARBORKNOX1* (*ARK1*), *P. tremula* x *P. alba*, Groover *et al*., 2006), is expressed at the SAM, as well as in stems undergoing secondary growth, a key perennial feature (Du and Groover, 2010). *ARK1* expression occurs primarily in the cambial zone of *Populus* (*P. tremula* x *P. alba*) stems during secondary growth and regulates cambial functions and cell differentiation (Groover *et al*., 2006). Other poplar *KNOX1* genes (*PttKNOX1*, *PttKNOX2*, and *PttKNOX6*) are also expressed within the SAM and cambial zone, but expression of the closest homologue of *AS1* is restricted to the SAM, which suggests that in poplar *AS1* is associated with initiation of leaf primordia, not vascular tissue differentiation (Schrader *et al*., 2004; Baucher *et al.,* 2007).

Independently from *STM*, the *WUSCHEL* (*WUS*) gene plays a key role in maintaining structural and functional integrity of the SAM, as well as regulating stem cell fate in indeterminate shoots and identity in determinate floral meristems (Laux *et al.,* 1996; Mayer *et al.,* 1998). *CLAVATA*

(*CLV1*, *CLV2* and *CLV3*) genes also play vital roles at the SAM and promote the progression of stem cells toward organ initiation while limiting the size of the shoot meristem (Schoof *et al.*, 2000). Studies have shown an interactive relationship between *WUS* and *CLV* activity through regulatory feedback loops (Figure 1.1). The *CLV* signalling pathway restricts the size of the stem cell population and maintains SAM size by negatively regulating the expression of WUS protein; *WUS* expression promotes *CLV3* transcription, whilst *CLV3* limits *WUS* expression (Ohmori *et al.*, 2013; Lee and Clark, 2015). *CLV1* also has a regulatory influence on *WUS*, with activation of *WUS* transcription restricted through the binding of *CLV3* to *CLV1* (Yadav and Reddy, 2012).

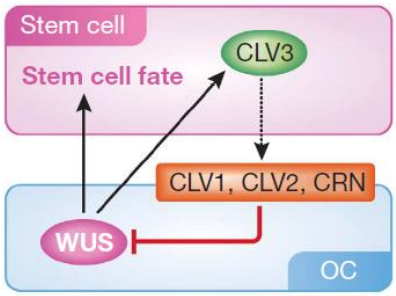

Figure 1.1 Developmental regulation of structural and functional integrity in the SAM in *Arabidopsis* (Miyashima *et al.*, 2013)

The *WUS*-*CLV* pathway was initially described over 15 years ago in *Arabidopsis*, and it has since been shown to be at the centre of meristem function in a range of annuals and perennials, including: *Antirrhinum*, *Petunia*, rice, maize and tomato (Butenko and Simon, 2015; Galli and Gallavotti, 2016). In the model perennial *P. trichocarpa*, a *WUS*-like orthologue has been implicated in the regulation of meristem function at the apex and in axillary buds (Rinne *et al.*, 2015); whilst in *P. tremula* *CLV1*-like *PttRLK3* and *WUS*-like *PttHB3* have been shown to balance cell proliferation and maintain undifferentiated cells during active growth (Schrader *et al.*, 2004).

Stem cell maintenance is not confined to the SAM, with shoot meristem regulators (*CLV1* and *STM*) playing a role in vascular stem cell maintenance in *Arabidopsis* and *P. tremula* (Aichinger *et al.*, 2012). In *P. tremula*, some orthologues of *CLV3* (*PttCLV3*) and *WUS* (*PttWUS*) are limited to the shoot apex and not expressed in the vascular cambium, whilst other *WUS*-like (*PttHB3*) and *CLV1*-like (*PttRLK3*) transcripts have been detected in the cambial zone (Schrader *et al.*, 2004; Caño-Delgado *et al.*, 2010). This suggests that *CLV1*-like and other related genes may be upregulated in stems undergoing secondary growth (Baucher *et al.*, 2007).

The transition to flowering occurs at the SAM and is co-ordinated in *Arabidopsis* by the floral meristem identity genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*) via two distinct pathways: SUPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) acts in a complex with AGAMOUS-LIKE 24 (AGL24) to promote *LFY* expression (Posé *et al.*, 2012). *AP1*, on the other hand, is mainly regulated by *FLOWERING LOCUS T* (*FT*) (Wigge *et al.*, 2005); which is expressed in the leaves in response to optimal flowering photoperiod (typically long days) (Krzymuski *et al*., 2015). *LFY* and *AP1* positively regulate each other, reinforcing floral identity, and share partially overlapping functions (Denay *et al.*, 2017). They also have specific functions, with *LFY* involved in the initiation of flower development and *AP1* conferring floral identity in the primordium (Okamuro *et al.*, 1996; Mizukami and Ma, 1997; Andrés and Coupland, 2012).

TERMINAL FLOWER1 (*TFL1*) is a member of the CETS (*CENTRORADIALIS*/*TERMINAL FLOWER 1*/*SELF-PRUNING*) protein family (McGarry and Ayre, 2012). In contrast to the closely-related gene *FT*, *TFL1* is a flowering repressor. *TFL1* and *FT* have been shown to compete to bind with *FLOWERING LOCUS D* (*FD*) and as a result either activate (*FT*) or repress (*TFL1*) flowering (Wigge *et al.*, 2005; Ahn *et al.*, 2006). *TFL1* has also been shown to repress flowering by negatively regulating the floral promotors *LFY* and *AP1* (Ratcliffe *et al*., 1999; Sung *et al*., 2003). The effect of *TFL1* depends on the stage of development; during early development, *TFL1* delays commitment to flowering, whereas at later developmental stages, *TFL1* acts to maintain inflorescence meristem identity (Bradley *et al*., 1997). It was suggested that this varied regulation allowed *TFL1* to regulate the duration of the growth phase and, as a result, overall plant architecture (Ratcliffe *et al*., 1998). In this way, *TFL1* regulates the transition from vegetative to reproductive development and may be considered to mark the transition from juvenile to adult form in *Arabidopsis* (see Battey and Tooke, 2002).

Those perennials which have been studied appear to be similar in the basic regulation of their transition to flowering to *Arabidopsis*, at least with regard to floral meristem identity genes. For example in *Citrus sinensis* (orange), quantitative RT-PCR studies showed a positive correlation of *LFY*-like *CsLFY* and *AP1*-like *CsAP1* expression with flowering; transcripts of *CsLFY* and *CsAP1* became highly accumulated in adult tissue during floral induction, and this was not observed in juveniles (Pillitteri *et al.*, 2004). Orthologues of *FT* have also been identified in perennials, with *VvFT* expression in grapevine associated with seasonal flowering induction and inflorescence development (Carmona *et al.*, 2007, 2008). In *Populus* species, two *FT* genes have been identified which regulate reproductive onset (*FT1*) and vegetative growth (*FT2*) (Hsu *et al*., 2011; Pin and Nilsson, 2012; Ding and Nilsson, 2016), with low abundance of *FT2* transcript during juvenility presumed to constrain flowering in juvenile plants (Hsu *et al*., 2006). The role of *FT* has also been studied in *Fragaria*, with a flower-promoting effect of *FT* (*FvFT1* in *F. vesca*)

identified in everbearing accessions (Rantanen *et al*., 2014) and a temperature-photoperiod interaction regulating *FT*/*TFL1*-like gene expression associated with seasonal flowering (Iwata *et al.,* 2012; Koskela *et al.,* 2012; Nakano *et al*., 2015; see also below).

TFL1 homologues in perennials appear to play a role in juvenility, by preventing flowering in juvenile plants. In *P. trichocarpa*, the length of the vegetative growing period before first flowering was shortened with reduced *TFL1* function (Mohamed *et al*., 2010). In *C. sinensis*, the pattern of expression of *TFL1*-like *CsTFL1* contrasted with that of *CsLFY* and *CsAP1*: *CsTFL1* RNAs were more abundant in juvenile plants than in adults, with levels negatively correlated with flowering and consistent with the opposing action of *LFY* and *TFL* in *Arabidopsis* (Pillitteri *et al.,* 2004). Similarly, suppression of *TFL1*-like genes (*MdTFL1*) in apple was shown to reduce the juvenile phase and enable earlier flower initiation (Kotoda *et al.,* 2006). In *A. alpina*, Aa *TFL1* prevented flowering in young plants, even after exposure to vernalization; in older plants, Aa *TFL1* increased the duration of vernalization required for expression of the floral meristem identity gene (Aa *LFY*) (Wang *et al.,* 2011).

Flower development follows on from floral induction and initiation. The ABC(D)E model was proposed to explain how floral organ identity is controlled by five classes of regulatory genes, A, B, C, D and E (Kramer and Irish, 1999; Causier *et al*., 2010; Irish, 2010). The ABC model was originally inferred using *Arabidopsis* and *Antirrhinum* (Coen and Meyerowitz, 1991) and proposed that three gene functions (A, B and C) worked together to determine organ identity in each whorl of the developing flower (Krizek and Fletcher, 2005). Alvarez-Buylla *et al*. (2010) summarised previous work, showing how these three gene classes interact to produce the floral organs: A genes specify sepals; A and B together, petals; B and C together, stamens; and C genes, carpels. Further genetic studies have extended the floral model to include class D and class E genes. Class D genes were found to confer ovule identity on tissues that develop within the carpels; these D functional genes were originally identified in *Petunia* (Angenent *et al.,* 1995) and have since been shown in a number of other species: *Arabidopsis* (Pelaz *et al.,* 2000; Ditta *et al.,* 2004), wheat (Zhao *et al.,* 2006) and rice (Yoshida *et al.,* 2011; Yun *et al*., 2015). The addition of class E genes to the ABC model greatly extended the understanding of floral development (Rijpkema *et al.,* 2006), because these genes are required for the development of all floral organs (Krizek and Fletcher, 2005). Class E genes are a group of closely related and functionally redundant MADS-box genes, *SEPALLATA1/2/*3/4 (*SEP1/2/3/4*) (Pelaz *et al*., 2000; Ditta *et al*., 2004). Mutants in class E activity show impaired class A, B and C function, resulting in a loss of floral determinacy (Honma and Goto, 2001; Krizek and Fletcher, 2005). Similarly mutations of the C function gene, *AGAMOUS* (*AG*), results in the absence of stamens and carpels (Yanofsky *et al*., 1990); with *AG* shown to integrate stamen identity, carpel identity and floral meristem

determinacy through repression of *WUS* expression within the floral meristem (Smaczniak *et al.*, 2012; Dreni and Kater, 2014; Liu *et al.*, 2014).

The ABC(D)E model has also been explored in perennials. Although there is overlap in genes between the model proposed for *Arabidopsis* and perennial species, this model is not sufficient to account for variability in flowering across species (Barcaccia *et al*., 2012). Nevertheless, ABC(D)E model genes have been identified in a range of perennial species, including: apple, peach, olive, poplar, grape and *Eucalyptus* (Sreekantan *et al*., 2006; An *et al*., 2011; Barcaccia *et al*., 2012; Vining *et al*., 2015).

Flower development may be considered to transition to fruit development at the moment of ovule fertilisation by the male (pollen) nucleus. Ovules become seeds, and the surrounding floral tissues begin to follow the developmental fates associated with fruit tissues. Fruit structure is plant family and sub-family specific, so that the precise fate and role of floral tissues varies enormously (Figure 1.2). For example, in *Malus* and *Prunus*, the position of insertion of the petals on the receptacle differs, so that the condition of the ovary transitions from superior (cherry) to inferior (apple). This means that the fruit is derived from the bases of petals and sepals in apple, but only from carpellar tissue in cherry. In *Fragaria*, the strawberry is an aggregate accessory fruit, with the botanical fruit made up of achenes (single indehiscent seeds derived from individual carpels) embedded in a fleshy receptacle, which is a false fruit originating from receptacle tissue in the flower (Liston *et al.*, 2014).

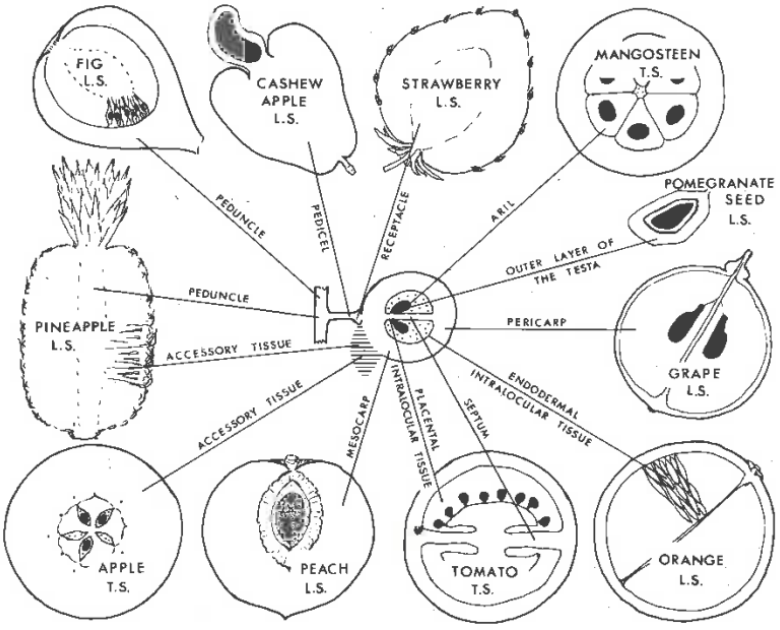

Figure 1.2 Diversity of tissues which can develop into fruit flesh for 11 species (Coombe, 1976)

Given this complexity, at the stage of fruit development the relevance of work with the model *Arabidopsis* may be limited. However, a very significant role of auxin in directing fruit tissue development has been found in *Arabidopsis*. The PIN-FORMED (PIN) family of proteins has been shown to control the efflux transport of auxin (Roeder and Yanofsky, 2006; Křeček *et al.*, 2009); with *PIN1* and *PIN3* being particularly important in the regulation of fruit development (Sorefan *et al*., 2009; Ceccato *et al.*, 2013). A key role of auxin is also found in *Fragaria* fruit (Nitsch, 1970); auxin derived from the developing achenes is essential for expansion of the surrounding receptacle (Given *et al.*, 1988).

1.2 Environmental regulation of growth and development in annuals and perennials

Environmental conditions play a vital role in regulating growth and development in both annuals and perennials, which occurs at all growing points throughout the plant. The subject of environmental regulation is discussed in more detail in relation to its morphological effect in *Fragaria* in Chapter 3; the regulation of terminal and axillary growth and development is the focus of Chapter 4.

The regulation of flowering has been studied in detail in the model species *Arabidopsis,* with five main flowering pathways identified: photoperiod, vernalization, autonomous, age-related and gibberellin (GA); although there is some overlap between these pathways (Simpson and Dean, 2002; Crevillén and Dean, 2011) (Figure 1.3).

In *Arabidopsis*, which is a quantitative (or facultative) long day plant (Thomas and Vince Prue, 1997), in short days (SD) vegetative growth is promoted at the SAM, while long day (LD) conditions promote flowering through the function of FT protein, which is expressed in the leaves within 24 hours of exposure to LD (Jaeger and Wigge, 2007; Wigge, 2011). *FT* expression is directly induced by *CONSTANS* (*CO*), whose basic expression pattern is controlled by the circadian clock (Golembeski *et al.*, 2014; Romera-Branchat *et al.*, 2014; Putterill and Varkonyi-Gasic, 2016). FT is a mobile florigen and once synthesised in the leaves is translocated to the SAM where it interacts with FD (Amasino, 2010; Ding and Nilsson, 2016). This FT-FD interaction activates downstream floral meristem identity genes and floral promoters, such as *AP1* and *SOC1* (Amasino and Michaels, 2010), which causes cells at the SAM to differentiate to form floral meristems (Amasino, 2010). Transfer to LD results in the observation of floral primordia after approximately three-five days (Albani and Coupland, 2010).

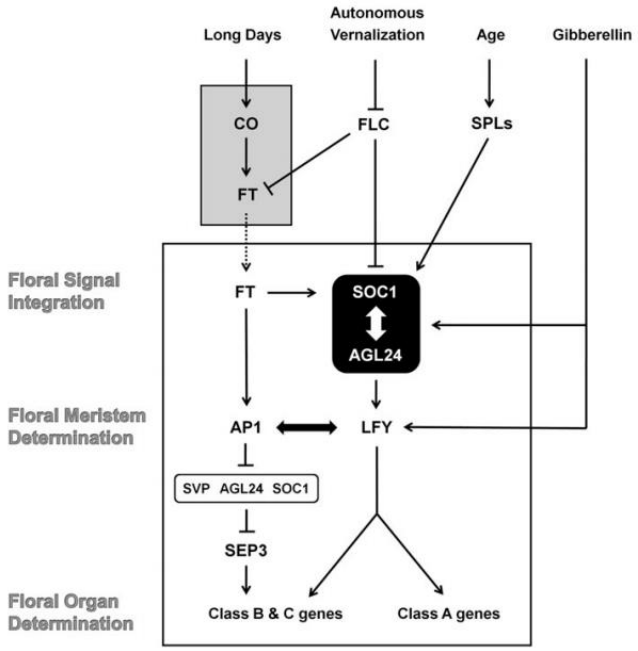

Figure 1.3 Diagrammatic representation of the five main pathways to flowering: long days (photoperiod), vernalization, autonomous, age and GA, and the interactions between pathways to restrict and/or promote flowering. CO-FT induction as a result of photoperiod occurs in the leaf, as shown by the grey box, whereas the majority of the flowering process occurs at the SAM, indicated by the open rectangle (Lee and Lee, 2010)

Photoperiod also regulates flowering time in the perennial model *Populus*. Studies show an increase in the abundance of *FT*-like transcripts *FT1* and *FT2* under LD, which promotes flowering in competent buds (Zhang *et al.*, 2010; Ding and Nilsson, 2016). The means by which *FT* induces flowering differs between perennials: grafting experiments on tomato and tobacco have shown that *SFT* (*SINGLE FLOWER TRUSS*), a *FT* homologue, is transported from the leaves to the apex to promote flowering (Zhang *et al.*, 2010; Xu *et al.*, 2012). It has been suggested, however, that in some trees the FT protein may be less mobile; FT has not been detected in the phloem or xylem sap suggesting that it may not be transported from source leaf into the shoot apex, but may be sourced from closer, stronger sinks (Putterill and Varkonyi-Gasic, 2016). This phenomenon has been specifically observed in *Populus* and *Malus*, with no flowering in non-transgenic scions once grafted onto transgenic FT over-expressing rootstocks (Tränkner *et al.*, 2010; Zhang *et al.*, 2010).

Accessions of *Fragaria* show contrasting responses to photoperiod: those with seasonal flowering (the focus of this thesis) are facultative short-day (SD) plants (Heide and Sønsteby, 2007) (Figure 1.4). In SD *F. vesca*, inductive SD down-regulate the flowering repressor *FvTFL1*, and under these conditions floral meristem identity genes *FvAP1* and *FvFUL1* (*F. vesca*

homologues of *AP1* and *FRUITFULL1* (*FUL1*)) are up-regulated; with up-regulation of *FvTFL1* only detected once LD conditions return (Koskela *et al.,* 2012). Flowering activation by *FT1* is overridden by the repressing effect of *FvTFL1*; so flowering occurs as a result of a down-regulation of *FvTFL1* rather than an up-regulation of *FT1* (Koskela *et al.,* 2012; Kurokura *et al.,* 2013). Similarly to *Arabidopsis, FT* homologues in *Fragaria* (*FvFT1* in *F. vesca* and *FaFT1* in *F.* x *ananassa*) are also regulated by photoperiod, and *FvCO* (*CO*- like) mRNA expression has been linked to the regulation of *FvFT1* (Kurokura *et al.,* 2017).

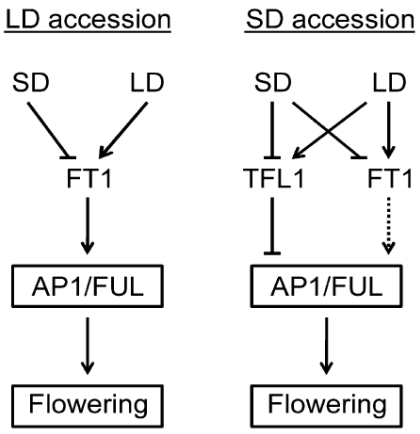

Figure 1.4 Photoperiodic control of flowering in *F. vesca* SD and LD accessions. Arrows indicate activation and bars repression. The dashed line represents the activation of *FvAP1*/*FUL* by *FvFT1* in SD accessions of *F. vesca* (Koskela *et al.,* 2012)

Temperature is another environmental factor regulating flowering, with vernalization (a period of cold temperature) necessary to overcome flowering restraint in winter annual ecotypes of *Arabidopsis* (Bratzel and Turck, 2015). In the absence of vernalization in these ecotypes, FLOWERING LOCUS C (FLC) (a MADS box protein) directly binds to the floral promoting genes, *SOC1* and *FT*, blocking transcription and repressing flowering (Andrés and Coupland, 2012). SHORT VEGETATIVE PHASE (SVP) (a MADS box protein related to FLC) physically interacts with FLC to delay flowering and also binds to *SOC1* and *FT*, resulting in repression (Mateos *et al.,* 2015). Vernalization epigenetically regulates flowering by permanently suppressing *FLC*, thus enabling flowering (Sung and Amasino, 2004). The response to vernalization shown by winter annuals is due in part to the dominant gene *FRIGIDA* (*FRI*), which is required for the winter annual habit: *FRI* and *FLC* act together to prevent flowering in the non-vernalized shoot apex by preventing the transcription of *FT* in leaves and *SOC1* in the SAM (Bouché *et al.*, 2017). Other accessions of *Arabidopsis*, considered to be rapid cycling/summer annuals, do not require vernalization for flowering, with the independent evolution of this habit in a number of accessions often a consequence of *FRI* mutations (Shindo *et al.,* 2005). A reduction

of *FLC* and/or *FRI* function has been linked with early flowering in summer annuals (Crevillén and Dean, 2011; Jarillo and Piñeiro, 2015). The autonomous pathway acts independently of environmental cues but regulates the expression of *FLC* through a similar mechanism to vernalization (Albani and Coupland, 2010; Amasino and Michaels, 2010; Kim and Sung, 2014). In summer annuals particularly, gene products from the autonomous pathway keep *FLC* levels low, which enables flowering (Koornneef *et al.*, 1991).

Arabis alpina has been used as model perennial, to explore the regulation of flowering in comparison to that shown in *A. thaliana* (Castaings *et al*., 2014; Kiefer *et al.*, 2017). *A. alpina* also shows a regulatory effect of vernalization on flowering, with the *FLC* orthologue *PERPETUAL FLOWERING 1* (*PEP1*) downregulated during vernalization. This promotes the transition to flowering in adult shoot meristems, independently of photoperiod (Wang *et al.*, 2009b). In contrast to *Arabidopsis*, in which *FLC* is permanently supressed in response to vernalization, in *A. alpina PEP1* levels are restored in flowering and vegetative meristems following vernalization; this helps maintain perenniality and allows vegetative growth following flowering (Wang *et al.*, 2009b). Accessions of *A. alpina* have shown variation in their *PEP1* activity, linked to phenotypic differences in flowering, with some accessions not requiring vernalization (Albani *et al.*, 2012). *PERPETUAL FLOWERING 2* (*PEP2*) has also been identified in *A. alpina* and acts to enhance expression of *PEP1* (Bergonzi *et al.*, 2013). In many horticultural, temperate deciduous tree species, flower initiation is not associated with photoperiod and vernalization (Wilkie *et al.*, 2008); for example, in apple autonomous regulation is thought to have a dominant role in flower initiation (Nishikawa, 2013). In *Fragaria* however, cool temperatures (below 13°C) have a flower inducing effect by downregulating *FvTFL1*, an effect which is independent of photoperiod; although this regulation is not considered to be a vernalization pathway and the molecular mechanism is not yet known (Rantanen *et al.*, 2015). Cold temperatures also play a crucial role in regulating perennial growth and development through dormancy (see below).

In *Arabidopsis*, plant age has also been shown to affect flowering, a process referred to as the age-related pathway (Albani and Coupland, 2010). One influence of developmental age on the flowering response is an increase in *SOC1* expression with age, an increase considered to occur independently from photoperiod and FT-FD regulation (Lee and Lee, 2010). This independent increase in *SOC1* expression occurs as a result of other age-related processes, with *SQUAMOSA BINDING FACTOR-LIKE* (*SPL*) transcription factors playing a role in the age-related regulation of *SOC1* (Wang *et al.*, 2009a; Lee and Lee, 2010; Wang, 2014). The expression of *SPL* is also regulated by an age-related interaction with miR156: high miR156 abundance limits *SPL* expression, but as miR156 decreases with time (and age), *SPL* expression is promoted and

subsequent floral promotion occurs as a result of increased expression of *SOC1* and other MADS-box genes (*AP1*, *LFY* and *FUL*) (Wang, 2014). miR156 has a crucial role in plant developmental transitions, as observed in *Arabidopsis*, *A. alpina*, *Cardamine flexuosa*, Chinese cabbage, maize, rice, tobacco, tomato and poplar; overexpression of miR156 prolongs the juvenile phase and delays flowering (Yu *et al.*, 2015).

The effect of plant age, which manifests as juvenility in perennials and represses flowering in incompetent plants, has been previously discussed (Chapter 1, Section 1.1). Interestingly, and in contrast to traditional dogma (see, for example Thomas and Vince Prue, 1997), an interaction between juvenility and environmental regulation, in particular vernalization, has been discovered in the perennials *A. alpina* (Wang *et al.*, 2011) and *Cardamine flexuosa* (Zhou *et al.*, 2013). Quantification of the length of the juvenile phase in plants is crucial to understanding age-related regulation. In perennials, establishing the duration of juvenility is key for predicting production and yield of food crops. Reciprocal transfer experiments have long been used as a method of quantifying the duration of juvenility in annuals and perennials, with plants transferred between inductive and non-inductive conditions and growth and flowering responses then assessed (Matsoukas, 2014). In an experiment using *Arabidopsis*, plants were transferred daily from SD to LD conditions from seedling emergence until flowering in order to determine the length of the (photoperiod-insensitive, vegetative) juvenile phase before (photoperiod-sensitive) floral induction. A number of accessions were included in this experiment and showed variation in the length of the juvenile phase, ranging from 0.3-7.6 days until floral competence (Matsoukas *et al.*, 2013).

The final pathway regulating flowering in *Arabidopsis* operates through GA. GA promotes flowering and is an absolute requirement for flowering in SD (Wilson *et al.*, 1992; Mutasa-Göttgens and Hedden, 2009). The GA flowering pathway interacts with two downstream floral genes, *SOC1* and *LFY* (Blázquez *et al.*, 1998; Moon *et al.*, 2003). The molecular mechanism by which the regulation of *SOC1* occurs is not completely clear (Lee and Lee, 2010; Hedden and Thomas, 2016), although subunits of a nuclear factor Y (NF-Y) complex have been found to control flowering time by directly regulating *SOC1* expression in response to flowering signals from the photoperiod and GA pathways in *Arabidopsis* (Hou *et al.*, 2014). The mechanism of GA influence on *LFY* differs from that of *SOC1* and was previously considered to be more clear (Blázquez *et al.*, 1998), with GA_4 found to be a direct activator of *LFY* transcription, under SD conditions (Eriksson *et al.*, 2006).

GA appears to have a predominantly inhibitory effect on flowering in perennials, which contrasts with the work on *Arabidopsis* (Wilkie *et al.*, 2008). In *C. sinensis*, application of GA_3 during

flower induction repressed *CiFT* expression in the leaves and thus repressed flowering. GA_3 was not shown to modify relative expression of *SOC1* expression, which contrasts to work in *Arabidopsis* (Muñoz-Fambuena *et al*., 2012). Similar GA inhibition has been shown in rose, with GA_3 acting as a floral repressor during floral transition; a proposed model for this regulation showed GA to positively regulate a floral repressor (*TFL1* homologue, *RoKSN*) (Randoux *et al.*, 2012). In general, GAs promote vegetative growth in perennials at the expense of reproductive development (Mutasa-Göttgens and Hedden, 2009). The response of *Fragaria* is consistent with this effect, with GA promoting vegetative growth through runnering (Hytönen *et al.*, 2009) (see also Chapter 4).

There are a number of developmental phases in plant life cycles that are closely regulated by the environment; one such event, which is also one of the most noticeable differences between perennials and annuals, is senescence. In annuals, due to their monocarpic nature, senescence and plant death are observed following reproductive development. In contrast, the morphology of perennials and their frequently polycarpic nature enables a switch back to vegetative growth following flowering and fruiting (Battey and Tooke, 2002; Friedman and Rubin, 2015).

The regulation of senescence in annual *Arabidopsis* may be widespread in other annuals. Inflorescence development in *Arabidopsis* is indeterminate, with all effective shoot meristems transitioning to flower (Bleecker and Patterson, 1997). Following flowering, inflorescence meristems degenerate to tissue showing no sign of continued proliferative activity (Hensel *et al.*, 1994); this is a result of global arrest throughout the plant of inflorescence meristems and therefore the plant is unable to produce new vegetative tissue (Bleecker and Patterson, 1997). This form of response has been viewed as evidence for a global systemic signal in *Arabidopsis*, with all shoot meristems responding co-ordinately regardless of the physiological process (Hensel *et al.*, 1994; Ay *et al.*, 2014).

Perennials differ from annuals in their senescence behaviour, with senescence not leading to total plant death, and the nature of this senescence and its effects on plants differs with life history strategy. In perennials, meristems do not behave in the same fashion, as observed in many annuals; not all meristems become induced to flower and subsequently senesce, therefore some meristems persist post-flowering senescence and enable continued vegetative growth (Townsend *et al.*, 2006; Amasino, 2009). There are a number of theories which describe the process of senescence in perennials. Thomas *et al.* (2000) described it as a wave of cell senescence and death, following behind the proliferating shoots and meristems; provided growth and development continued to outpace this wave of senescence then the plant would persist. Perennials generally maintain vegetative growth through two processes, either by conserving

vegetative meristems regardless of flower initiation, or by reverting some meristems back to vegetative development following flower induction (Tooke *et al.*, 2005; Wang *et al*., 2009b). *Cheiranthus cheiri* (Ravenelle wallflower) is a perennial by means of the latter process, in which the terminal meristem cycles between a state of vegetative and inflorescence growth; this inflorescence reversion maintains perenniality (Townsend *et al.,* 2006). *A. alpina* is a model perennial which maintains perenniality by conserving vegetative meristems, with vegetative shoots developing from meristems that were either not present or in early developmental stages during vernalization (Wang *et al*., 2009b).

Unlike in most annuals, where plant death follows senescence, in many perennials, senescence is observed through the loss of vegetative material and/or a significant reduction in active vegetative growth. As a result of this, some perennials maintain leaf cover regardless of senescence (are evergreen), whilst others annually lose leaf cover (are deciduous). In the model perennial, *Populus* spp., photoperiod has been identified as the most important trigger for leaf senescence, with phytochrome A playing a key role (Munné-Bosch, 2008).

1.3 The annual cycle in perennials

The preceding section has shown that annuals and perennials appear to share common regulatory genes and processes underlying establishment and growth at the SAM. There are also shared regulatory elements controlling the transition to flowering: in *Arabidopsis SOC1* and *AGL24*, as well as FT-FD interactions, are responsible for promoting flowering, through expression of floral meristem identify genes (*LFY* and *AP1).* Orthologues of *FT* and other key regulators have been described in perennials such as grapevine, strawberry and the model species *Populus*; it is also clear, however, that there are important differences in the way in which the core regulators interact to create pathways linking environment to development. *F .vesca* is itself a good example of this: *CO* and *FT* orthologues are both important in flowering regulation, but their interaction differs significantly from that in *Arabidopsis* (Kurokura *et al*., 2017). *TFL1* is a floral repressor in both annual *Arabidopsis* and perennial species, such as strawberry and citrus. But *TFL1* also appears to have perennial-specific functions: it underpins perenniality through the maintenance of vegetative shoots (in *A. alpina*), and by promoting spring vegetative growth following flower initiation (in *F. vesca*). In annuals, such as *Arabidopsis*, *TFL1* must be down-regulated to enable flower initiation, but at later stages of development it acts to maintain the inflorescence meristem. In *Arabidopsis*, however, *TFL1* does not promote vegetative growth following flowering; as a result plants senesce and die. Global senescence following flowering in annuals is not observed in polycarpic perennials, due to the availability of vegetative meristems.

There are a number of differences, including senescence, which are particularly important in contrasting the life cycles of perennials and annuals. In annuals, the typically monocarpic nature and global senescence results in a relatively simple life cycle; plants germinate from seeds; grow vegetatively (the duration of which may be controlled by age-related processes); become induced to flower (in response to endogenous and/or environmental signals); flower; set seed; senesce and die (Figure 1.5).

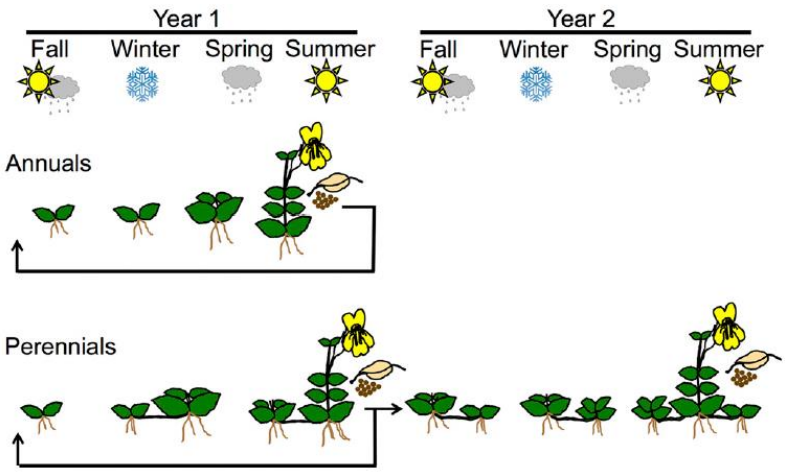

Figure 1.5 Comparison of life cycles of typical (winter) annuals and perennials (Friedman and Rubin, 2015)

Annuals vary in their timing of flowering, with some species and cultivars requiring vernalization to flower; they are often referred to as winter annuals (e.g. some Arabidopsis ecotypes) or biennials (e.g. *Beta vulgaris*) (Figure 1.5). The typical cold requirement in these plants can vary, with biennials having obligate and winter annuals often having a quantitative requirement (Amasino, 2004). Polycarpic perennials by comparison have a repeated annual cycle, in which they flower, fruit/set seed and senescence annually (Figure 1.5). It is also noteworthy, however, that some perennials have a monocarpic life history, such as bamboo and cacti, like *Agave*, growing vegetatively for years before eventually flowering, which is followed by plant death (Townsend *et al.*, 2006).

F. vesca has a characteristic cycle of annual growth and development (Figure 1.6) (Darrow, 1966; Guttridge, 1985; Carew and Battey, 2005; Kurokura *et al.*, 2013). In the spring, under favourable growth conditions, *F. vesca* resumes vegetative growth following a period of winter dormancy. Spring growth is characterised by the emergence of leaves from the main crown SAM (Arney, 1955a). Following the resumption of vegetative growth, inflorescence emergence is observed;

these inflorescences were initiated the previous autumn and overwintered in the crown (Perrotte *et al.*, 2016b). Fruit set and development follows (Kurokura *et al.*, 2013).

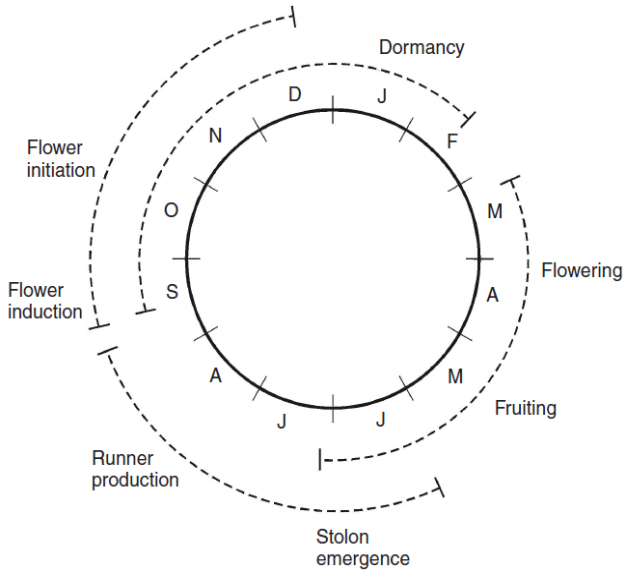

Figure 1.6 Annual cycle of seasonal flowering *Fragaria* (Carew and Battey, 2005)

F. vesca can also reproduce asexually, predominantly through the production of runners. Runner emergence is generally observed from late spring and continues throughout the summer, under optimal vegetative growing conditions, and into the autumn (Figure 1.6) (Darrow, 1966). Branch crowning is another form of asexual reproduction and is typically observed when conditions are less favourable for runner production (e.g. from late summer and through the autumn) (Guttridge, 1955; Darrow, 1966). Flower induction occurs alongside branch crowning in the autumn, in response to declining temperature and shortening photoperiod, and results in the initiation of floral primordia (Heide, 1977; Guttridge, 1985). Induction and progression of semi-dormancy also occurs during the time of flower initiation. Semi-dormancy is broken by cold winter temperature so that plant growth resumes with full vigour in the spring (Sønsteby and Heide, 2006).

There are a number of key traits that are characteristic of *F. vesca*. One is the development of runners from axillary meristems during vegetative growth. Another is that overwintering *Fragaria* plants are considered to become semi-dormant and do not lose their leaves (Guttridge, 1985; Sønsteby and Heide, 2006; Kurokura *et al*., 2013), unlike most deciduous rosaceous trees of temperate origin which experience true dormancy. True dormancy acts to repress vegetative growth (budburst) (Kurokura *et al*., 2013); in this dormant state, meristems are unable to resume growth even if conditions are favourable (Rohde and Bhalerao, 2007). In contrast to this true or absolute dormancy, the relative/semi-dormant nature of *Fragaria* results in a restraint on growth

but it is more limited; with a resumption in growth being possible under favourable conditions, although growth vigour is restrained (Heide *et al.,* 2013; Kurokura *et al.,* 2013).

In general, therefore, dormancy enables temperate perennials to overwinter before vegetative growth is resumed in the spring and the annual cycle repeated, and is a crucial physiological process that enables plant survival when conditions are not favourable for growth. Dormancy will now be reviewed with particular focus on rosaceous species, as background to the subsequent discussion of the role and nature of semi-dormancy in *F. vesca*.

Dormancy is a complex process with three distinct forms proposed: paradormancy, associated with bud position; endodormancy, or true dormancy associated with winter and broken by chilling; and ecodormancy, in which buds require only the correct environmental conditions to enter growth (Lang *et al.*, 1987). Bud set and budburst are two morphological events that coincide with the induction and release of dormancy in many tree species (Basler and Körner, 2014); photoperiod and temperature are primary environmental cues controlling these events (Cooke *et al.,* 2012; Pletsers *et al.*, 2015). Recent research has identified important regulatory mechanisms by which dormancy is regulated (Shim *et al.,* 2014) and peach (*Prunus persica*) has proved pivotal to molecular and genetic progress in the Rosaceae. Three strong QTLs have been associated with chilling requirement (linked with dormancy), one of which mapped close to the *EVERGROWING* (*EVG*) locus (Fan *et al.*, 2010; Romeu *et al.*, 2014; Bielenberg, 2015). An *evg* peach mutant, which lacks dormancy, showed the deletion of a tandem repeat of six *SVP/AGL24*-like MADS-box genes, called *DORMANCY ASSOCIATED MADS-BOX* (*DAM*) genes, with this deletion proposed as the likely cause of the evergrowing phenotype (Bielenberg *et al.*, 2008). The timing of expression of different *DAM* genes in peach has been linked with various elements of dormancy: *DAM 1*, *2* and *4* were associated with a role in bud set, while *DAM 3*, *5* and *6* were considered more likely to be associated with the establishment and/or maintenance of dormancy (Li *et al.*, 2009). *DAM 4*, *5* and *6* were found to be differentially expressed during dormancy transitions by Leida *et al.* (2010), and expression studies of *DAM5* and *DAM6* confirmed their likely involvement in mediating the chilling requirement by inhibiting bud growth (Yamane *et al.*, 2011). Changes in histone methylation correlated with the transcriptional activity of *DAM6* (Leida *et al.*, 2012). Intronic insertions in *DAM5* and *DAM6* were found to be associated with a low chill QTL (Zhebentyayeva *et al.*, 2014).

DAM genes have been identified in other rosaceous species; in *Prunus mume*, *PmDAM6* was proposed to be involved in endodormancy induction and endodormancy release of lateral buds (Yamane *et al*., 2008). A physical interaction has been reported between *PmDAM6* and *PmSOC1* proteins in *P. mume*, suggesting an association between their expression and dormancy release,

as well as providing evidence for their involvement in dormancy and flowering time regulation (Kitamura *et al*., 2016).

An understanding of dormancy release is also crucial. *PpDAM1* and *PpDAM3* have been identified as targets for degradation by an increase in miR6390 expression, which resulted in increased *FT2* mRNA levels and dormancy release in *Pyrus pyrifolia* (Niu *et al.*, 2016). Another recent transcriptome profiling study revealed that an *FLC*-like gene localizing to the QTL on linkage group 9 was induced in association with dormancy release and linked to fulfilment of the chill requirement (Porto *et al.*, 2015); expression of an *FLC*-like gene was also shown to be upregulated towards the end of endodormancy in *P. pyrifolia* (Niu *et al.*, 2016). The subject of chill requirement and accumulation is discussed in detail in Chapter 6.

Populus is a key non-rosaceous model perennial and studies have identified dormancy-associated *DAM* genes, such as *PtMADS7* and *PtMADS21* which are differentially regulated during the growth-dormancy cycle (Shim *et al*., 2014). Other genes have also been shown to have an effect on dormancy in *Populus;* for example, overexpression of *CEN*- and *FT*-like genes have been associated with failure of buds to enter endodormancy (Horvath, 2009). Downregulation of *FT1* and *FT2* influences timing of growth cessation and bud set and a decrease in *CENTRORADIALIS-LIKE1* (*CENL1*) expression is observed during this period (Ruonala *et al*., 2008). The nature of semi-dormancy in *Fragaria* is discussed in detail in the next Section.

1.4 Developmental cycles and environmental regulation in selected rosaceous species

The Rosaceae family, which can be divided into six subfamilies (Datta, 1988; Longhi *et al*., 2014), is of economic importance, because it includes a number of commercial fruit species. To provide a focused description of the developmental cycles and environmental regulation within this family, three Rosaceae sub-families will be considered here: Maloideae, Amygdaloideae and Rosoideae. One genus from each sub-family will be discussed in detail in relation to environmental regulation: *Malus, Prunus* and *Fragaria* (Figure 1.7). The reason for focusing on one core species within each sub-family is that more similarities would be expected within each sub-family than between them. For example, *Fragaria* and *Rubus* are both within Rosoideae and are more similar than *Malus,* which is within the Maloideae sub-family (Illa *et al*., 2011).

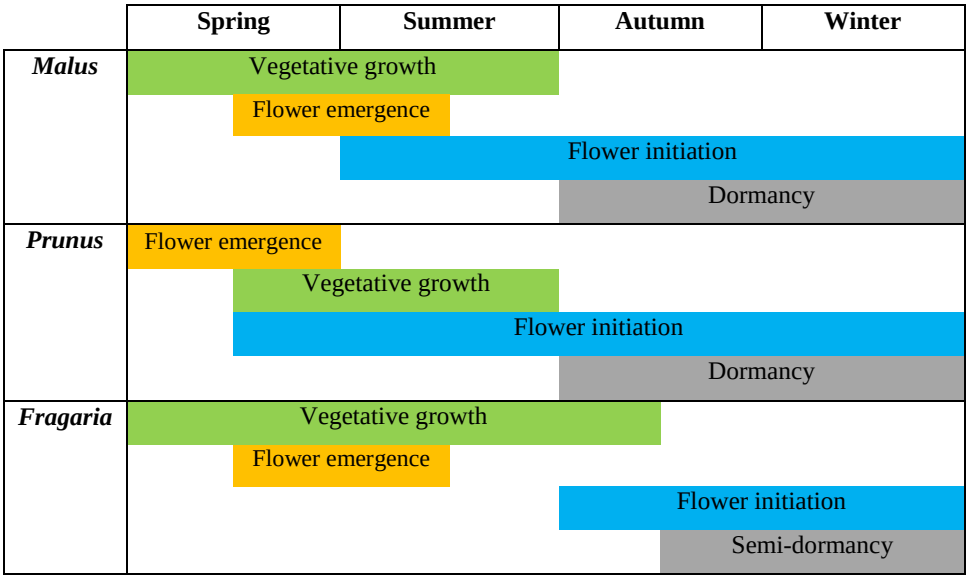

Figure 1.7 Typical annual cycle for *Malus*, *Prunus* and seasonal-flowering *Fragaria* showing the timing of flower emergence, vegetative growth, flower initiation and dormancy

Malus

Flower initiation in *Malus* x *domestica* occurs within 50 days of full bloom (Foster *et al*., 2003) (Figure 1.7). As a result, the environmental conditions following flowering (first four-five weeks) are of great importance for flower formation (Tromp, 1980), although the specific environmental regulation, in particular the influence of photoperiod, is still not clearly understood (Wilkie *et al*., 2008; Kurokura *et al*., 2013). Studies have provided conflicting evidence with regards to the effect of photoperiod: some have suggested that apples are generally day-neutral (Zieslin and Moe, 1985; Thomas and Vince-Prue, 1997). Others have, however, found that intermediate photoperiod (12-14h) increased initiation, in comparison to SD (8h), while long photoperiod (16h) had a repressive effect (Stahly and Piringer, 1962).

Temperature has also been reported to affect flower initiation; an increase in temperature (13 to 20°C over six-seven weeks) enhanced flower bud formation, although high temperature (24°C) had an inhibitory effect and delayed floral development (Tromp, 1976, 1980; Gur, 1985). Temperature effects can be observed by the number of flower clusters per spurs, with a greater number of clusters for plants grown at 20°C, in comparison to those at 13°C (Zhu *et al*., 1997). Naturally fluctuating temperatures (average of 14.5°C) favoured flower initiation in comparison to a constant temperature (of 14.5°C) (Abbott *et al*., 1974).

As well as regulation by environmental conditions, autonomous factors also control flower initiation. A critical number of leaf nodes must be present in a bud before flower initiation occurs,

with the threshold in node number varying with cultivar (Luckwill, 1975; Wilkie *et al*., 2008). This requirement can modulate the effect of temperature on initiation, as flowering is restrained until the meristem becomes generative (Zhu *et al.*, 1997). Another regulator of flower initiation is plant age, with the juvenile phase (which can be greater than six years) preventing flower initiation (Kotoda *et al*., 2006).

Flower initiation and development does not occur at a continuous rate until emergence; during the autumn, relatively high temperature resulted in accelerated flower development, although a day temperature of 11°C and night temperature of 7°C during the autumn were found to be optimal during this period (Gur, 1985). Floral development has also been shown to continue during winter dormancy, although it was slower (Kuroda and Sagisaka, 2001). It is generally accepted, however, that continued active floral development recommences after dormancy release, between bud burst and anthesis (Sung *et al*., 2000; Wilkie *et al*., 2008).

Temperature plays a primary role in the induction of growth cessation and dormancy in apple, with no effect of photoperiod (Nitsch, 1957; Heide and Prestrud, 2005; Kurokura *et al*., 2013). Heide and Prestrud (2005) studied the effect of temperature and photoperiod on growth cessation and dormancy induction and showed growth cessation to occur in response to low temperature (9°C), regardless of photoperiod and cultivar. These authors also showed variation in the specific temperature response between cultivars, with growth cessation observed at 12°C for some cultivars. Continued exposure to growth cessation-inducing conditions (at temperatures less than 12°C) led to the formation of winter buds and a reduction in leaf lamina size (which had previously been visually associated with growth cessation – Abbott, 1970) (Heide and Prestrud, 2005). The same conditions inducing cessation resulted in buds entering true dormancy (Heide and Prestrud, 2005). Previous studies had suggested that dormancy onset was endogenously controlled and therefore apple was considered to be day-neutral (Thomas and Vince-Prue, 1997; Battey, 2000); the work of Heide and Prestrud (2005) also showed no photoperiodic influence on dormancy induction, but low temperature did have a promotive effect.

At low temperatures, such as those associated with growth cessation and dormancy induction, chill accumulation occurs, which acts to break dormancy and restore bud growth potential (Heide and Prestrud, 2005). Low chilling temperatures varied in their effectiveness at releasing dormancy, with 6°C being most effective and 12°C only marginally effective (Heide and Prestrud, 2005); although Thompson *et al.* (1975) found that for some apple cultivars, the optimal temperature for chilling was 2°C. There is also variation in the chilling requirement of this species with a reported range of 200-1400 chill units (Atkinson *et al.*, 2005). Hawerroth *et al*. (2013) studied the effect of chill temperature on subsequent spring response and showed that chilling at

5°C and 10°C was more effective for breaking dormancy than 15°C in 'Royal Gala'. The influence of higher chill temperature (15°C) was less pronounced in 'Castel Gala', which was assumed to be associated with its low chill requirement (Hawerroth *et al*., 2013).

In apple, leaf emergence (budburst) occurs in the spring before flower emergence, and as a result the species is described as hysteranthous (Figure 1.7). Spring bloom is the culmination of the reproductive process which starts with flower initiation the preceding summer (Abbott, 1970); and time of bloom is greatly dependant on spring temperature (Swartz and Powell, 1981). Temperature also regulates vegetative growth, with warm temperatures (15°C) having a more promotive influence on budburst than low temperatures (5-10°C) (Hawerroth *et al*., 2013). Photoperiod also promotes vegetative growth, with shoot elongation significantly increased by long photoperiods (Heide and Prestrud, 2005).

Prunus

The genus *Prunus* contains a number of commercially important species, including cherry, plum, peach, apricot, nectarine and almond. To exemplify the principles of environmental regulation of flower initiation, dormancy, chilling and spring growth in this genus, cherry (*Prunus avium* and *Prunus cerasus*) forms the focus of the following summary.

As in *Malus*, flower initiation in *Prunus* occurs after the emergence of flowers in the spring; but unlike in *Malus*, in sweet cherry (*Prunus avium*) it follows fruit harvest (Westwood, 1993). Fruit and shoot growth typically occur at the same time (Predieri *et al*., 2003), with continued shoot elongation coinciding with flower initiation. Flower initiation has been regarded as regulated by endogenous plant hormones, including gibberellin inhibitors, auxins, cytokinins and ethylene (Hoad, 1984; Webster and Looney, 1995; Engin and Ünal, 2007); and vegetative growth appears to be a prerequisite for flower initiation (as described for apple), with a critical number of nodes required to enable the transition from a vegetative to a floral state (Koutinas *et al.*, 2010). Juvenility has been reported as a major restricting factor of flower initiation for cherry, with *P. avium* and *P. cerasus* having long juvenile periods (average of six years) (Gur, 1985; Wang *et al.*, 2015).

Environmental conditions affect the timing of flower initiation and differentiation (Wilkie *et al*., 2008), but the way environment influences these processes has not yet been described comprehensively (Koutinas *et al.*, 2010). Warm temperatures promote flower initiation and differentiation (Li *et al.*, 2010), although the optimum temperature for development typically varies with cultivar. For example, in *P. avium* cultivars optimal temperature for pollen

germination and pollen tube growth varied between 20-30°C (Hedhly *et al*., 2004). However, high temperature becomes inhibitory because of competition with vegetative growth and can also result in malformed flowers (Gur, 1985; Hedhly *et al.,* 2007; Li *et al.,* 2010). Unlike the conflicting reports of photoperiod for apple, daylength is not considered to affect flower initiation in cultivated cherry (Wareing, 1956); and flower initiation and differentiation continues over the summer and into the winter (Figure 1.7).

Prunus species also show growth cessation and dormancy and there is a pronounced interaction of photoperiod and temperature controlling these growth responses; although sensitivity to environmental regulation varies with temperature and cultivar (Heide, 2008). Heide (2008) showed that at high temperature (21°C) growth was continuous regardless of photoperiod; a few weeks in SD caused growth to cease at intermediate temperatures (12°C and 15°C); while at low temperature (9°C) SD were required for induction of growth cessation in *P. avium* but not in *P. cerasus*. Heide (2008) also reported that dormancy induction followed growth cessation, with the formation of terminal winter buds; plants reached a state of relatively deep dormancy, with shoot elongation and leaf formation significantly reduced after extended exposure to dormancy inducing conditions. This suggested that the environmental conditions inducing growth cessation also induced dormancy (Heide, 2008). Winter chilling followed dormancy induction and actively promoted gynoecium and ovule development (Liu *et al*., 2015); insufficient chilling accumulation caused abnormality of the female floral organs (Wang *et al*., 2016).

Mahmood *et al*. (2000a) studied chilling in three sweet cherry cultivars and showed very little variation in the optimum temperature for satisfying chilling requirements (3.2-3.7°C); chill accumulation occurred over a wide range of temperatures but those above 12°C and below -4.5°C were ineffective. The duration of chilling influenced flower number, with a greater number of flowers associated with increased chilling (Mahmood *et al*., 2000a). Chill duration also influenced vegetative growth: with increased chilling duration, time to budburst was reduced and the frequency of budburst increased (Mahmood *et al*., 2000b). Cherry varieties, much like apple, also vary in their chill requirement, with variation between 800-1700 chill hours reported (Atkinson *et al.,* 2005). In the absence of sufficient chilling, prolonged dormancy has been observed (Mahmood *et al*., 2000b).

In the spring, under favourable conditions active growth and development resumes with the emergence of flowers and vegetative growth (Figure 1.7). In contrast to apple, flower emergence occurs before vegetative growth; as a result cherry is described as precocious, and emerging flowers are those which were initiated during the previous growing season. Temperature

conditions during late floral development (February and March) have been shown to influence flowering time, with warmer temperatures advancing flowering (Aono and Kazui, 2008).

Fragaria

There are eight key papers which address the issue of flower initiation, dormancy, chilling and spring response in *F.* x *ananassa* and *F. vesca* (see Table 1.1 for detailed information). The conclusions for each physiological process will be summarised, focusing first on *F.* x *ananassa*, then *F. vesca*. The natural transition to flowering occurs in early autumn as flower initiation is activated in response to SD and decreasing temperature (Darrow, 1966; Guttridge, 1985; Mouhu *et al.*, 2013) (Figure 1.7). The exact time of flower initiation depends on environmental conditions and cultivar and typically occurs between September and October in the temperate zone in the Northern Hemisphere (Jahn and Dana, 1970a and b). Heide *et al.* (2013) reviewed the optimal conditions for induction in the genus *Fragaria* and concluded that under SD, temperatures up to 18°C promote induction (Heide, 1977; Le Mière *et al.*, 1996; Heide and Sønsteby, 2007); although the critical photoperiodic requirement for induction varies between cultivars (*F.* x *ananassa*) and populations (*F. vesca*) (Heide *et al.*, 2013). Heide (1977) studied floral induction in Scandinavian *F.* x *ananassa* cultivars and showed that their response to photoperiod was mediated by temperature: at 12°C plants were indifferent to photoperiod (ranging from 10-24h); at 18°C, more flowers were initiated under SD but some cultivars were still induced to flower at this temperature under continuous light (24h); at 24°C plants remained vegetative at photoperiods above 14-16h (Heide, 1977). In other cultivars, photoperiod has been shown to play a key role in promoting flower induction at 18°C; for example, flower initiation in cv. 'Korona' occurred at photoperiods between 12-15h at 18°C, with the number of flowers reduced at 15h and falling to zero at 18 hours (Konsin *et al.*, 2001).

Table 1.1 Key studies which address the issues of flower initiation, dormancy, chilling and spring response (vegetative growth and flower emergence) in *F.* x *ananassa* and *F. vesca*

Study	Species	Experiment conditions	Findings
Guttridge, 1958	*F.* x *ananassa*	Transfers from field environments at a variety of locations to controlled forcing.	Cultivars varied in their chill requirement. Prior to chilling, dormant plants had a distinct growth habit with short petioles and reduced runnering; although they continued flower initiation. Post-chilling, plants were unable to initiate flowers even under favourable conditions and displayed more vigorous growth, with an initially high rate of leaf emergence, longer petiole length, larger leaves and greater runnering.
Jonkers, 1965	*F.* x *ananassa*	Plants grown at 15, 18 and 21°C either in the field (from August) or controlled LD (16h).	Photoperiod was the dominant cue for growth cessation, regardless of temperature. Shortening photoperiod decreased above-ground growth and this response was accelerated by low temperature.
		Plants transferred from the field to forcing (18°C/LD), every month from August-March.	Inflorescence emergence was shown for all transfers, which implied no absolute dormancy and emergence was most rapid following transfers in November. There was a minimal growth response between mid-October – mid-November: these plants had the smallest trusses and shortest petioles, which favoured the term relative dormancy. Long periods of chilling promoted inflorescence elongation.
Heide, 1977	*F.* x *ananassa*	Five week induction treatment: combination of photoperiod (10, 12, 14, 16 and 24h) and temperature (12, 18 and 24°C), followed by forcing at 24°C/24h.	The optimum photoperiod for growth parameters (leaf area, petiole length and runnering) was typically 16h. High temperatures stimulated runnering regardless of photoperiod, while temperatures of 12°C and less strongly reduced petiole length. This implied that broadly the conditions stimulating growth and initiating flowering inhibition are independent. However, in some cultivars, 16h/18°C caused maximal petiole length and was also inductive for flowering.
Tehranifar *et al.*, 1998	*F.* x *ananassa*	Exp1: plants lifted on 06/11 and 06/12; controls transferred to forcing (15°C/LD); others cold stored at -2 or 3°C for varying intervals, then forced. Exp2: plants lifted in November; cold stored at -2°C for 0, 4, 8 or 12wks; forced at 10, 15 or 20°C.	Exp1: a greater duration of chilling resulted in plants with longer petioles and more runners, once forced. Lifting date had a significant effect on vegetative growth: plants lifted in December had longer petioles and more runners, than those lifted in November. Exp2: there was no significant interaction between chill duration and forcing temperature. Petiole length increased with forcing temperature, and at all temperatures a longer chill duration increased petiole length. Chilled plants generally had more flowers across forcing temperatures and flower emergence was more rapid; chilling was also shown to cease flower initiation. Vegetative vigour was positively related to chilling and this effect occurred regardless of subsequent forcing conditions.

Study	Species	Experimental conditions	Findings
Konsin *et al.*, 2001	*F.* x *ananassa*	Induction treatments, combination of photoperiod (12, 13.5, 15 or 18h) for either (21, 35 or 49 days), followed by forcing at 18h. The temperature was 18/16°C (day/night) throughout.	The longer the duration of the induction treatment the more significant the effect of photoperiod on petiole length; but after 10 weeks forcing differences between treatments had diminished. Photoperiod influenced leaf area, with smaller leaves under shorter photoperiod. The length and number of runners was affected by photoperiod and duration: 18 hours produced the most and longest runners; while shortening photoperiod inhibited runnering, especially after 49 days at 12/13.5 hours. Plants did not exhibit any clear signs of endodormancy. Although increased duration at sub-optimal growth conditions reduced petiole length, leaf area and runner production, this dwarf-like growth habit was not maintained after prolonged forcing. This suggested that dormancy induction might require photoperiod shorter than 12 hours, temperature lower than 18°C and/or longer duration than 49 days.
Sønsteby and Heide, 2006	*F.* x *ananassa*	Exp1: five weeks induction under SD (10h) and 15°C, followed by chilling at 2°C for 0, 2, 4 or 6 weeks; then forced at 18°C SD and LD treatment. Exp2: varied duration of SD induction at 6 or 15°C, followed by forcing at 18°C SD and LD treatment. Exp3: induction under SD or LD for five weeks at 9, 15, 21 or 27°C, then forced at 20°C/24h.	Exp1: during induction plants developed a compact growth habit with smaller leaves and shorter petiole length. Following induction, all plants flowered but emergence was delayed under SD in comparison to LD. In LD forcing, chilling did not affect flowering time or inflorescence length, although there were a greater number of emerged flowers with increased chilling; and inflorescence and petiole length were greater under LD forcing than SD. In SD forcing, the effect of chilling was greater and runnering was stimulated by previous chilling. Exp2: extended SD increasingly constrained growth, especially at 15°C. Five weeks at SD did not have an inhibitory effect on subsequent petiole and inflorescence length, but extended SD (10/15 weeks) at 15°C did strongly restrain growth (observed by petiole, inflorescences and runners). By contrast, extended SD (10/15 weeks) at 6°C did not have an inhibitory effect under subsequent forcing with active leaf and inflorescence growth; more runners were also stimulated following induction at 6°C. This suggested that 10/15 weeks at SD/15°C induced a semi-dormant state, with reduced growth potential under subsequent forcing. Induction at 6°C showed no dormancy-induced effect. Runnering was more sensitive to chilling than leaf or inflorescence elongation. Runner restraint was fully reversed by chilling, whereas under subsequent SD forcing the restraint on leaf and inflorescence elongation was only partially reduced by chilling. Exp3: there was a strong growth restraint under SD at 9 and 15°C, with no indication of subsequent dormancy-related growth inhibition once forced. This suggested that SD exposure for 5 weeks at temperatures greater than 9°C did not induce dormancy.

Study	Species	Experimental conditions	Findings
Heide and Sønsteby, 2007	*F. vesca*	Exp1: floral induction for five weeks, at either 9, 15 or 21°C under SD or LD. Exp2: plants induced at 15°C, SD for 2, 3, 4 or 5 weeks. Range of populations included. Exp3: plants exposed to a range of photoperiods at 15 or 18°C for five weeks; and then transferred to forcing (20°C/LD).	Exp1: flower induction occurred at 9°C under SD and LD; at 15°C, induction only occurred under SD; and at 21°C there was no initiation regardless of photoperiod. The greatest number of flowers was produced following exposure to 15°C/SD. Exp2: exposure to 15°C/SD resulted in flowering in most populations after 4 weeks, but 5 weeks exposure was required for full flowering across populations. Exp3: the critical photoperiod for 50% flowering at 15°C was 16h.
Sønsteby and Heide, 2011	*F. vesca*	Exp1: variety of populations. Plants were maintained in the field from September and transferred to forcing (20°C/24h) at intervals throughout autumn/winter of 2005 and 2006. Exp2: plants maintained at either 6 or 15°C under SD and at intervals transferred to forcing (21°C/24h).	Exp1: plants showed markedly different results between 2005 and 2006. In 2005, there was a warmer average temperature (9°C) at the start of autumn (October – November); in 2006 the average at the start of the autumn was 0°C and in the following four weeks it was warmer in 2006 than 2005. In 2005, the deepest dormancy was attained by the end of November with the least number of stolons, leaves and petiole length, although flower emergence in subsequent forcing was more rapid. In 2006, there was a gradual increase in growth measures throughout the experimental process; and flower buds showed slow and gradual loss of dormancy through the autumn, measured by emergence. Exp2: vegetative growth continued throughout induction but at a greater rate at 15°C. The capacity for runnering under forcing was reduced for plants grown at 15°C with increasing duration; following induction at 6°C, runnering capacity remained fairly constant. There was a similar but less pronounced response between induction temperatures for petiole length. This suggested that a semi-dormant state was attained at 15°C but not at 6°C.

Dormancy follows floral induction and in *Fragaria* similar conditions regulate both physiological processes (Figure 1.7). Dormancy in *Fragaria* is considered to be quantitative and it has been described as semi-dormancy (Guttridge, 1985; Konsin *et al.*, 2001; Sønsteby and Heide, 2006; Kurokura *et al.*, 2013). Guttridge (1985) reviewed work demonstrating semi-dormancy on *Fragaria* and described the effect on morphology as: vegetative growth being restrained, but still ongoing; emerging leaves being small with short petioles, no stolons formed, and the plant exhibiting a stunted, dwarf-like habit. Continued growth during this period has been observed through leaf initiation, during autumn and winter (Arney, 1955b). Nevertheless, the partial restraint on growth indicates that *Fragaria* does become dormant (to some degree), and this form of dormancy does reinvigorate growth; hence the term semi-dormant.

Jonkers (1965) studied dormancy induction in *F.* x *ananassa*, and showed a general response of decreasing above-ground growth with shortening photoperiod; although this response was accelerated by low temperature, photoperiod was the dominant cue for growth cessation. Plants were grown in the field and transferred to forcing conditions at regular intervals, in order to quantify the state of dormancy. Throughout the autumn and winter, transfers to forcing conditions resulted in leaf and inflorescence emergence, with plants retaining the ability to recommence growth, implying a lack of absolute dormancy (Jonkers, 1965). Jonkers (1965) showed that under natural environmental conditions the deepest state of semi-dormancy in *F.* x *ananassa* was attained in November, with plants transferred to forcing at this time having minimal inflorescence elongation, but, perhaps unexpectedly, flowering being most rapid following this transfer.

Subsequent work has shown that temperature interacts with photoperiod during the dormancy phase and can aid dormancy induction or act to release dormancy in *F.* x *ananassa*. Extended SD at intermediate temperatures (9 and 15°C) have a strong inhibitory effect on growth in comparison to low temperature (6°C), which was not shown to inhibit leaf and inflorescence growth, suggesting that the dormancy-inducing effect of SD is nullified by low temperature (Sønsteby and Heide, 2006). Temperature during dormancy induction also has a direct effect on subsequent vegetative growth, and growth parameters vary in their responses to dormancy-inducing temperatures. Sønsteby and Heide (2006) showed that extended exposure to SD (10-15 weeks) was necessary for dormancy induction, consistent with Jonkers' (1965) conclusion that photoperiod was the dominant cue for growth cessation. Insufficient exposure to SD appears not to induce semi-dormancy in *F.* x *ananassa*, with plants transferred to forcing rapidly losing any growth restraint (showed after induction for seven weeks, Konsin *et al.*, 2001; five weeks, Sønsteby and Heide, 2006).

Chilling is another physiological process that has been studied for its effect on subsequent growth and development, typically in conjunction with dormancy. The dwarf-like growth habit induced through semi-dormancy is released by chilling, which restores long petioles, runner production and leaf emergence (Guttridge, 1958). Chilling accumulated during the dormancy phase has been directly linked to increased subsequent vegetative growth, with a quadratic relation between petiole length and chill hours (Robert *et al*., 1997); petiole length and runner number also increased with chill duration (Tehranifar *et al.,* 1998). In *F.* x *ananassa,* chill requirement to release dormancy and reset growth vigour varies between varieties (Guttridge, 1958). Chilling also acts to restrict flower initiation even under subsequent favourable conditions (Guttridge, 1958; Tehranifar *et al.,* 1998), which is crucial to the maintenance of perenniality and seasonal flowering.

Sønsteby and Heide (2006) studied the effect of chilling on subsequent growth and development in *F.* x *ananassa* and showed that growth parameters varied in their response to chilling. Runner restraint induced by dormancy and unfavourable autumn/winter conditions was more sensitive to release by chilling than leaf and inflorescence emergence, with a total reversal of runner restraint during forcing (at 18°C) regardless of photoperiod. In comparison, restraint on leaf and inflorescence emergence was only released in LD forcing conditions, with more rapid emergence of inflorescences resulting in a greater number of flowers (Sønsteby and Heide, 2006).

Following winter dormancy and chilling, spring temperatures and photoperiod interact to promote active vegetative and inflorescence growth, and plants are insensitive to conditions which were previously inductive for flowering (SD and temperatures around 15°C) (Battey *et al.,* 1998); flower initiation therefore appears to be restricted for a time, as a result of chilling (Tehranifar *et al.,* 1998). Flower emergence follows the resumption of active vegetative growth in the spring in seasonal flowering *Fragaria,* with the rate of progress to flowering linearly related to temperature (Le Mière *et al.,* 1998). Fruiting follows flowering, and although increasing temperature accelerates the rate of progress to flowering and fruiting, high temperatures reduce yield (Le Mière *et al.,* 1998). Runnering is another growth response observed in the spring/summer; the conditions promoting this process are cultivar specific, but typically LD (16h) and warm temperatures (24°C) are optimal (Heide, 1977).

There are relatively few studies that primarily focus on environmental regulation in *F. vesca* (see Table 1.1). Heide and Sønsteby (2007) studied the interaction between temperature and photoperiod on flower initiation in *F. vesca*, showing the effect of temperature to be modified by photoperiod: at low temperature (9°C) initiation occurred under SD and LD conditions; at intermediate temperatures (15 and 18°C), SD were required for initiation; and at high temperature

(21°C) initiation did not occur regardless of photoperiod. Exposure to optimal conditions for a minimum of four weeks was required for floral induction (Heide and Sønsteby, 2007).

Sønsteby and Heide (2011) presented results from a series of experiments to establish the environmental regulation of dormancy. Their first experiment considered environmental induction of dormancy over two consecutive years (2005-2006) under natural conditions in *F. vesca* and in the first year (2005) showed a similar temperature-photoperiod interaction to that in *F.* x *ananassa* (Jonkers, 1965; Sønsteby and Heide, 2006). In the first year of this study the temperature at the start of the autumn was relatively warm (average 9°C) and the deepest state of semi-dormancy was reached by the end of November, with plants showing minimal vegetative growth (few runners, leaves and small petioles) but the most rapid inflorescence emergence following transfer to forcing. The same result was not, however, observed in the following year (2006) when autumn temperatures were significantly colder (average 0°C, in comparison to 9°C in 2005). This cold autumn temperature was not associated with deep dormancy; instead it was suggested that plants had an early and continued loss of dormancy across the autumn, associated with a gradual increase in leaf, runner and inflorescence number, and petiole length (Sønsteby and Heide, 2011).

These authors also used controlled environments in order to identify the specific influence of low and intermediate temperatures on dormancy induction. Intermediate temperature (15°C) under SD induced dormancy, with extended exposure to inductive conditions leading to: reduced petiole length and runner number in subsequent LD forcing; but advanced time to flowering. Maintaining plants at 6°C did not restrict subsequent growth under LD forcing; extended exposure to this temperature resulted in a higher number of runners and consistent petiole length. Sønsteby and Heide (2011) suggested that these results showed that a semi-dormant state was attained at 15°C, but not at 6°C, as shown in *F.* x *ananassa* (Sønsteby and Heide, 2006).

The papers described here highlight some key issues in understanding dormancy in *Fragaria* and its response to the environment. Temperatures of 15°C under SD conditions induce a dwarf-like growth habit, with extended exposure leading to a decline in leaf size in both *F.* x *ananassa* and *F. vesca* (Sønsteby and Heide, 2006, 2011). Photoperiod has been suggested as a dominant cue for growth cessation in the autumn, with a reduction in petiole length and leaf size correlated with shortening photoperiod (Jonkers, 1965). The effect of temperature is more complex, with a temperature of 6°C thought to nullify dormancy induction in both species, yet still inducing the stunted growth habit associated with semi-dormancy. Sønsteby and Heide (2011) concluded that "*when the temperature in the periods* [of dormancy induction] *was close to zero, there was an early and continuing loss of dormancy during the entire autumn*" (p. 47). This suggests that they

considered the plants to have become dormant to some degree, even though cold temperature was considered non-inductive for semi-dormancy: "*dormancy did not develop at 6°C, while the normal semi-dormant state was attained at 15°C*" (p. 47).

Given the natural fluctuations in temperatures within and between years during dormancy induction, it appears that a better understanding of environmental regulation of semi-dormancy, especially under natural conditions, is required. Controlled environment studies enable optimal conditions for induction to be established, but do not account for field responses. If the work of Sønsteby and Heide (2011) should be interpreted to mean shallow semi-dormancy is induced, even under cold temperatures, which had previously been suggested to counteract dormancy induction, then a more coherent understanding of semi-dormancy regulation is clearly required. Identifying a molecular marker of dormancy could prove beneficial, in order to establish semi-dormancy rather than relying on transfer experiments and observations of the typical semi-dormant growth habit which may be hard to quantify precisely. But there is also a need for more systematic analysis of *F. vesca* response to autumn/winter conditions. In contrast to the detailed research on the conditions inducing flowering in *Fragaria* (the optimal and range of temperatures and photoperiods) (Guttridge, 1985; Battey *et al.*, 1998; Heide *et al.*, 2013), dormancy induction has not been sufficiently analysed. It is likely that cultivars and populations vary in their semi-dormancy induction, as well as, perhaps, the depth of semi-dormancy experienced. This topic is of importance, particularly in the context of predicted climatic changes. It should be noted, however, that the semi-dormancy observed in *Fragaria* appears not to be typical of commercially exploited Rosoideae; for example, in another member of the Rosoideae, *Rubus idaeus* (raspberry) growth cessation and dormancy induction is absolute. Dormancy induction occurred most rapidly at low temperature (less than 12°C), was delayed at intermediate temperature (15°C) and was not observed at warm-high temperature (18°C) (Sønsteby and Heide, 2008). The conditions inducing flower initiation and dormancy appear to be similar in both *Fragaria* and *Rubus*.

1.5 Climate change and perennial growth and development

Many of the processes which form the annual cycle are typically referred to as phenological events, with phenology defined as 'the timing of recurrent biological events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species' (Badeck et al., 2003). There are a number of external, environmental factors reported to influence phenological timing, including: temperature, photoperiod and precipitation (Forrest and Miller-Rushing, 2010). As highlighted by the multiple pathways regulating flowering in *Arabidopsis* (Albani and Coupland, 2010), these regulatory mechanisms often

overlap and/or interact to create a complex symphony of influencing factors. Sensitivity to temperature and photoperiod are considered to be dominant themes in the regulation of seasonal changes and for well-timed phenology (Wilczek *et al.*, 2010), which is particularly important given the static nature of plants (Larcher, 2003).

This sensitivity to temperature, and its interaction with photoperiod signals, leads to concern over the potential effects of predicted climate change. Average global temperature has been increasing since the turn of the twentieth century and climate warming is predicted to continue, with a global mean surface temperature increase of between 0.3°C to 0.7°C from 2016–2035 (IPCC, 2014). As a result, physiological processes that are highly regulated by temperature are vulnerable to change and identifying the influence of warming temperature on such processes is vital to establish how predicted climate might affect responses. In *Arabidopsis* for example, previous research suggested that autonomous pathway mutants and high-*FLC* genotypes are relatively temperature insensitive, but more recent work has shown that under warm temperatures floral repressors (including *FLC*) are increased leading to delayed flowering, even in these previously assumed 'temperature insensitive' genotypes (Burghardt *et al.*, 2016). Changes in temperature can also have more widespread implications; levels of many key phytohormones, including GA and auxin, can either be reduced or increased by heat stress, and can alter plant responses causing, for instance, premature plant senescence (Bita and Gerats, 2013). In *Arabidopsis* elevated CO_2 can delay flowering time by minimising the down-regulation of *FLC* which in turn influences regulation of *SOC1* and *LFY*, resulting in delayed flowering (Springer *et al.*, 2008). A subsequent study suggested *MOTHER OF FT AND TFL1* (*MFT*) as a potential candidate gene for the influence of CO_2 on flowering time (Ward *et al.*, 2012).

If such effects can be clearly observed in *Arabidopsis*, they may be even more significant in long-lived perennial species, which are however, much more difficult to study experimentally. This emphasises two needs: for good model perennial species; and for detailed understanding of these developmental/morphological responses to environment, against which knowledge of mechanisms can be juxtaposed.

1.6 Scenarios of climate warming impacts on rosaceous species and examples of temperature influence in: Malus, Prunus and Fragaria

The following examples focus on climatic, and climate change, effects on rosaceous species, with phenological impacts as the primary focus. Resumption of spring growth in rosaceous species following dormancy occurs through floral and vegetative budburst; given the horticultural

importance of these species many studies have focused on the effect of climatic changes on flowering time and the response of fruiting and yield. A meta-analysis of changes in phenological timing in Mediterranean ecosystems in response to climate, which included a number of important rosaceous species (*Malus, Prunus, Pyrus* and *Rosa*), showed an advance in time of flowering (-6.47 days per 1°C increase) and leaf unfolding (-6.99 days per 1°C increase), which highlights the sensitivity of both of these phenophases to climate warming (Gordo and Sanz, 2010).

Miller-Rushing *et al.* (2007) studied flowering time in species and hybrids of cherry (*Cerasus* spp. and *Prunus* spp.) and showed an advance in timing of flowering of approximately 5.5 days over 25 years; this was correlated with an increase in mean temperature of 1.8°C during February and March over the same time period. The variation in the time of average flowering was proposed to be a general result of temperature change. Miller-Rushing *et al.* (2007) also suggested that species variation in responsiveness to temperature may lead to a divergence of flowering times, which could have significant biological consequences. However, this study also showed that in the species studied the duration of flowering lengthened at higher temperatures, an additional 0.5 days for every 1°C increase, which might at least in part offset the effect of diverging flowering times.

Flowering time has not only been correlated with early spring temperatures but is also influenced by temperatures over winter, as a result of chill accumulation (see Introduction to Chapter 6). In years of low-chilling accumulation, often as a result of warm winter temperatures, flowering time of peach can be delayed by one-two weeks for some cultivars; although flowering duration has also been observed to increase (by three-five days) another potential consequence of low chilling (Ghrab *et al.*, 2014). A possible method of mitigation for climatic effects on horticultural crops is the development of low chill requirement cultivars, an approach which has been notable in apple (see Vysini *et al.*, 2011) and peach (Fan *et al.*, 2010). There are a number of key studies which highlight the potential impacts of increased temperature on physiological processes, which differ from those which assume more of a phenological focus, and these are summarized for *Malus* x *domestica, P. avium/P. cerasus* and *F.* x *ananassa/F. vesca* in Table 1.2.

Table 1.2 Key studies showing the implications of increased temperature on developmental processes during the annual cycle in *Malus, Prunus* and *Fragaria*

<table>
<tr><th></th><th>Malus x domestica</th><th>P. avium/P. cerasus</th><th>F. x ananassa/ F. vesca</th></tr>
<tr><td>Jan</td><td>In Europe, modelling has shown that spring warming has been mainly responsible for advances in flowering time; winter warming can potentially delay flowering time (Guédon and Legave, 2008; Legave et al., 2013, 2015; El Yaacoubi et al., 2014).

Warm temperatures over the chill period led to inadequate chilling and resulted in uneven and prolonged budburst (Cook and Jacobs, 2000).</td><td>Increased heat accumulation advanced flowering, whereas reduced chilling can be associated with reduced fruit set (Erez, 2000; Castède et al., 2014). Generally, chill is considered to dominate over heat in the determination of flowering time (Dirlewanger et al., 2012; Castède et al., 2014).
Plants exposed to high temperature (20°C) (early forcing) had advanced bloom and harvest, which also enabled earlier floral initiation and differentiation (Beppu et al., 2001).</td><td>Warm winter temperatures decreased spring vegetative vigour and reduced fruiting capacity (Tehranifar et al., 1998).</td></tr>
<tr><td>Feb</td><td rowspan="3">Cool spring temperature (13°C) resulted in better fruit set than plants maintained at 19°C (Tromp and Borsboom, 1994). Low temperature (5°C) during this period delayed time to full bloom; plants required less time to complete pollen-tube growth and fruit set was greater (compared to 10°C during this period) (Jackson et al., 1983). Warm temperatures during the same period have been correlated with poor yield (Beattie and Folley, 1977, 1978).</td><td rowspan="3">High pre-blossom (one month before anthesis) temperatures (20-25°C) reduced fruit set: ovary growth was suppressed and small ovules were induced (Beppu et al., 1997).</td><td></td></tr>
<tr><td>Mar</td><td></td></tr>
<tr><td>Apr</td><td></td></tr>
<tr><td>May</td><td></td><td></td><td></td></tr>
<tr><td>Jun</td><td>Plants maintained at high constant temperature (20-25°C) over the summer/autumn (June-November) showed delayed bud break and fruit bloom the following spring (February-March) (Jonkers, 1979).</td><td rowspan="2">Exposure to high temperatures (35°C) (June-August) increased double pistil formation (Beppu et al., 2001).

Higher average temperature resulted in a greater proportion of malformed flowers (Li et al., 2010).</td><td rowspan="2">High temperatures (>26°C) (June-September) reduced germination, pollen viability, pollen tube growth and fruit set (Karapatzak et al., 2012).

High temperature (30/25°C) reduced the number of emerged inflorescences and fruit. Fruit grown under these conditions had reduced size and weight and there was a greater percentage of malformation (Ledesma et al., 2008).</td></tr>
<tr><td>Jul</td><td></td></tr>
<tr><td>Aug</td><td>High temperature (24°C) reduced flower abundance and cluster quality the following spring, compared to plants grown at 17°C (Tromp, 1980).</td><td></td></tr>
<tr><td>Sep</td><td rowspan="3">High temperature (24°C) (September-November) retarded spring development and delayed bloom (Tromp and Borsboom, 1994).

There was an absolute requirement for low temperature (<12°C) for induction of growth cessation and dormancy (Heide and Prestrud, 2005). In the absence of low temperature, declining photoperiod was insufficient for induction.</td><td></td><td></td></tr>
<tr><td>Oct</td><td rowspan="2">A pronounced photoperiod x temperature interaction regulated growth cessation and dormancy; intermediate/low temperatures (9-15°C) promoted induction of both processes induced under SD (Heide, 2008).</td><td rowspan="2">A pronounced photoperiod x temperature interaction regulated growth cessation and dormancy; intermediate temperatures (15°C) promoted induction of both processes induced under SD, but semi-dormancy was not attained at low temperatures (Sønsteby and Heide, 2011).</td></tr>
<tr><td>Nov</td></tr>
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</table>

All three genera show a negative effect on fruit set of increased temperature during flower emergence and anthesis. This can be associated with higher proportions of malformed fruit (e.g. in *Fragaria*) and may lead to reduced yields. High temperatures during flower initiation and development in *Malus* and *Prunus* can lead to malformed flowers and reduced flower quality the following spring. It has been argued (e.g. Heide, 2008, 2011) that regulation of developmental transitions by both temperature and photoperiod should make species less vulnerable to increased temperature. For example, growth cessation and dormancy induction is promoted by a temperature x photoperiod interaction in *Fragaria* and *Prunus*, with SD a dominant driver of such processes under intermediate temperatures (approximately 15°C); this may mean that even under extreme climate warming predictions, in these genera the onset of dormancy remains unaffected. It is important to note, however, that the relevant experiments, subjecting these genera to a range of climate change scenarios over several years, have not yet been carried out. *Malus* differs from both *Prunus* and *Fragaria* in that growth cessation and dormancy induction is independent of photoperiod and controlled by an absolute low temperature (<12 ºC) requirement (Table 1.2). This lack of photoperiod regulation means that *Malus* may be more vulnerable to climate warming than *Prunus* and *Fragaria*.

1.7 Aims and objectives of the research described in this thesis

The research described in this thesis represents an integrated approach to the annual cycle of development in the model perennial *F. vesca*, with the key overarching objective of better understanding the environmental regulation of development. The focus was on quantifying responses that manifest themselves at the morphological level, in order to provide a basis for anticipating the effects of climate and climatic change on the annual cycle in this species, and in perennials more broadly.

The experimental chapters (Chapter 4-6) each aimed to address a specific developmental process in the annual cycle; in Chapter 3, morphological processes during the annual cycle were described. In Chapter 4, the way in which runners and branch crowns become determined was investigated. Chapter 5 focused on the role of temperature and photoperiod in the regulation of flowering in a range of ecotypes. The influence of chilling and spring forcing temperature on flowering and runnering was quantified in Chapter 6. In Chapter 7, the data are integrated and interpreted in order to provide insight into the annual cycle of development in *F. vesca* and the implications for future responses to environment are explored. The results provide better understanding of how spring vegetative growth and flower emergence are co-ordinated in the natural environment and in response to defined winter chill/spring forcing regimes; and the nature

of between population variation in flowering and runnering behaviour. The pronounced correlative inhibition of the terminal SAM by axillary (runner) development is highlighted, along with its implications for the processes of runner/branch crown determination and *in situ* development of *F. vesca*. The value of a future focus on 'ecological development' is stressed.

Chapter 2: General Materials and Methods

2.1 Plant materials

2.1.1 Ecotypes

Eight *F. vesca* ecotypes from a range of latitudes (50.24-60.37°N) were used, four from Finland and four from the UK (Table 2.1).

Table 2.1 *F. vesca* ecotypes, arranged in order of latitude. F ecotypes (F6, F7, F50 and F53) were collected from Finland and UK ecotypes (UK2, UK9, UK11 and UK12) from England

Ecotype	Origin	Latitude	Longitude	Collection date	Chapter
F7	Salo, Halikko	60.37 °N	22.98 °E	June 2013	5
F53	Lohja	60.21 °N	23.81 °E	2013	5
F50	Raasepori, Karjaa	60.12 °N	23.68 °E	2013	5
F6	Hanko, Tvärminne	59.84 °N	23.24 °E	August 2013	5
UK11	Humble Jumble Gill, Lake District	54.73 °N	3.21 °W	April 2014	5
UK2	Thackthwaite, Lake District	54.60 °N	3.32 °W	April 2014	3, 5
UK9	Mapledurham, Reading	51.49 °N	1.03 °W	May 2014	4, 5, 6
UK12	Batson Creek, Salcombe	50.24 °N	3.78 °W	September 2014	5

2.1.2 Propagation

F. vesca plants were primarily obtained through vegetative propagation. For the plants used in Chapter 3, 4 and 6, plants were originally collected from natural populations and at each location a number of plants were selected and removed, with roots and runners intact. These plants were subsequently referred to as mother plants. There was some variation in timing of collection between ecotypes (Table 2.1), but once collected these plants were transferred to the University of Reading, potted individually (see Chapter 2, Section 2.2) and grown under glass or in the field (Experimental Grounds, University of Reading). Once a number of runners had emerged and become sufficiently developed (with numerous plantlets along the runner, referred to as daughter plants), runners were removed from mother plants for propagation. Daughter plants were removed from the runners and individually potted into plug trays (see Chapter 2, Section 2.2.2). Trays were placed in a propagating tunnel, which was shaded (white polythene), with a gravel (basal heated) bed and mist irrigation. Daughter plants were maintained under propagation

conditions for a minimum of two weeks to encourage rooting and promote growth. Following propagation, daughter plants were individually potted and grown either under glass or in the field to enable continued growth, prior to experimentation.

For all Finnish ecotypes and the UK plants in Chapter 5, experimental plants were propagated from established plants supplied by the University of Helsinki (kindly provided by Dr. Timo Hytönen, University of Helsinki). Plants of all Chapter 5 ecotypes were sent in June 2015, while original collection date varied (detailed information is provided where possible, Table 2.1). The same method of propagation from runner plantlets was used as described above.

Some plants were also propagated sexually, details are provided in Chapter 5 where this material was used.

2.2 Growing media and materials

2.2.1 Growing media

A number of growing medium mixes were used over the course of experimentation. Mixes were altered in response to issues that arose. Initially (July 2014 – July 2015) plants were grown in a growing medium mix of J. Arthur Bowers John Innes No. 2 soil-based compost (Attgrow Ltd, Esher, UK) and Vitax Grower traditional potting compost (Vitax Ltd, Leicester, UK) (at a 1:2 ratio) – referred to as Growing Mix 1. This mix was, however, prone to waterlogging and so a second growing medium mix was devised consisting of Clover professional potting/bedding compost (Clover, Dungannon, Ireland) and Sinclair medium vermiculite (William Sinclair Horticulture Ltd, Lincoln, UK) (at a 4:1 ratio), used from August 2015 – September 2016 and referred to as Growing Mix 2. For the last stages of experimentation (from September 2016), vermiculite was replaced with perlite to prevent pots from drying out so rapidly, with a growing medium mix of Clover professional potting/bedding compost (Clover, Dungannon, Ireland) and Sinclair perlite (William Sinclair Horticulture Ltd, Lincoln, UK) (at a 4:1 ratio) – referred to as Growing Mix 3. The same growing medium mix was used in individual pots and plug trays (for propagation) during these time periods.

2.2.2 Pots and trays

Daughter plants were propagated using plug trays (21ml square cells, LBS Horticulture, Lancashire, UK). Plants were placed individually within each plug and once sufficiently rooted they were potted up individually. Established plants were grown in 9-10cm pots (Optipot, LBS Horticulture, Lancashire, UK), with consistency in pot size within experimental cohorts. Plants were maintained in these pots throughout the experimental period.

2.3 Growth facilities

Plants were grown in a number of facilities at the University of Reading, as follows.

2.3.1 Glasshouses

Heated multi-factorial glasshouse compartments were used to promote growth throughout the year, sustaining vegetative growth over the winter (2016-2017) and for experimentation (Chapter 4 – treatments; Chapter 5 – forcing). Plants under glass were exposed to fluctuating temperatures and natural photoperiod. Temperature conditions were monitored using data loggers (TinyTag Extra TGX-3020, Gemini Data Loggers), with hourly temperature recorded. Day length extension (where required) was provided by supplementary photosynthetic lights (OSRAM SON-T 400W high pressure sodium lamps; 150 μmol m^{-2} sec^{-1}). Plants were regularly watered with tap water as required.

2.3.2 Controlled environments

Two types of controlled environment facilities were used. Saxcil growth cabinets (R.K. Saxton Ltd., Cheshire, UK) were illuminated by a combination of fluorescent and tungsten lights (270-310 μmol m^{-2} sec^{-1}). Sanyo cabinets (Sanyo Gallenkamp, Leicester, UK) were illuminated by a combination of fluorescent and tungsten lights (160 μmol m^{-2} sec^{-1}). In both cases plants were irrigated manually as necessary.

2.3.3 In the field

Plants were also maintained in pots outside in the Experimental Grounds, University of Reading and grown under natural conditions; irrigation was provided manually as needed. During favourable conditions (spring-autumn), plants were placed on staging for ease of maintenance. For staging plants, temperature data were obtained from Reading University Atmospheric Observatory, with maximum/minimum daily temperature recorded. Over the winter, plants maintained under natural conditions were transferred to cold frames to provide protection from adverse conditions. In the cold frames, temperature conditions were recorded through the use of data loggers (TinyTag Extra TGX-3020, Gemini Data Loggers), with hourly temperature recorded.

2.4 Plant maintenance

Plants were sprayed against pests and diseases as required using a variety of pesticides: Calypso (Bayer CropScience Ltd, Cambridgeshire), Aphox (Syngenta UK Ltd, Cambridgeshire), AQ10 (Fargo Ltd, West Sussex), Serenade (Bayer CropScience Ltd, Cambridgeshire), Equity (Dow AgroSciences Ltd, Cambridgeshire) and Encarline (Bioline, Syngenta UK Ltd, Cambridgeshire). All chemicals were applied at the manufacturers' recommended rate. A number of sticky PE traps (HORIVER, Koppert, Suffolk) were placed at intervals throughout plants under glass and in controlled environment facilities, to monitor and trap pests; these were replaced as required. Plants were fed weekly across experiments and growing conditions (Vitafeed Standard 1:1:1, Vitax Grower (Vitax Ltd, Leicester, UK), with fertilisers diluted and applied at the recommended rate.

2.5 Morphological observations

A number of morphological parameters were measured during the experiments described in this thesis. There was some variation in frequency and method of recording between experiments; detailed information is provided in specific experimental Chapters (3, 4, 5 and 6).

The main parameters recorded were:

- Leaf emergence – leaves were tagged (with Tip-Ex) and recorded as they emerged
- Petiole length – for chosen leaves (varied between experiments) the length of the petiole from the base of the leaf lamina to the point of insertion on the crown was recorded

- Runner production – either by removing all emerged runners from the plants, or counting and tagging emerged runners (with Tip-Ex)
- Branch crowns – the number of visible, emerged branch crowns were recorded throughout the main crown
- Flower emergence – the timing of first flower emergence and the number of emerged flowers were recorded.

2.6 Statistical analysis

Statistical analysis were performed using Minitab (Minitab 16 or 17), a detailed description of the method of analysis used us provided in the individual experimental Chapters. Means and standard errors were calculated using Excel (Microsoft Excel 2013).

Chapter 3: Annual cycle of *F. vesca* – control of growth and development at the meristem

3.1 Introduction

Perennials undergo seasonal changes that form the basis of their repeated annual cycles, and enable them to maintain perenniality. These seasonal changes in growth and development are often recorded as phenological timing and are driven by internal mechanisms, in combination with environmental responses. Phenological variation is often driven by climatic latitudinal differences; in *F. vesca*, for example, it has been reported that the interaction between photoperiod and temperature for flower induction shows variation across populations (Heide and Sønsteby, 2007). Reproduction in perennials can occur both sexually and asexually. With sexual reproduction, flowering is followed by seed set within the fruit, whilst asexual reproduction occurs through a number of mechanisms, one of which is the production of daughter plants along a runner, as seen in *Fragaria*.

The aim of the research described in this chapter was carefully to describe and analyse growth and development in the herbaceous perennial *F. vesca*, with visual observations complemented by frequent dissections to provide a comprehensive understanding of morphological changes at all above-ground growing points, through one growing season. The main objective was to determine where in the crown (meristems, axillary buds etc.) growth and development occurs in response to seasonal cues and how this varies over the course of the year. This chapter therefore provides a detailed morphological description of the plant, with a focus on shoot meristem fate in relation to position on the main axis. Much of the existing literature has focused on commercial strawberry (*F.* x *ananassa*), where the general morphological structure and processes are thought to be comparable with *F. vesca* (Guttridge, 1985). Here literature specific to *F. vesca* is comprehensively reviewed, in order to provide background information on morphological changes in the annual cycle, complemented by findings from *F.* x *ananassa* where appropriate.

3.1.1 Spring – vegetative growth

There are no papers which provide a comprehensive description of spring growth in *F. vesca.* The work of Arney was undertaken using the cultivar 'Royal Sovereign', which is now known as *F.* x *ananassa,* although Arney (1953a and b) states that the species is *F. vesca*. However, it is assumed in this chapter that the findings discussed by Arney relate to that for *F.* x *ananassa,* not

F. vesca. In *F.* x *ananassa*, initial spring growth results from the emergence of leaves initiated in late August/September and later, which over-wintered in the bud of each crown (see Table 3.1; Arney, 1955b; Darrow, 1966). These develop and elongate to become the first observed leaves to emerge at the start of the new growing season (Dana, 1980). Extension growth of the crown continues from the vegetative bud subtended by the uppermost leaf (Guttridge, 1969).

Table 3.1 Cell size and number of cells per leaflet. Numbers based on adaxial epidermal surface in 1951 (from Arney, 1955b)

Date of leaf initiation.	Date of leaf emergence.	Mean area of one epidermal cell (μ^2).	Mean cross-section area of one palisade cell (μ^2).	Number of epidermal cells per leaflet (in millions).
Late Aug.	Feb.	1,180	94	1·05
Sept.	Mar.	1,430	105	1·39
Early Oct.	Apr. 13–25	1,330	110	2·51
Oct.–Nov.	May 6–16	1,570	142	4·23
Feb.	May 21–30	1,850	166	3·80
Early May	June 22–30	1,620	129	4·10
Mid May	July 11–22	1,800	133	3·38
Early June	Aug. 1–4	2,050	156	3·96
June–July	Aug. 21–28	1,820	137	3·28
July–Aug.	Sept. 8–18	1,350	129	3·04
Early Aug.	Sept.–Oct.	1,270	102	3·14
Late Aug.	Oct.–Nov.	660	75	0·95

At this stage in the annual cycle there are approximately seven leaves enclosed within the stipules (Figure 3.1), compared to five-six later in the spring (see Table 3.2; Arney, 1955b). This difference arises because the rate of leaf initiation outstrips the rate of emergence during the dormancy period. There is an initial spurt in leaf emergence in early spring, as primordia accumulated in the terminal bud during winter rapidly expand. The rate of leaf emergence then slows; after this spurt leaves take nearly twice as long to complete expansion in the spring (and autumn) compared to midsummer (Arney, 1954).

Petiole length of emerged leaves of *F.* x *ananassa* is considered a measure of growth vigour (Tehranifar *et al.,* 1998), as petiole growth is particularly responsive to environmental conditions (Heide *et al.,* 2013). Petiole length increases by five-six times between emergence and full expansion (Arney, 1953a) and increases markedly in newly emerged and expanded leaves of fully chilled plants in comparison to plants with less (insufficient) chilling (Tehranifar *et al.,* 1998). Jahn and Dana (1970a) observed that the final size of leaves that first emerged in early spring with the resumption of vegetative growth was generally smaller than at other times, even though the length of leaves at emergence has been shown to be approximately constant throughout the year. This contrasts with research which indicates that maximum petiole length is achieved in the first leaves to emerge in fully chilled plants in the spring (Tehranifar *et al.,* 1998). Regardless of size or initiation time, leaves emerge in the sequence in which they were initiated and usually senesce in this same sequence (Darrow, 1966).

Figure 3.1 Unemerged leaf primordia at the apical meristem are enclosed within the stipules of the youngest emerged leaf

Table 3.2 Change in the number of enclosed leaf initials during the spring (from Arney, 1955b)

The values are the means of 10 or more apices. The emergent leaf is included the number of enclosed initials.

1951.		1952.		1953.		1954.	
Jan. 25	6·9	Jan. 29	7·0	Jan. 17	7·0	Jan. 9	7·1
				Feb. 17	7·4	Feb. 13	7·0
						Feb. 26	7·1
				Mar. 10	7·0		
				Mar. 23	6·6	Mar. 26	5·8
				Apr. 8	6·1		
				Apr. 30	5·9	Apr. 26	5·3
May 3	5·0	May 5	5·0	May 13	5·6	May 7	5·3
		May 26	5·2	May 20	5·6	May 19	5·9
				May 26	6·2	June 3	6·2

3.1.2 Spring – sexual reproduction: flower emergence

Flower emergence follows the resumption of active vegetative growth in the spring for seasonal flowering *Fragaria.* Emergence has been stated to occur in early May in *F. vesca* (Angevine, 1983) and the first observable sign of it is the elongation of the peduncle and the appearance of the terminal (primary) flower, which was initiated first and as a result is the largest (Guttridge, 1969).

In *F.* x *ananassa*, the emergence of an inflorescence from the protection of the sheathing stipule of the leaf immediately below it occurs with the expansion of this leaf; the axis of the inflorescence (the peduncle), terminates with a primary flower and supports two lateral branches, which form the two pedicels of the secondary flowers (Savini *et al.*, 2005). Commonly the first

branch arises several centimetres from the top of the primary peduncle (Guttridge, 1969) and further branching can be seen with the emergence of tertiary and quaternary flowers (Savini *et al.,* 2005). Due to the terminal flowering habit of the plant, the number of inflorescences is dependent on the number apical meristems (as a result of branch crowning) in the main crown (Hytönen, 2009).

Under suitable conditions, the inflorescences which were differentiated during the autumn expand and elongate to expose the flower clusters. The emerged inflorescence is a cyme, with variable structure (Guttridge, 1969) and emergence is observed with the opening of the primary flower first, followed by the secondaries, tertiaries and quaternaries (Dana, 1980), the number of which varies depending on the conditions during initiation. For inflorescences formed from axillary shoots below the terminal meristem, maturation to fruiting trusses can only occur where the axillaries develop to form established branch crowns (with flower initials) (Guttridge, 1985). For some cultivars (e.g. 'Elsanta'), even if axillary inflorescences are initiated, they may fail to survive the winter (Le Mière, 1997).

In *F. vesca*, the flower morphology is similar to that in *F.* x *ananassa*; there is a basic floral whorl number of five, with 10 sepals, five petals, numerous stamens and numerous carpels (Figure 3.2, Hollender *et al.,* 2012). However, *F. vesca* has an average of 20 stamens per flower, whereas *F.* x *ananassa* has an average of 25 stamens per flower (Hollender *et al.,* 2012).

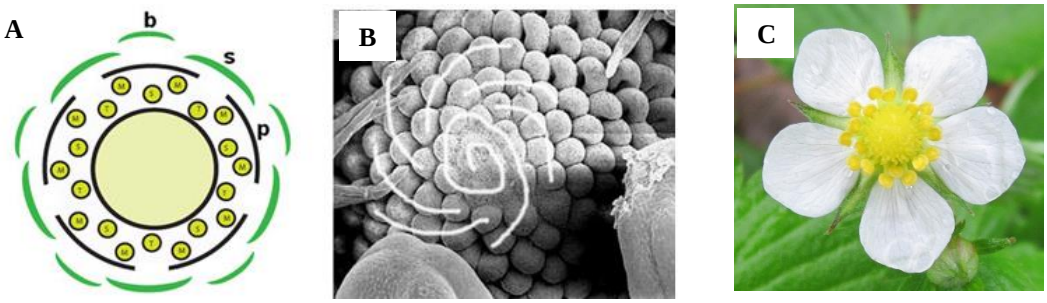


Figure 3.2 A) The arrangement of bracts (b), sepals (s) and petals (p) is shown, with two whorls of stamens interior to the petals, in a *F. vesca* flower; B) SEM showing the spiral arrangement of carpel primordia in the developing flower (Hollender *et al.,* 2012); C) *F. vesca* flower

During flower emergence, plants are observed to reach their 'minimal condition', meaning that the apical bud often contains around five leaf primordia (Arney, 1955b; Table 3.2). The assumption for *F.* x *ananassa* is that most plants pass through the minimal condition at some time during April or May (Arney, 1955b), as a result of leaf emergence outstripping initiation during early spring.

3.1.3 Spring/summer – asexual reproduction: runnering

As well as sexual reproduction through flowering and fruit set, *F. vesca* can also reproduce asexually through two vegetative structures: runners (stolons) and vegetative branch crowns. The first form of asexual reproductive development to be observed in the annual cycle is the emergence of runners. Angevine (1983) studied wild, mixed communities of *F. vesca* and suggested that runners typically emerge from early June to late August, although some literature suggests earlier runnering (Darrow, 1966). Runners reiterate the entire plant, including roots (Costes *et al.*, 2014), with most runner growth observed to occur some distance from the parent plant and therefore daughter plants from runners can be considered as separate growth units (Arney, 1954).

Runners originate from an axillary leaf bud in the main crown, as documented in *F.* x *ananassa* (Fang *et al.*, 2011); the first runner to emerge usually develops from the axil of one of the new leaves initiated in the spring (Darrow, 1966; Dana, 1980); it has been suggested that the first axillary bud to begin growth in the spring becomes the first runner (Guttridge, 1959; Dana, 1980). Leaves initiated in the new growing season (February) are likely to emerge from May (Arney, 1955b; Table 3.1); this can also be seen from runner emergence data, with observations of emergence reported from May/June, although cultivars show some variation (Darrow, 1966; Angevine, 1983).

A runner consists of successive units of two long internodes followed by a terminal daughter plant with roots from the underside and leaves and growing point at the tip (Darrow, 1966). After formation of the daughter plant from the terminal bud of the runner, and in favourable growing conditions, the second axillary bud of the runner continues runner elongation and produces the next sympodial unit (Darrow 1966; Hytönen, 2009); this enables runners to form a 'chain' (Savini *et al.*, 2005). The first axillary bud may produce a new runner, but it may also remain quiescent (Hytönen, 2009); should this bud develop into a runner it is usually much smaller than the main runner (Darrow, 1966). At the runner tip a new daughter plant is formed, the first leaf of which is a scale, or bract-like structure, but whose leaf traces arise in the apical meristem of the new plant (Darrow, 1966).

Runner production has been linked to growth rate, with rapid plant growth resulting in a higher number of runners being produced; runner size and length are also dependent on growth conditions and genotype (Darrow, 1966). Flowering potential has been shown to influence runnering, as plants with no flower buds in the spring start producing leaves and runners before those with flower buds, and those with few flower buds before those with many (Darrow, 1966).

Following this initial emergence, runners are produced all summer from buds in the axils of new leaves, and in succession as the leaves develop (Darrow, 1966).

3.1.4 Summer – asexual reproduction: branch crowning

Over the spring and summer, while axillary buds of *F.* x *ananassa* typically develop into runners, some may remain dormant or occasionally develop into a branch crown (Darrow, 1966). Branch crowns are another form of asexual reproduction in both *F.* x *ananassa* and *F. vesca*, and can be considered as axillary leaf rosettes (Mouhu *et al.,* 2013). They are morphologically identical to the main crown axis and runner daughter plants; except that unlike runners, branch crowns do not produce a separate root system from the mother plant (Dana, 1980). Regardless of this lack of independent root system, once established branch crowns function independently of the main crown; with the presence of emerged branch crowns within the main crown not affecting its rate of leaf production (Jahn and Dana, 1970b). It is assumed that in *F.* x *ananassa* differentiation into either a runner or branch crown occurs during formation of the first two internodes (Guttridge, 1955), depending on the conditions to which the plant is exposed. However, there is little published information on this point.

There are other differences between these two asexual structures, with regards to node and bud growth and development. In contrast with runners, there is no elongated tissue between nodes for branch crowns; and initial axillary bud behaviour also differs. In runners, the axillary bud in the second leaf continues runner elongation, whereas this is not observed in branch crowns, in which leaf primordia and their axillary meristems develop in a similar way to those on the main crown (Guttridge, 1955).

3.1.5 Autumn – asexual reproduction: branch crowning

During the autumn, environmental conditions are more conducive for branch crowning, runner initiation ceases and the upper axillary buds in the crown differentiate to form branch crowns (Mouhu *et al.,* 2013). In *F.* x *ananassa,* autumn conditions also cause reduced vegetative growth, characterized by decreased petiole elongation (Konsin *et al*., 2001). Previous runnering can affect plant size, with plants often observed to be smaller by this stage in the annual cycle than earlier in the year (smaller in September in comparison to June), presumably because energy is expended producing runners over the summer (Darrow, 1966). Branch crown emergence is most common during the autumn when the plant is not engaged in runner production, but emergence of

vegetative branch crowns initiated during the previous growing season can also be observed in spring (Guttridge, 1955).

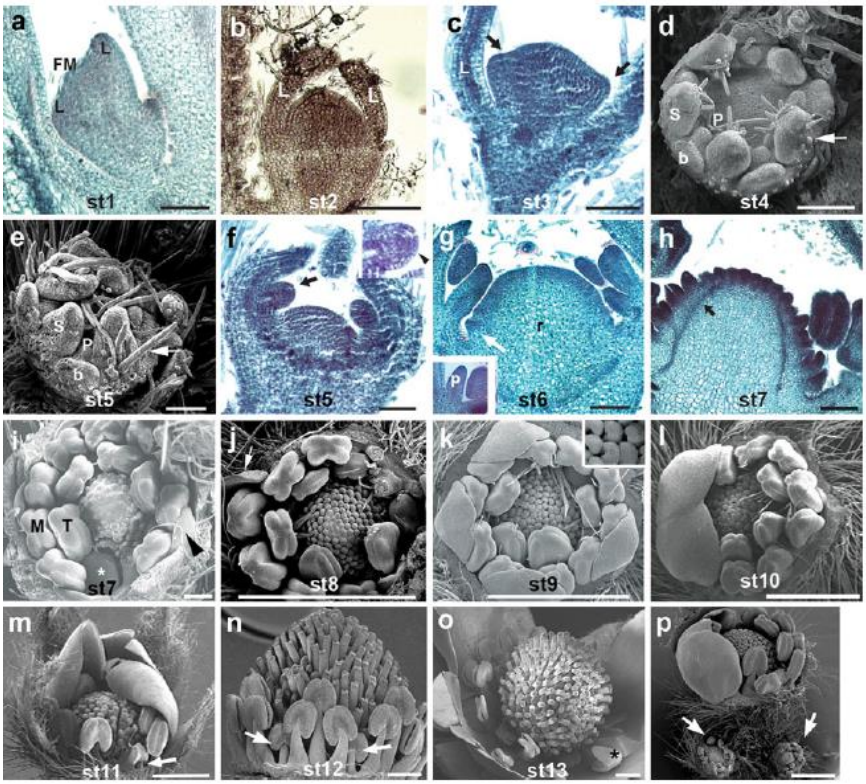
The environmental conditions experienced during the autumn, whilst restrictive of runnering still allow vegetative growth, in the form of leaf production. Leaves emerge from the main crown until late autumn when the last leaf emerges (Arney, 1953a). Leaves initiated during the branch crowning phase may emerge in the late autumn if conditions are still promotive for vegetative growth, otherwise they overwinter enclosed in the bud at the apical of the crown and emerge the following spring (Arney, 1954). As the plant progresses through the branch crowning phase, there is a general decrease in the rate of leaf initiation and a decrease in total shoot growth (Arney 1953a; 1954).

In *F.* x *ananassa*, the morphology of the first two leaves in the branch crown differs from that of leaves on the main crown, as they have reduced leaf blades. The final size of the laminae is dependent on the position of the branch crown in relation to the terminal inflorescence on the main axis; if a branch crown develops directly beneath the inflorescence, the laminae resemble normal size and shape, whereas further down the crown they are typically reduced (Guttridge, 1955).

3.1.6 Autumn – sexual reproduction: flower initiation

In seasonal-flowering *F.* x *ananassa*, flower initiation occurs under short days and cooling temperatures, conditions which are also stated to promote branch crowning; however, once flower initiation begins the plant is considered to be in a reproductive state (Darrow, 1966). In *F.* x *ananassa*, under flower inducing conditions, the terminal growing point is the first meristem to transition out of the vegetative state; this is first observable as a broadening and flattening of the apex (Guttridge, 1955). Floral development has been comprehensively studied in *F. vesca* by Hollender *et al.* (2012), with a detailed description of the key developmental stages observed at the meristem during floral development (Figure 3.3). The primary or terminal flower is initiated first, and is the largest (Guttridge, 1985). Jahn and Dana (1970a) stated that bracts of the inflorescence become evident prior to observable initiation of the flower, but this is not the general consensus; other literature states that enlargement of the apex and the observed development of floral organs occurs before bracts become distinguishable (Guttridge, 1955, 1985; Darrow, 1966). Under natural conditions in the UK, the first stages of flower initiation normally occur in September (Guttridge, 1985), when conditions become inductive (see Chapter 1). Variation has been shown in time of initiation depending on environmental conditions and

cultivar, with initiation shown to start from September – October (Arney, 1955a; Jahn and Dana, 1970a).



Floral stage	State of development
1	Rounded doming at the meristem
2	Continued doming
3	Sepal primordia emerge
4	Petal primordia emerge
5	Stamen primordia emerge
6	Continued development of stamen and petals, initiation of carpels
7	Carpels clearly observed
8	Carpels form thumb-like development
9	Extending petals
10	Petals overlap
11	Whitening petals, visible stamens
12	Closed, mature floral bud with fully developed organs
13	Fully developed open flower

Figure 3.3 Key flower developmental stages in *F. vesca* with SEM images showing floral bud with bracts and sepals removed to highlight the key events of each stage (adapted from Hollender *et al.*, 2012)

The *F.* x *ananassa* inflorescence is considered to be a dichasial structure, with two secondary flowers forming on the primary pedicel, followed by two tertiaries on each of the secondary branches (Guttridge, 1985); a similar structure was observed in *F. vesca* through preliminary dissections, as shown in Figure 3.4. Each inflorescence is subtended by two bracts (Jahn and Dana, 1970a) and the secondary flowers appear in the axils of floral bracts (Guttridge, 1952; Jahn and Dana, 1970a), while branches of the inflorescence arise from bract axils (Darrow, 1966). Although a general dichasial structure is suggested, cultivars vary in inflorescence structure with many shown to be irregular (Darrow, 1966; Guttridge, 1985). Some cultivars initiate several inflorescence branches from the base or along the peduncle, in these cases the primary axis may have one or two long internodes and several very short ones (Darrow, 1966). Most well developed inflorescences have fully developed tertiary flowers and branches, but not always quaternaries (Guttridge, 1985).

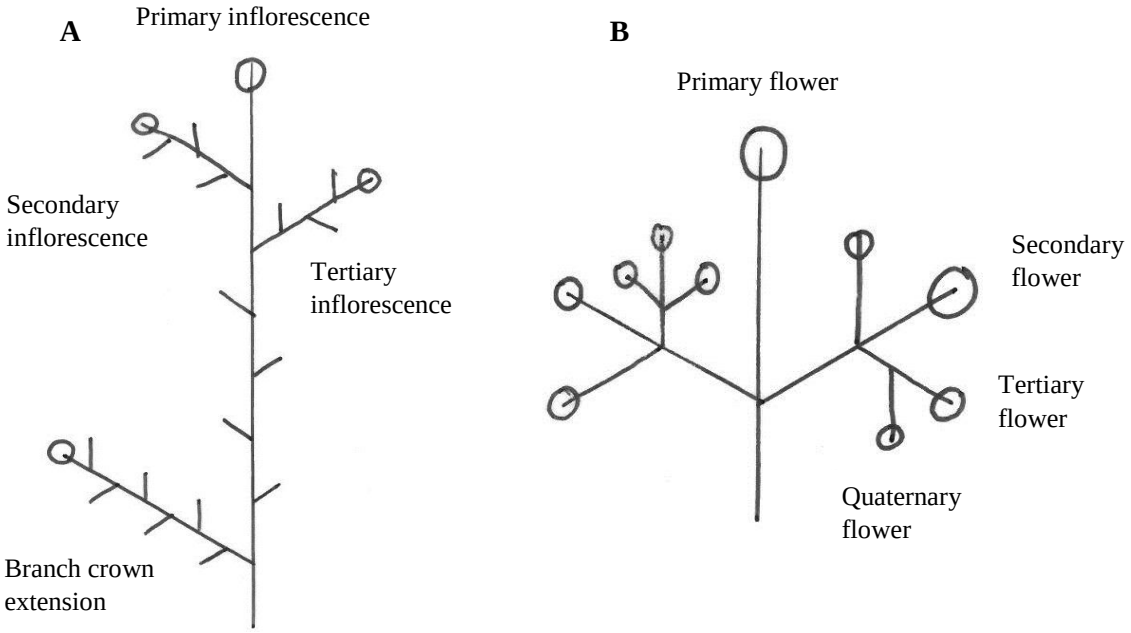


Figure 3.4 A) Diagrammatic representation of the structures of inflorescences in the main crown of *F. vesca*, showing the position of primary, secondary and tertiary inflorescences, as well as the presence of a branch crown extension. B) Diagrammatic representation of the floral structure within an inflorescence, showing the position of primary, secondary, tertiary and quaternary flowers

In *F.* x *ananassa*, once the terminal meristem has become floral, further growth and development is terminated at this meristem; consequently further extension of the crowns is by the uppermost lateral bud, which assumes dominance over lower laterals and displaces the inflorescence to one side (see Figure 3.5 B–C; Heide *et al.*, 2013).

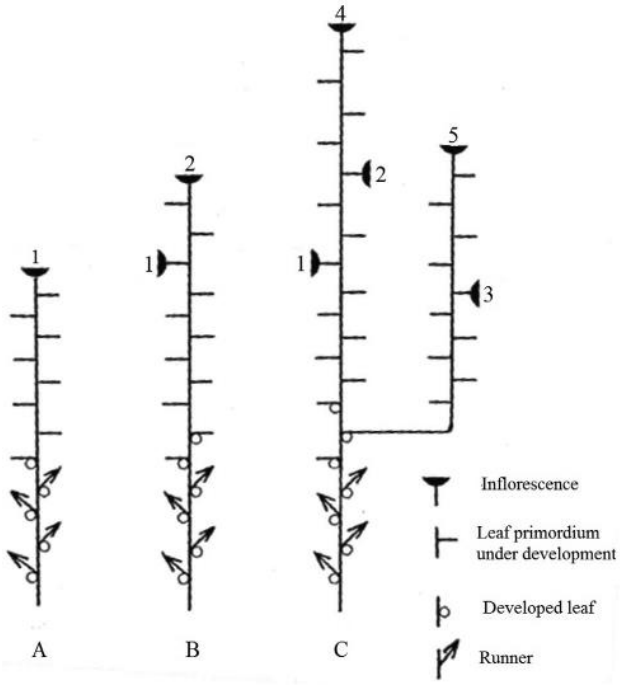


Figure 3.5 Successive stages of inflorescence development in a strawberry (*F.* x *ananassa*) crown (A-C). Numbers refer to the order of inflorescence initiation (modified from Guttridge, 1955; Heide *et al.*, 2013)

The continuation of reproductive growth occurs through development of the meristem in the uppermost axillary position (Jahn and Dana, 1970b), and leaf primordia produced in these axillaries (below an inflorescence) develop into normal trifoliate leaves rather than bracts, as in other axillary positions (Jahn and Dana, 1970a). These axillary buds often development to form extension crowns (Jahn and Dana, 1970b) which subsequently may initiate inflorescences (Arney, 1955a; Heide *et al.*, 2013). Inflorescences are initiated in these extension crowns in the same way as described for the main crown (Figure 3.5C; Heide *et al.*, 2013). The formation of inflorescences in these extension crowns occurs after the initiation of two-to-four new leaves (Figure 3.5C; Heide *et al.*, 2013).

Because of the terminal flowering habit, the total number of inflorescences is influenced by the number of apical meristems in the plant (Hytönen, 2009); for example, plants with a greater number of branch crowns have the potential to produce more inflorescences. This interaction between the number of apical meristems and inflorescences was stated in the early literature, but the relationship was considered to be influenced by the number of leaves. Darrow (1966) suggested that generally plants that possessed more leaves generated more flower clusters, due to the greater number of leaf axils in these plants in which inflorescence initials could develop.

Flower initiation in *F. vesca* follows the same pattern as described for *F.* x *ananassa* (Hollender *et al.*, 2012), but the specific inductive conditions required vary between species, just as between

populations/cultivars (see Chapter 1). In *F. x ananassa*, the flowers are larger than those of *F. vesca*, usually with more basic pentamerous multiples of floral parts; as observed in *F.* x *ananassa* inflorescence branching structure can vary (Darrow, 1966; Guttridge, 1985). *F. vesca* flower and inflorescence development has been described by Hollender *et al.* (2012), but this work does not discuss in detail the development of axillary flowers or the process of branch crown extension (floral development within branch crowns); neither does it consider the state of the other meristems within the crown during this phase.

Interestingly, because flowers, runners and branch crowns all develop from buds in the leaf axils, intermediate structures have been observed in *Fragaria.* For example, under moist conditions the inflorescence may root at one or more of its nodes and even give rise to an independent plant as a result (Darrow, 1966).

3.1.7 Winter – semi-dormancy

Fragaria is considered to be a semi-dormant perennial (Kurokura *et al.,* 2013) (see Chapter 1); the combination of dormancy and environmental conditions during the winter restrains vegetative growth, observed as a reduction in leaf size and petiole length shortening (Guttridge, 1985; Durner and Poling 1987). Arney (1955a) described winter growth in *F.* x *ananassa* ('Royal Sovereign'), and observed a general lack of leaf emergence during December and January; as a result total enclosed primordia was observed to be highest during the winter period (Arney, 1955a), in comparison to the rest of the annual cycle.

As a result of the semi-dormant nature of *Fragaria*, plants exposed to favourable growth conditions during the dormancy phase may resume growth, but growth rates may be slow and plants maintain a dwarf-like appearance (Arney, 1955b; Jonkers, 1965); leaves that do emerge are small with stunted petioles (Guttridge, 1985). Similarly to vegetative growth, flower emergence may also occur whilst the plant is in a semi-dormant state, but emergence is slow and flowers are often poorly developed (Konsin *et al.,* 2001).

In *F.* x *ananassa* chilling accumulated during the dormancy phase directly influences subsequent vegetative growth, an interaction which has been quantified, with a quadratic relation found between petiole length and chilling hours; additional chilling significantly increased petiole length (Robert *et al*., 1997; Figure 3.6). Other research, however, has suggested that some cultivars ('Elsanta' and 'Korona') under certain experimental conditions showed no indication of dormancy-related growth inhibition (as judged by petiole length) (Sønsteby and Heide, 2006).

This lack of dormancy inhibition may reflect chilling temperature, as Tehranifar (1997) showed that chill temperature influences petiole length, with chilling at -0.3°C resulting in the largest petioles, in comparison to chilling at 4°C, 3°C or -2°C.

Unlike many other perennials, which display deciduous seasonal behaviour, shedding their leaves and vegetative structures in the autumn, *Fragaria* maintains vegetative cover over the winter, although overwintering leaves often differ in colour to those during the growing season (scarlet, purple, green without a trace of purple, or intermediate) (Darrow, 1966). While emerged leaves are maintained during the winter, the embryonic leaves remain enclosed by stipules, which act to protect them from severe environmental conditions prior to emergence in spring under favourable conditions (Darrow, 1966). In *F. vesca*, over-wintering leaves show differences to those observed during the summer, with smaller leaflets, shorter petioles and a dense covering of long hairs (Åström *et al.*, 2015).

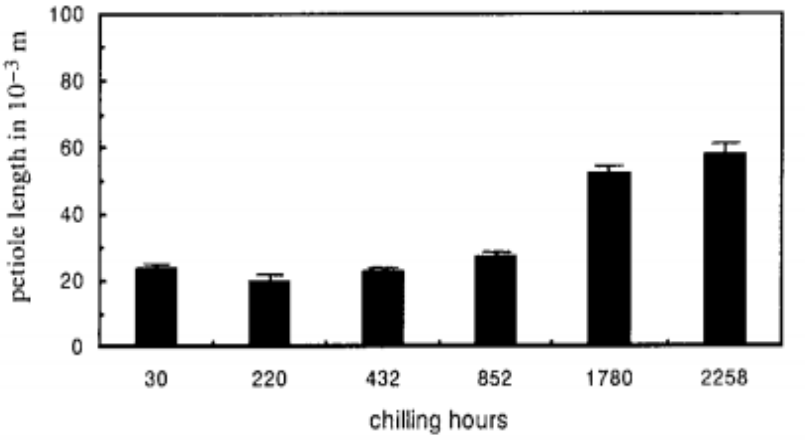


Figure 3.6 Average petiole length of plants transferred from chill treatments to forcing conditions (from Robert *et al*., 1997)

3.1.8 Aims of the research described in this Chapter

The overarching aim of the research described in this chapter was to document the changes at the meristematic level through the annual cycle in *F. vesca*; and to consider how meristem position influenced growth and development in response to the changing environment. It is apparent from the literature reviewed here that there is a lack of *F. vesca*-specific research which quantifiably describes morphological changes, especially at the meristem. Issues highlighted here but addressed in other Chapters are as follows:

- Chapter 4 (environmental control of meristem fate) – what are the environmental signals regulating runner or branch crown development?

- Chapter 5 (regulation of vegetative and reproductive growth and development) – do *F. vesca* from different populations vary in the environmental signals needed for flowering, and in their pattern of inflorescence development?

- Chapter 6 (the nature and progress of dormancy in *F. vesca*) – response of spring growth following chilling

3.2 Materials and Methods

Information concerning propagation, potting, growing material, early growth conditions, irrigation, nutritional regime and pest and disease management, can be found in Chapter 2.

3.2.1 Plant material

F. vesca plants from a single original location were used throughout the annual cycle to allow comparison between weeks and stages of growth and development. The founder plants were collected from Thackthwaite, Lake District (April 2014), and were known as UK2 (see Chapter 2).

In order to ensure a constant supply of plant material for dissections, cohorts of plants were propagated throughout the year. As a result, there was some variation in plant size and age between successive cohorts; these differences were recorded at the time of dissection by the total number of leaves and leaf nodes. The mother plants used for propagation were maintained in the field over the winter, and exposed to natural environmental conditions resulting in chill accumulation and restoration of spring growth vigour. The first cohort of plants was propagated from mother plants at the start of March and potted on at the end of March; at this time mother plants provided a limited number of runners and daughter plants as conditions were not optimal for runner emergence and development. At the start of the annual cycle data collection (19/04/2016), the plants had a single crown. Two further cohorts were propagated at the start of May (potted on 08/06/2016) and the end of June (potted on 03/08/2016), which were dissected from 19/07/2016 and 04/10/2016, respectively. A final cohort of plants was propagated at the start of August (potted on 29/08/2016) and dissected on 18/04/2017. Once potted on, all plants were fed weekly by hand.

3.2.2 Growth conditions

Plants were initially maintained under glass (14/04 – 01/07/2016), in an unheated, venting glasshouse, to ease irrigation, pest and disease management and to avoid potential frost damage or exposure to harsh conditions. Cohort one and two plants were transferred into the field from 01/07/2016. The third cohort of plants was transferred into the field from 01/08/2016, and the last cohort of plants was transferred into the field on 05/09/2016. Field temperature and photoperiod during the 2016 sampling period are summarised in Figure 3.7.

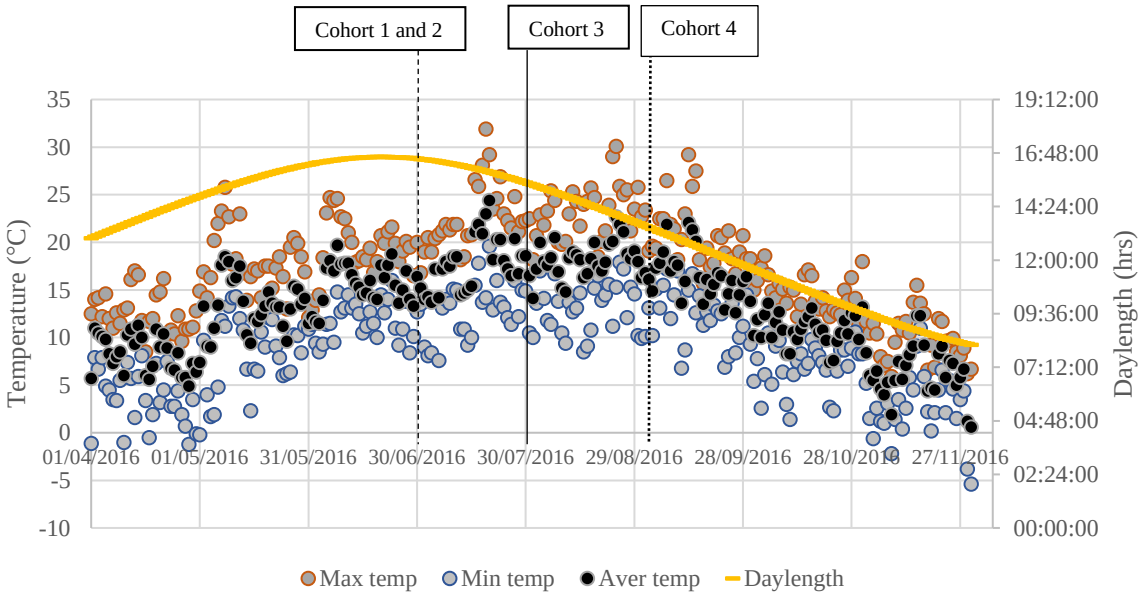


Figure 3.7 Temperature and photoperiod (daylength) in the field during the main sampling period. Temperature data extracted from the Reading Atmospheric Observatory (based at the University of Reading, Whiteknights Campus) and photoperiod data from: https://www.timeanddate.com/sun/uk/reading?month=11&year=2016. Vertical lines represent the time of transfer to the field for cohorts; dashed line = cohort one and two, solid line = cohort three and dotted line = cohort four

3.2.3 Parameters measured

Weekly recordings and dissections were undertaken for five plants, with visual observations of the following growth and development parameters recorded prior to dissection:

- Number of emerged leaves
- Crown diameter
- Number of emerged branch crowns
- Number of emerged runners.

Dissections were carried out using basic dissecting equipment: tweezers (Biology Tweezers 5 Stainless Steel, Dumont; Agar Scientific Ltd, Essex) and a dissecting blade (BD Beaver micro sharp blade 3.0mm. 15°; Agar Scientific Ltd, Essex). The state of the meristems were recorded individually using visual observations or a dissecting microscope (Leica MZ9 5), and where appropriate photographs were taken, using a digital camera (Lumix DMC-TZ55, Panasonic).

Axillary buds were found to form either runners or branch crowns, or they remained undetermined or were arrested. The following definitions were devised to allow the four types of axillary bud to be distinguished prior to emergence:

Runner

Axillary buds which showed prominent elongation at the base to form the first node of the stolon. The tip was flat and thin in comparison to branch crown and arrested buds (Figure 3.8A). Distinct leaf primordia were not as clear as observed for branch crown and arrested buds, so the tip of a runner contained fewer leaf primordia than observed for branch crowns.

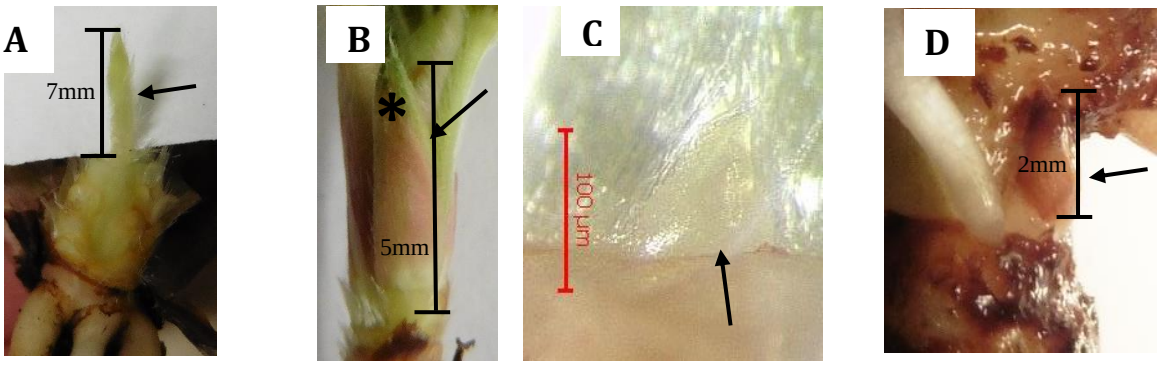


Figure 3.8 Illustration of the four axillary bud types prior to emergence: A) runner showing characteristic elongation at the base of the bud and pointed, thin tip. B) Branch crown, showing bulking at the base of the bud and an emerging leaf. C) Undifferentiated bud, where the axillary bud is visible but the character of the bud is not clear. D) An arrested bud from the base of the main crown; this bud contained two leaf primordia; and the leaf in the axil of which this bud had developed had abscised, hence the discolouration of the bud exterior

Branch crown

Axillary buds were generally wider at the base in comparison with runners, and some showed evidence of lengthening and curved leaf tips (Figure 3.8B, specifically *). When dissected they were found to contain distinct leaf primordia, which were clearly observable from an early stage.

Undifferentiated bud

Axillary buds generally near the apical meristem, in which bud identity could not be determined, most likely due to the small size of the bud at this stage (Figure 3.8C).

Arrested bud

Axillary buds generally observed at the base of the main crown, whose identity was not clear (until dissected) (Figure 3.8D); these buds contained leaf primordia (typically up to three).

For each of the plants the following data were recorded at each node:

- Node number (from the base up) – the number of total leaves and leaf primordia which included senescent leaves that had not abscised. Once leaves had abscised their presence could be inferred from the remaining stipules and axillary buds and so they were also included in the total
- Presence or absence of an axillary bud
- Character of the axillary bud (if observable)
    - For branch crowns: the height and width of the bud and the number of leaves and the axillary bud character
    - For runners: the number of daughter plants and internodes within the runner, as well as overall length
    - For arrested buds: the height and width of the bud, as well as the number of leaf initials within
    - For axillary buds without discernible character: the height and width of the bud (where possible).

These descriptive data were collated to provide information on the modal number and character of nodes and axillary structures for each dissection date (see Chapter 3, Section 3.3; Figure 3.10). Average values were also calculated for the total number of leaves (unemerged, emerged and senescent), crown diameter, petiole length, number of emerged runners and number of branch crowns. Linear regression analysis was performed on each recorded cohort using Minitab 17, to analyse the changes in growth response over time. Data distribution for all recorded parameters was tested separately for each cohort using the Ryan-Joiner test for normality. All recorded parameters were normally distributed with the exception of runners (for cohort 1). A Poisson regression was undertaken for runner data and showed comparable levels of significance with the linear regression analysis across the cohorts, so the changes in growth parameters over the sampling period were analysed using linear regression analysis for all growth measures.

3.3 Results

The data collected during April-November 2016 are presented in two sections. General plant morphology is described first, highlighting growth and changes in overall structure both of the main plant (number of leaves and leaf nodes) and its axillary buds (axillary bud number and character). Specific phases of growth and development, such as the development of the axillary buds, are then described; these findings provide essential background for subsequent experimental chapters.

As described in Section 3.2 (Materials and Methods) in this study, three cohorts of plants were sampled and dissected over the course of the growing season and while there were some differences in overall plant size between the cohorts, associated with differences in plant age, the overall developmental state of all cohorts was similar, with comparable plant architecture.

3.3.1 Changes in morphology of the main crown

Here general changes in plant morphology are presented using modal information and schematic illustrations. To enable comparison between sampling dates, individual plant data were used to calculate the modal character of each axillary structure at each leaf node and the number of total leaves and leaf nodes. In instances where modal character could not be simply calculated, the character chosen was that which best represented the typical character at this node position. In the following month-by-month account, schematic illustrations showing modal character of axillary buds and the typical number of leaves and leaf nodes are presented (Figure 3.9). At the base of the main crown, in the oldest and lowest (one-three) nodes, axillary buds were generally either branch crowns or arrested axillary buds (which were assumed to be arrested branch crowns, as they contained developed leaf initials); Section 3.2 (Chapter 3) provides a definition and full description of axillary bud character. This basal group of nodes was referred to as Group 1, and was present throughout the year. Above Group 1, axillary buds were classified as Group 2; the majority of buds in this group were runners and the number of internodes in this category increased over the growing season as the plants grew and increased in size. Those closest to the base (and Group 1) contained axillary buds that had typically emerged, and in many cases were well developed; for example, runners contained a number of nodes and daughter plants. The axillary buds in nodes higher up the crown, closer to the apical meristem, were less developed or had often not emerged, but their character was discernible. Above Group 2 came Group 3, in which axillary buds were either observable but their character undiscernible, or not yet visible (Figure 3.9). Most plants contained about one node that had an observable axillary bud with

undiscernible character, and above this node between two and five leaf primordia which did not contain observable axillary buds.

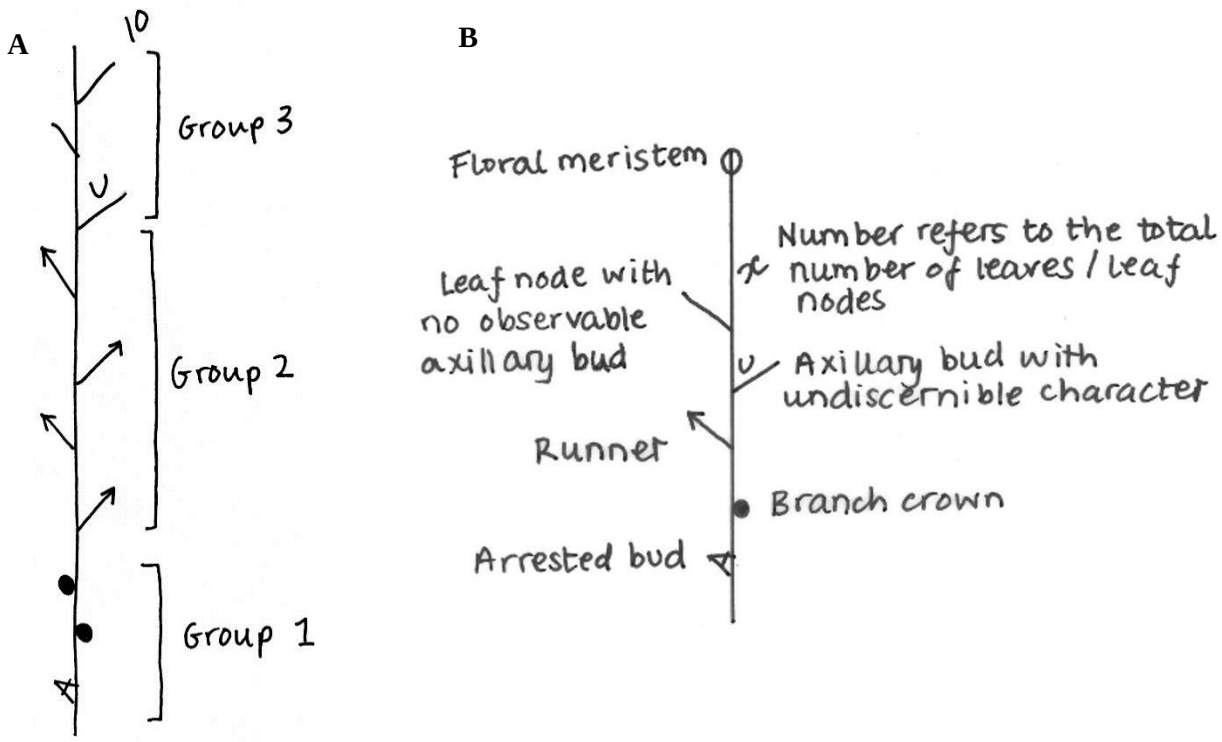


Figure 3.9 A) The general architecture of plants over the dissection period, with node groupings from the base of the main plant to the SAM; B) key for symbols of terminal and axillary bud character and leaf nodes during vegetative growth

April 2016

The plants dissected in April 2016 had been recently propagated (in March) from runners, so had not initiated flowers the previous autumn; as a result, no flower emergence was recorded in the spring/summer of this annual cycle, as would be expected in more mature plants (see April 2017). A typical plant at the end of April 2016 had nine leaves and leaf nodes in the main crown (Figure 3.10A). Leaves at the lowest five nodes had typically emerged and although axillary buds were visible at these node positions, bud character was undiscernible. Axillary buds were not observed in the nodes of the most recently initiated leaf primordia, typically the upper four nodes (Figure 3.10A).

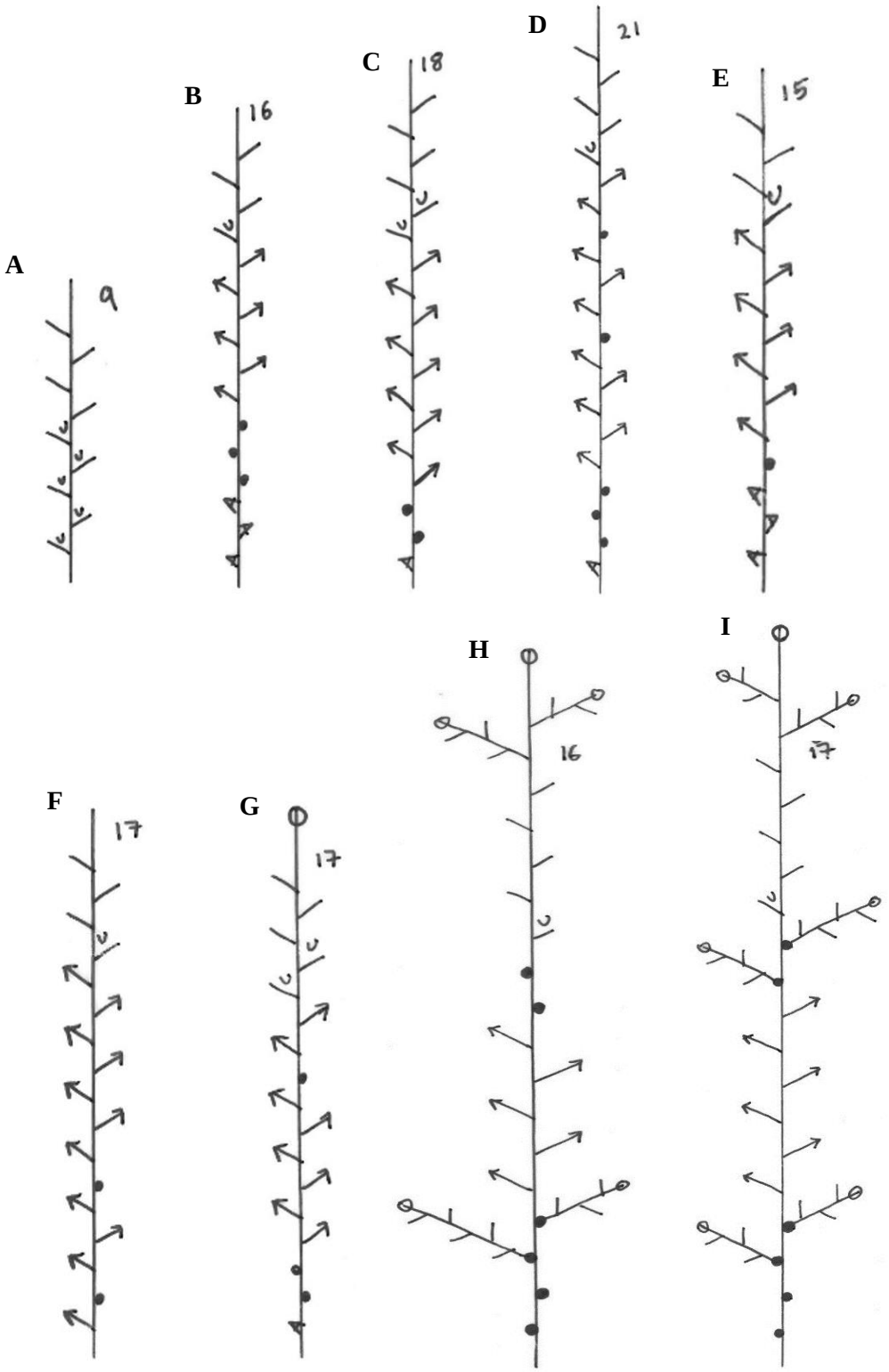


Figure 3.10 Modal *F. vesca* plant by: (A) 26/04/2016 (1); (B) 31/05/2016 (1); (C) 28/06/2016 (1); (D) 12/07/2016 (1); (E) 19/07/2016 (2); (F) 30/08/2016 (2); (G) 27/09/2016 (2); (H) 25/10/2016 (3); (I) 15/11/2016 (3). The number in brackets following the date shows plant cohort. The modal number of nodes and character of axillary buds in the main crown are shown in the diagrams

May 2016

By the end of May the number of nodes in the main crown had increased, typically to 16 (Figure 3.10B). This rapid increase in node number was accompanied by an increase in emerged leaves. Active growth was observed in the axillary buds throughout most of the main crown.

The axillary buds in the lowest nodes, of which there were typically two to three, were assumed to be arrested branch crowns, as when dissected they had leaf primordia similar to those in a branch crown and their shape resembled branch crowns, being relatively wide at the base. The definition of arrested came through subsequent dissections, when it was observed that these buds had not developed significantly, i.e. shown leaf emergence or initiation; this also led to the presence of arrested buds at the base of the main crown being characterised as a typical feature of *F. vesca* morphology (Figure 3.9).

Runners and branch crowns were observed in nodes above these basal arrested buds, with runners being dominant. Plants at the end of May typically had six runners (Figure 3.10B), of which four had emerged, showing internodes and daughter plants. The character of all axillary buds at the youngest node with discernible fate was a runner. Branch crowns were typically observed towards the base of the main crown, between the arrested buds and the runners above (Figure 3.10B). The accumulation of branch crowns at the base of the plant, even though they had not all emerged, resulted in a general widening of the plants (described further in Chapter 3, Section 3.3.2).

Plants during this dissection period typically contained four unemerged leaves at the shoot apex. Axillary buds were generally not observed in the axils of any of the uppermost leaf primordia, but in some cases an axillary bud with undiscernible character was observed in association with the lowest (oldest) unemerged leaf primordium (Figure 3.10B). Active vegetative growth, as observed through leaf emergence and an increase in node number, also resulted in leaf senescence, with leaves generally senescent at the lowest nodes. By the end of May, there were approximately three senesced leaves. The axillary buds at these node positions did not senesce in response to this leaf death; the way senescence occurred typically resulted in these axillary buds remaining protected by the stipules of the associated leaf.

June 2016

Plants in June had arrested buds at the very base of the main crown, with branch crowns in the nodes above (Figure 3.10C). There were typically less arrested buds and branch crowns (Group 1 nodes) by the end of June than at the end of May. Plants typically accumulated two leaves and

leaf nodes during June, fewer than during May. The increase in the total number of leaves and leaf nodes was accompanied by an increase in the typical number of runners (Group 2 nodes), with nine runners per plant by the end of June (Figure 3.10C). Previously emerged runners continued to grow and develop, observed as an increase in the number of internodes and daughter plants. Typically by the end of June plants had two leaf nodes containing axillary buds with undiscernible character and three leaf nodes with no observable axillary buds (Figure 3.10C).

July 2016

The last of the first cohort of plants were dissected on 12/07/2016 and had Group 1 nodes (arrested buds and branch crowns) at the base of the main crown, whilst at the shoot apex, typically five Group 3 nodes were observed (Figure 3.10D). There were however, some differences in overall plant morphology as branch crowns were not confined to Group 1, with occasional branch crowns in higher Group 2 node positions. In previous dissections, runners were typically observed at Group 2 nodes (e.g. Figure 3.10D). The exact nodal position of these upper branch crowns was not consistent across plants, but an upper branch crown was included in the schematic illustration to indicate their typical presence at this time.

From mid-July until September the plants sampled had been propagated in May. These plants were generally comparable with cohort one, with typically four Group 1 nodes (arrested buds/branch crowns), followed by seven runners, and four nodes with buds which were either not visible or had undiscernible character (Group 3 nodes) (Figure 3.10E). The plants from this second cohort were smaller than those from cohort one, having 15 nodes in total on 19/07/2016. They also lacked the upper branch crowns found in cohort one plants.

August 2016

By the end of August plants typically showed a total of 17 nodes, with this increase in plant size seemingly observed by an increase in the number of discernible runners. These runners were typically observed in Group 2 node positions, but as described for cohort one plants in July, branch crowns were no longer confined to Group 1 node positions and were also sporadically present in upper node positions (Figure 3.10F). In Group 1 nodes, a runner was observed in the first axillary bud position followed by one to two branch crowns/arrested buds (Figure 3.10F).

September 2016

By the end of September, flower initiation was observed at the shoot apex, with a floral meristem typically in the early stages of development associated with the development of a primary inflorescence (see Chapter 3, Section 3.3.2). Aside from the development of a floral meristem, plant morphology by the end of September was comparable with previously dissected plants and there was no change in the total number of nodes present (Figure 3.10G). There were typically three Group 1 nodes followed by eight runners (Group 2). Similar to observations in August, branch crowns were observed in upper node positions between Group 2 runners (Figure 3.10G). Below the floral meristem, Group 3 nodes were still observed, with typically five leaf nodes either containing no observable axillary buds, or buds with undiscernible character.

October 2016

Plants dissected from October onwards were from the third cohort of plants, propagated in June. Typically cohort three plants at the end of October had a total of 16 nodes, comparable with cohort two plants at the end of September (17 nodes, see Figures 3.10H and 3.10G respectively). By this time branch crowns were clearly visible above the Group 2 runners indicating that branch crowns were differentiated in newly developing axillary buds during October. Plants were also floral, with primary, secondary and tertiary inflorescences typically observed at the shoot apex, as well as floral development in lower branch crowns, referred to as branch crown extensions (nodes 3 and 4 – see Figure 3.10G; Figure 3.3A) (see also Section 3.3.2).

November 2016

By mid-November, floral development was found throughout the plant, both at the shoot apex and in the branch crown extensions (Figure 3.10I). At the shoot apex, secondary and tertiary flowers on the secondary and tertiary inflorescences were typically observed. Floral development through branch crown extensions was observed in upper branch crowns, typically at nodes 11 and 12; and subsequent development with secondary flowers in lower branch crown extensions (Figure 3.10I). Plants typically had five Group 3 buds beneath the primary inflorescence, as at the end of October. Further, quantitative information on floral development is provided in Section 3.3.2 (Chapter 3).

April 2017

A fourth cohort of plants was transferred to the field (on 05/09/2016) and overwintered under these conditions. These plants were assumed to reflect the natural timing and order of flower and runner emergence (in the spring), following winter chilling. Dissections were not carried out on this cohort of plants between November 2016 and April 2017 because of differences in the state and stage of floral development compared to cohort three plants; this was assumed to be because of the difference in time of transfer to the field. Nevertheless these plants were still considered suitable for dissection in order to study the re-establishment of vegetative growth in the spring. A sample of plants was dissected in mid-April 2017, by which time the plants typically showed the emergence of flowers and runners. Floral development was observed at the apices of branch crowns throughout the main crown, including the terminal SAM, although occasional branch crowns remained vegetative typically either at the base of the main crown (assumed to have previously been arrested buds) or in mid-crown positions (Figure 3.11). Emerged inflorescences were typically observed from floral buds at the apex of the main crown (the previous SAM), and some plants also had emerged basal inflorescences.

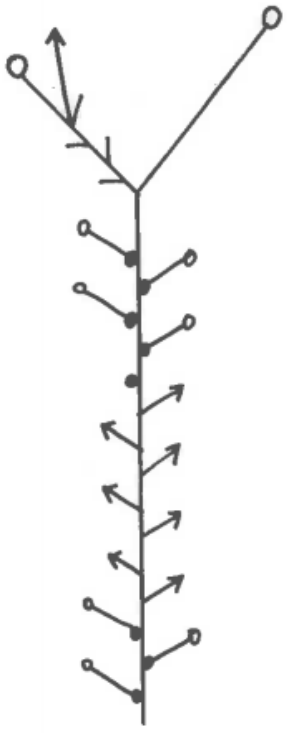


Figure 3.11 Typical *F. vesca* plant by 18/04/2017, showing the modal number of nodes and character of axillary buds in the main crown. The arrow at the apex of the plant indicates the continuation of vegetative growth

Vegetative growth was re-established at the apex of the main crown and at the apices of well-developed, typically basal branch crowns, contemporaneous with inflorescence emergence (Figure 3.11; highlighted in Figure 3.12); vegetative growth resumed from the axillary bud beneath the terminal inflorescence(s). In some plants only a primary inflorescence appeared to have been initiated, while in other plants (especially at the SAM) a secondary inflorescence was also observed (as shown in Figure 3.12A). From the dissections it was not clear whether the first emerged leaf beneath the terminal inflorescence(s) was the leaf containing the axillary bud from which vegetative growth continued, or the first leaf initiated within this bud. Axillary buds beneath the terminal inflorescence(s) typically gave rise to two-four leaves/leaf initials (Figure 3.12B); in some cases an inflorescence was observed at the apex, which may or may not have emerged (see shoot apex in Figure 3.11). Figure 3.12B highlights the typical morphology of an extended axillary bud beneath the terminal inflorescence(s), which itself had also become floral: some of the leaves within this axillary bud had emerged and the axillary buds of these emerged leaves had developed as runners, branch crowns or had undiscernible character. The axillary bud subtended by the leaf below the inflorescence was a branch crown with several leaf initials and it was assumed it was from this axillary bud that vegetative growth continued (Figure 3.12B). In plants that had a number of well-developed basal branch crowns, vegetative growth was re-established at the shoot apices of these branch crowns, in a similar manner to the main crown.

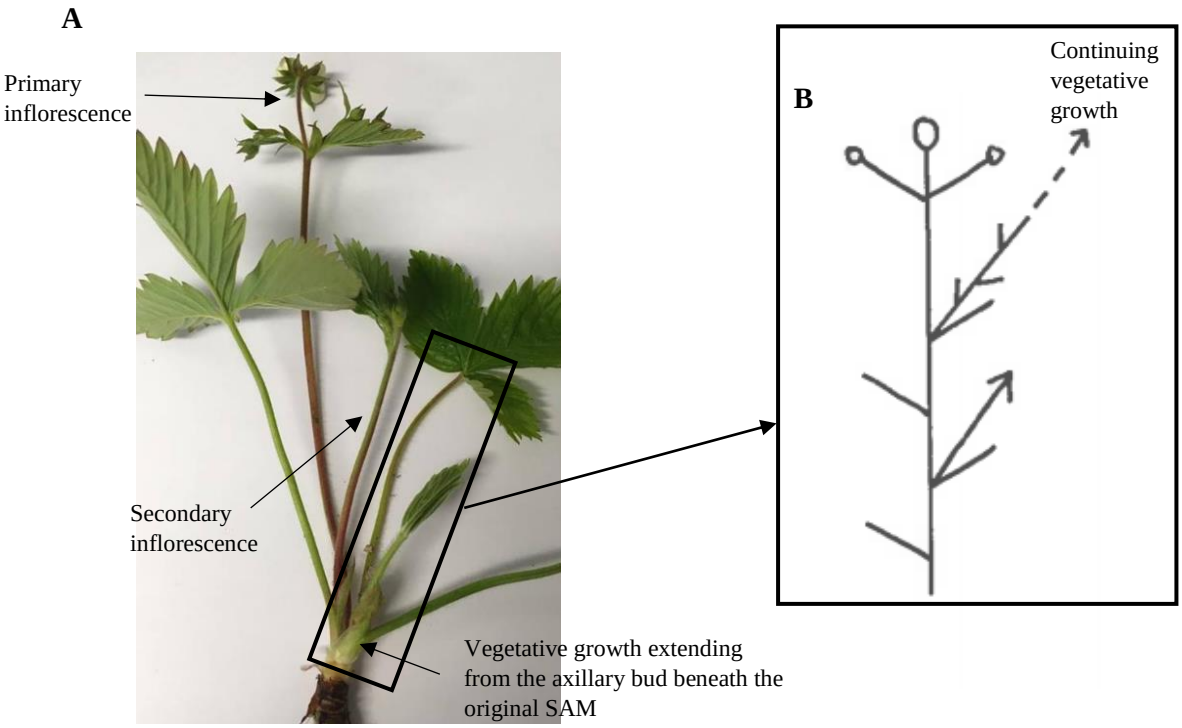


Figure 3.12 (A) Typical re-establishment of a vegetative apex at the top of the main crown or well-developed branch crowns; (B) Typical morphology of the axillary bud beneath the terminal inflorescence(s) from which vegetative growth continued

Inflorescences typically showed the presence of two different leaf/bract-like structures highlighted in Figure 3.13: A type structures were typically observed at the top of the peduncle; and were typically more leaf-like than bract-like; B type structures were typically observed at the junction of secondary or tertiary flowers and appeared more bract-like (Figure 3.13i). In some cases both a primary and secondary inflorescence were observed at the shoot apex (Figure 3.13ii and iii).

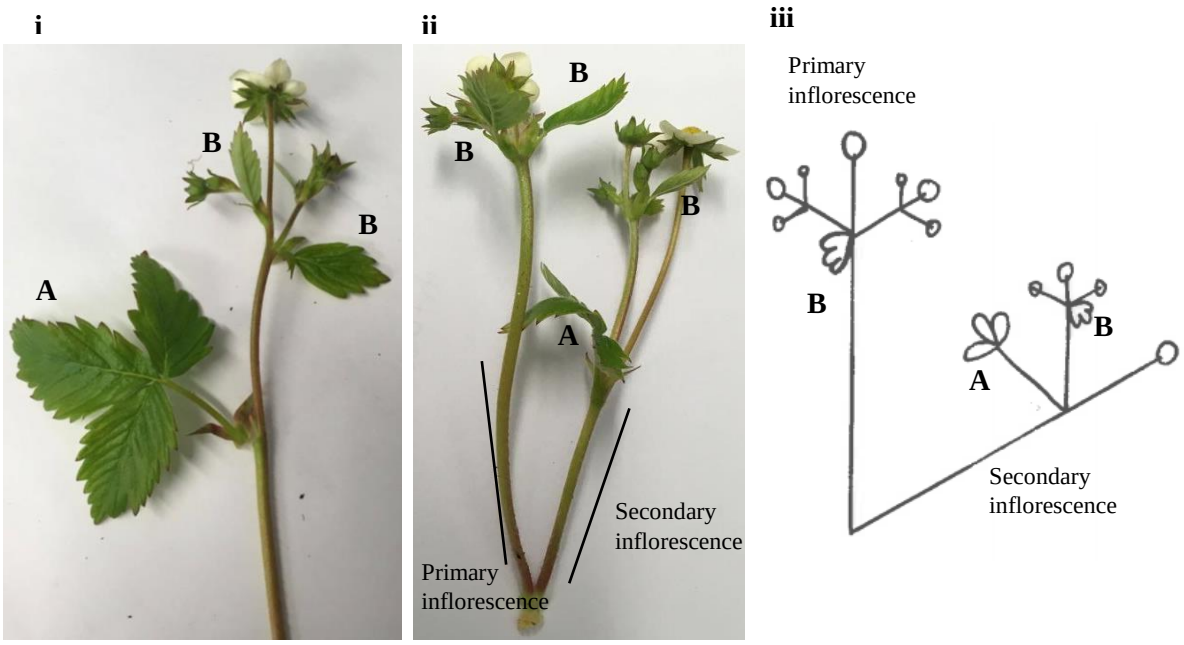


Figure 3.13 Typical inflorescence(s) structures observed on 18/04/2017, highlighting the emergence of structures A and B

Emerged runners were also observed on 18/04/2017. Inflorescences were typically visible before runners, but the timing of these events was close and the order in some plants was reversed. Runners typically were formed by the axillary bud subtended by the leaf immediately below the primary inflorescence, or from emerged branch crowns (Figure 3.14).

Figure 3.14 Plants showing the emergence of runners and inflorescences, from emerged branch crowns (A) and from the axillary bud subtended by the leaf below the primary inflorescence (B)

3.3.2 Changes in plant growth and development parameters

In this Section growth and development over the main sampling period (April-November 2016) are quantified; key interpretations are highlighted in italics.

Crown diameter

As well as apical growth, lateral growth also occurred and was associated with a change in crown diameter, as measured at the base of the main crown. The three cohorts of plants included within the main sampling period were initially analysed separately; all cohorts showed an increase in average crown diameter over the growing season (Figure 3.15). The increase in crown diameter was, however, not significant across cohorts, because of differences in plant size at the transition points between cohorts. The first cohort showed the most rapid and significant increase in crown diameter, at a rate of 0.7 mm a week from 19/04 – 12/07/2016.

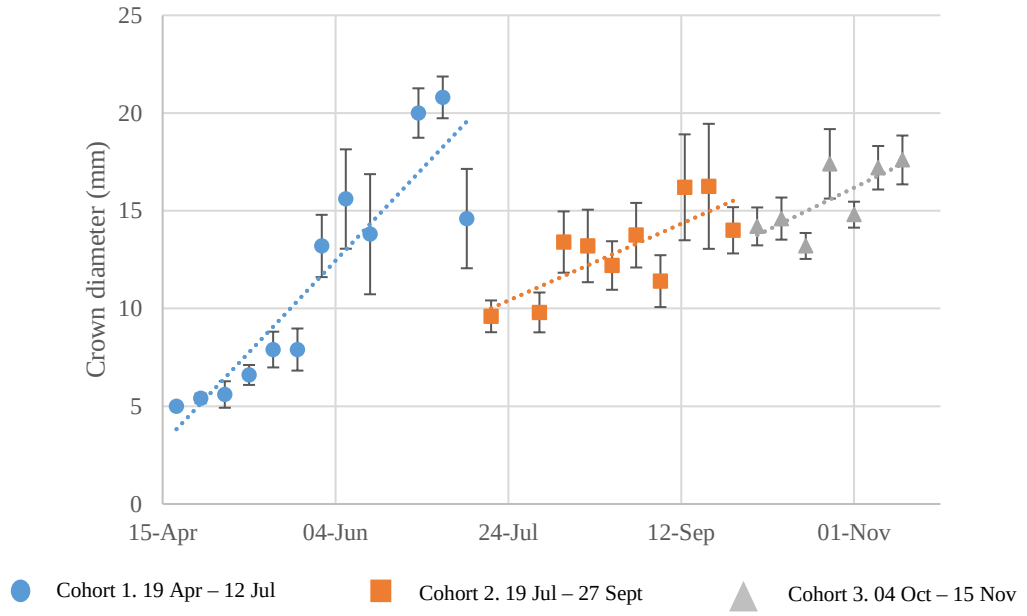


Figure 3.15 Mean diameter of the main crown during the growing season. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.1874x - 8026.7	$R^2 = 0.83$, d.f. = 11, $p = 0.00$
s.e.	(0.0268) (1151)	
Cohort 2 =	0.0786x - 3364.1	$R^2 = 0.59$, d.f. = 9, $p = 0.01$
s.e.	(0.0233) (1000)	
Cohort 3 =	0.0867x - 3716.9	$R^2 = 0.54$, d.f. = 6, $p = 0.06$
s.e.	(0.0359) (1544)	

Total leaves and leaf nodes

The total number of leaves and leaf nodes in the main crown of cohort one plants showed a significant increase over the spring and early summer (19/04 – 12/07/2016), at a rate of 1.2 nodes/week (Figure 3.16). The rate of leaf production of the subsequent cohorts was much reduced, 0.1 nodes/week and 0.2/week for cohorts two and three respectively, and there was no significant change in the total number of nodes with sampling date for cohort two or three. This implied a more rapid rate of apical growth during the early growing season.

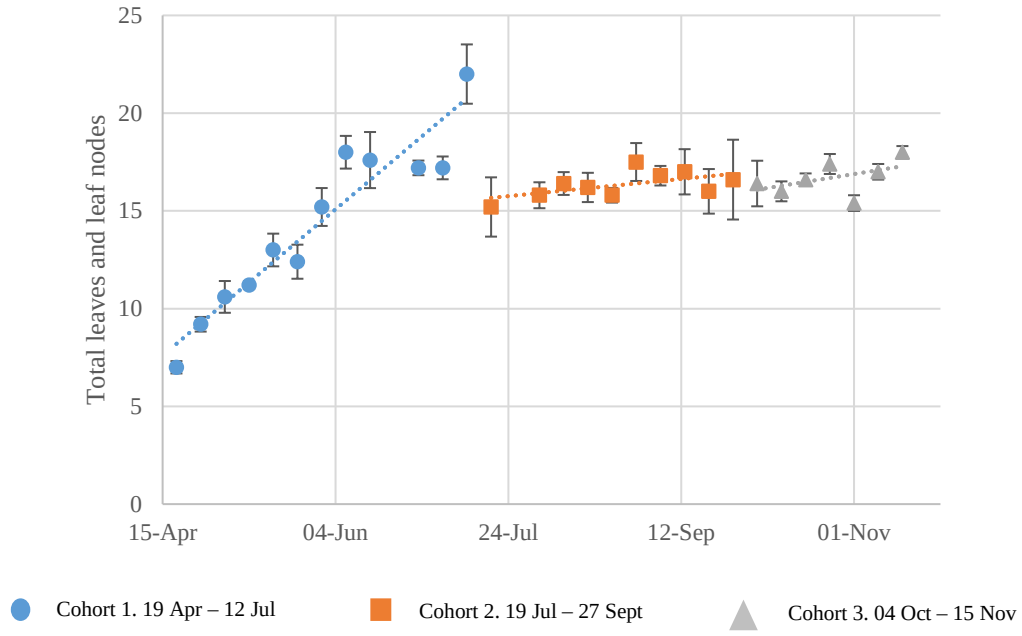


Figure 3.16 Mean number of leaves and leaf nodes in the main crown during the sampling period. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.1495x - 6396.6	$R^2 = 0.90$, d.f. = 11, $p = 0.00$
s.e.	(0.0157) (672)	
Cohort 2 =	0.0176x - 739.75	$R^2 = 0.34$, d.f. = 9, $p = 0.08$
s.e.	(0.0086) (370)	
Cohort 3 =	0.0286x - 1212.8	$R^2 = 0.25$, d.f. = 6, $p = 0.26$
s.e.	(0.0224) (962)	

Leaves

The number of leaf primordia enclosed at the apex was relatively constant, with no significant change over the sampling period (Figure 3.17).

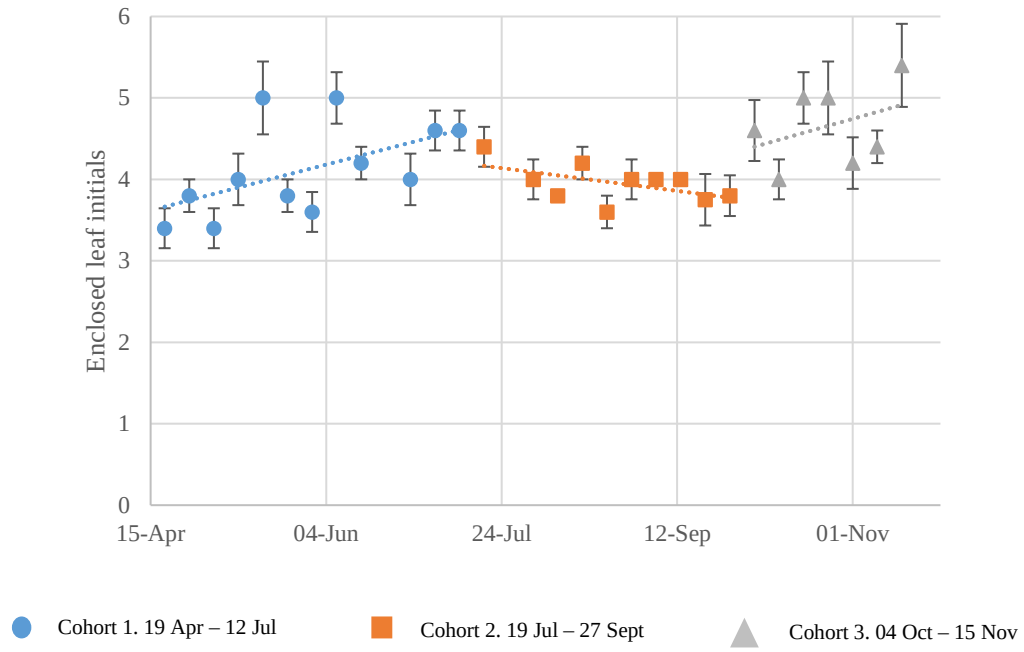


Figure 3.17 Mean number of enclosed leaves at the shoot apex of the main crown during the growing season. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.0112x - 477.81	$R^2 = 0.30$, d.f. = 11, $p = 0.07$
s.e.	(0.0054) (233)	
Cohort 2 =	-0.0056x + 244.46	$R^2 = 0.29$, d.f. = 9, $p = 0.10$
s.e.	(0.0031) (132)	
Cohort 3 =	0.0122x - 522.28	$R^2 = 0.14$, d.f. = 6, $p = 0.41$
s.e.	(0.0137) (590)	

The number of emerged, complete leaves of the first cohort of plants increased significantly over time, at a rate of 0.4 leaves/week (Figure 3.18). Following this initial increase, cohort two plants showed no significant change in the number of emerged leaves over the summer and autumn, with a reduced rate of increase in comparison to cohort one (0.1 leaves/week). Over the autumn, there was an apparent decrease in the number of emerged leaves of cohort three plants (-0.2 leaves/week), but this trend was not significant (Figure 3.18).

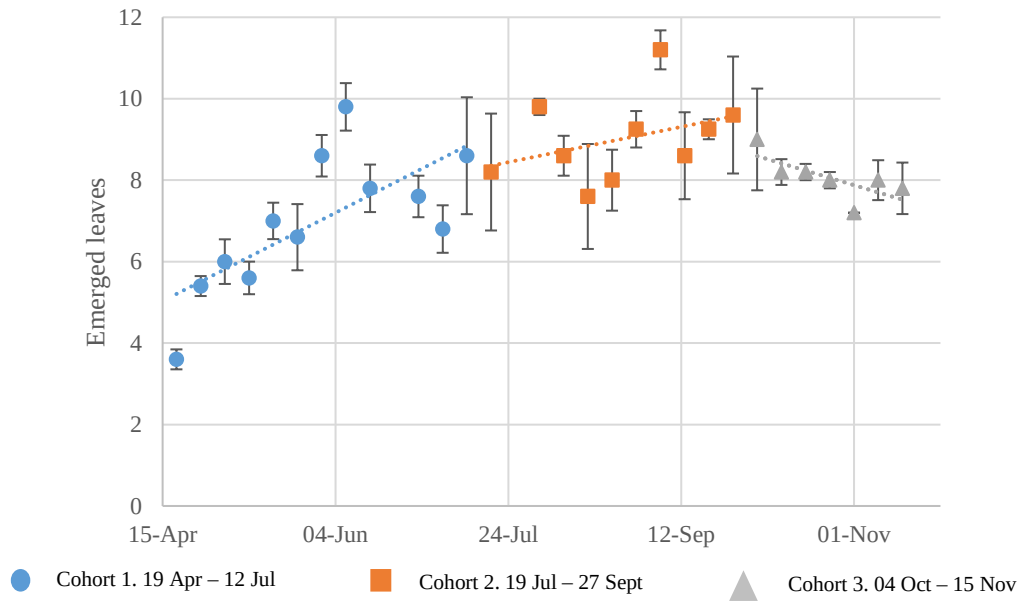


Figure 3.18 Mean number of emerged leaves on the main crown during the sampling period. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.0433x - 1851.4	$R^2 = 0.51$, d.f. = 11, $p = 0.01$
s.e.	(0.0135) (579)	
Cohort 2 =	0.0173x - 735.77	$R^2 = 0.14$, d.f. = 9, $p = 0.29$
s.e.	(0.0153) (659)	
Cohort 3 =	-0.0255x + 1105.8	$R^2 = 0.51$, d.f. = 6, $p = 0.07$
s.e.	(0.0111) (477)	

From April until mid-July, the number of senesced leaves in cohort one plants increased significantly, at a rate of 0.7 leaves/week (Figure 3.19). This was not maintained over the summer and cohort two showed no significant increase in the number of senescent leaves. However, from October until the end of the sampling period, leaf senescence significantly increased in cohort three plants but at a reduced rate compared to that of cohort one (0.4 leaves/week) (Figure 3.19). Leaf senescence was primarily observed at the base of the crown; at these basal nodes the stipules of the senesced leaves often remained and provided some protection to the associated axillary bud (typically unemerged branch crowns or arrested buds). Some senescent leaves were found in upper nodal positions, where senescence was assumed to be due to damage.

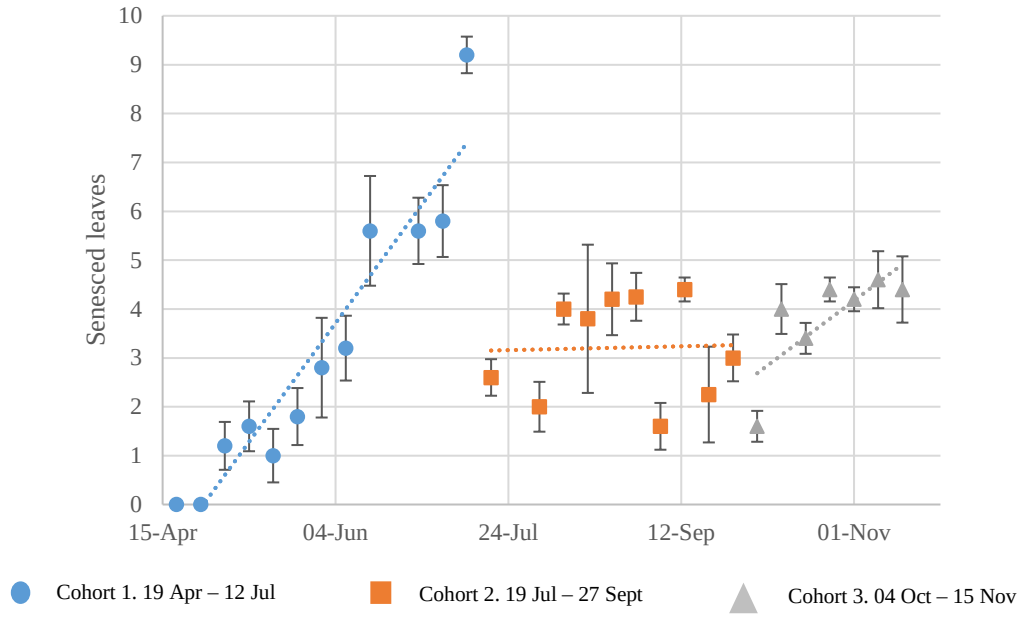


Figure 3.19 Mean number of senesced leaves on the main crown during the sampling period. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.0971x - 4159.9	$R^2 = 0.90$, d.f. = 11, $p = 0.00$
s.e.	(0.0102) (436)	
Cohort 2 =	0.0016x - 63.893	$R^2 = 0.00$, d.f. = 9, $p = 0.93$
s.e.	(0.0165) (708)	
Cohort 3 =	0.0531x - 2279.6	$R^2 = 0.59$, d.f. = 6, $p = 0.04$
s.e.	(0.0198) (853)	

Key interpretations

The main increase in total leaf number occurred during April and May, and was paralleled, although at a lower rate, by an increase in emerged and senesced leaves (Figure 3.20). A regression analysis was performed for leaf emergence, senescence and initiation, combining cohort responses from June onwards and showed no significant change in the total number of leaves and leaf nodes, emerged and senesced leaves (P > 0.05). This indicates rapid growth at the start of the sampling period, followed by a plateau from June-November (Figure 3.20); which was also reflected by changes in crown diameter. The lack of change in the number of enclosed leaves is also consistent with this interpretation, which suggests a real (cohort-independent) change in growth in early June. This change appeared to be independent of the specific growing environment, with a reduced rate observed from June while plants were still grown under glass (Figure 3.20).

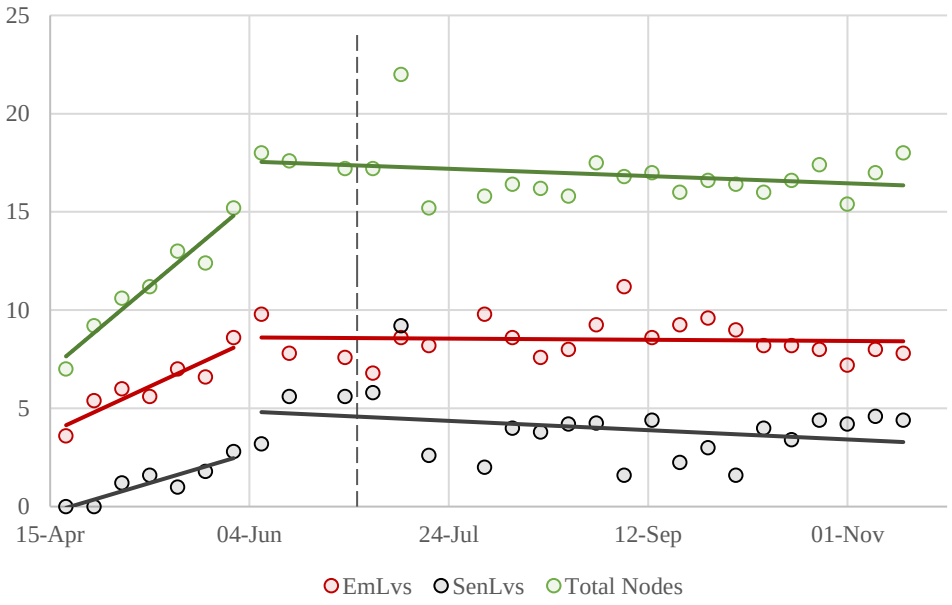


Figure 3.20 Mean change in the total number of leaves/leaf nodes, senesced and emerged leaves, with regression lines fitted combining cohort data from April-May and June-November. Vertical dashed line represents the time of transfer to the field for cohort one and two plants. The equations fitted by regression analysis for April-May data were:

Emerged leaves =	0.657x + 3.486	$R^2 = 0.85$, d.f. = 6, $p = 0.00$
s.e.	(0.126) (0.563)	
Total nodes =	1.193x – 6.457	$R^2 = 0.93$, d.f. = 6, $p = 0.00$
s.e.	(0.142) (0.636)	

Runners

Runners were observed on the main crown of all plants, regardless of cohort or sampling time; however, a significant increase in runner number was only observed from 19/04/2016 – 12/07/2016 (0.7 runners/week; Figure 3.21). This increase in runner number did not continue after July. The number of runners/plant declined from a maximum of between eight and nine in the summer to around five at the end of the sampling period, an effect most likely associated with cohort because even if runners had senesced or abscised their original presence could still be deduced by their remnants, and they were therefore included in the analysis.

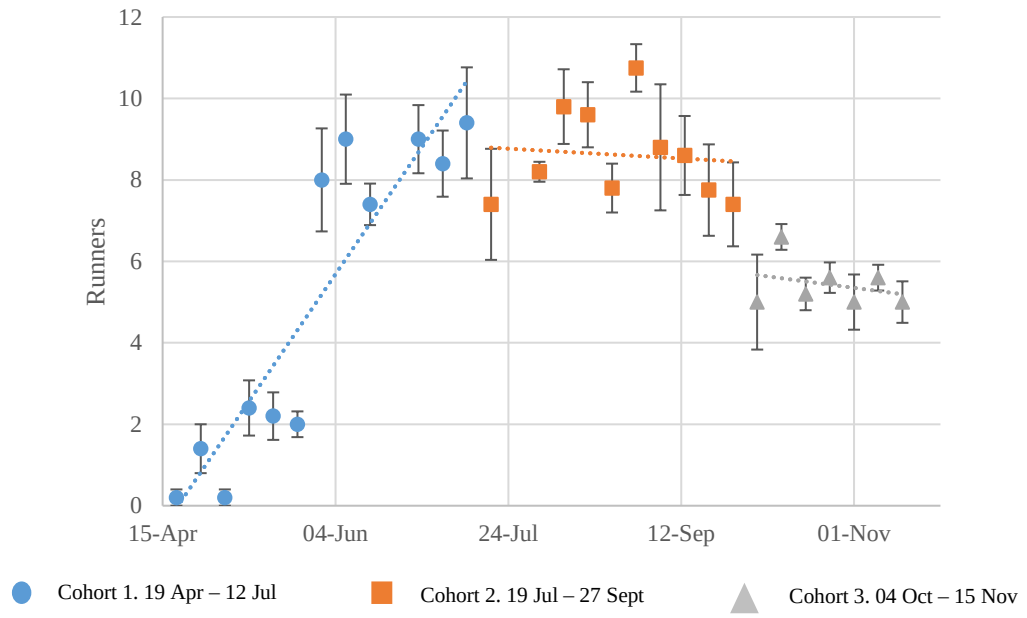


Figure 3.21 Mean number of runners on the main crown during the sampling period. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.1248x - 5347.8	$R^2 = 0.82$, d.f. = 11, $p = 0.00$
s.e.	(0.0185) (792)	
Cohort 2 =	-0.0048x + 213.91	$R^2 = 0.01$, d.f. = 9, $p = 0.80$
s.e.	(0.0177) (762)	
Cohort 3 =	-0.0112x + 488.45	$R^2 = 0.09$, d.f. = 6, $p = 0.53$
s.e.	(0.0165) (709)	

Branch crowns

Arrested buds at the base of the main crown were a typical feature and characteristic of some of Group 1 nodes. There was an absence of arrested buds at the start of the sampling period (in April), associated with a lack of discernible axillary bud character throughout the plant at this time. The number of arrested buds did not change significantly within cohorts throughout the sampling period ($P > 0.05$) (Figure 3.22). However, there was a difference in the average number of arrested buds between cohorts. Plants from October had an average of 0.4 ± 0.1 arrested buds, in comparison with plants over the summer and autumn (19/07 – 27/09/2016), which had an average of 1.1 ± 0.2 arrested buds. There was a significant decrease across the whole sampling period ($P < 0.05$) associated with an increase in active branch crowns.

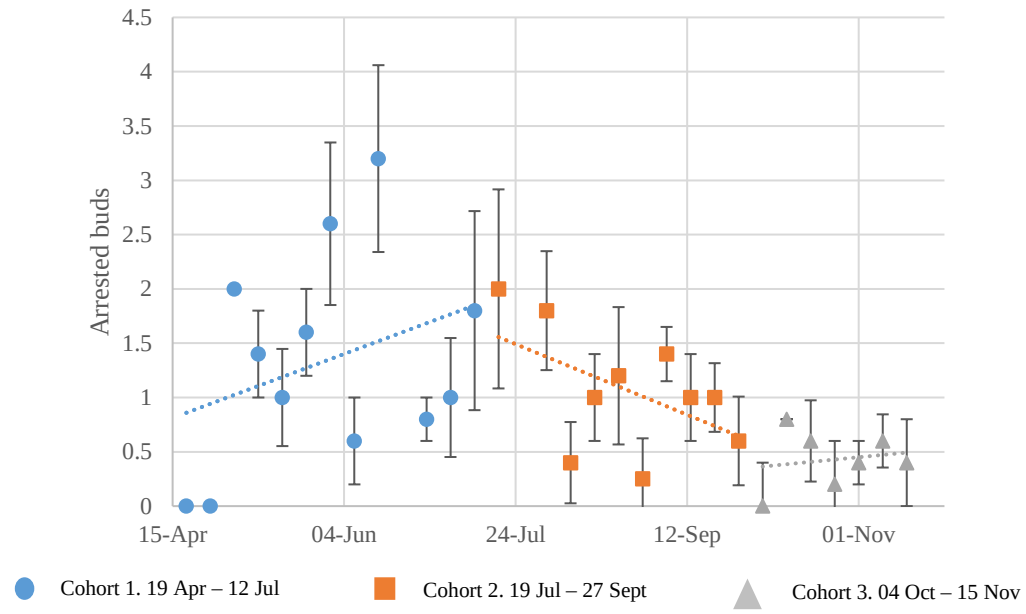


Figure 3.22 Mean number of arrested buds on the main crown during the sampling period. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.0118x - 503.71	$R^2 = 0.11$, d.f. = 11, $p = 0.29$
s.e.	(0.0105) (449)	
Cohort 2 =	-0.013x + 559.82	$R^2 = 0.27$, d.f. = 9, $p = 0.13$
s.e.	(0.0076) (328)	
Cohort 3 =	0.0031x - 131.31	$R^2 = 0.03$, d.f. = 6, $p = 0.71$
s.e.	(0.0078) (337)	

The presence of active branch crowns at the base of the plant, alongside arrested buds, was a typical feature of *F. vesca* plants. The number of branch crowns across all cohorts significantly increased with time (Figure 3.23). There was also a difference in the number of branch crowns between cohorts, with cohort three plants showing a greater number of branch crowns than those earlier in the sampling period. This increase in branch crowns was associated with the differentiation of branch crowns in newly developing axillary buds at the shoot apex during the autumn and the resumption of growth and development in arrested buds (previously inactive branch crowns) at the base of the main crown.

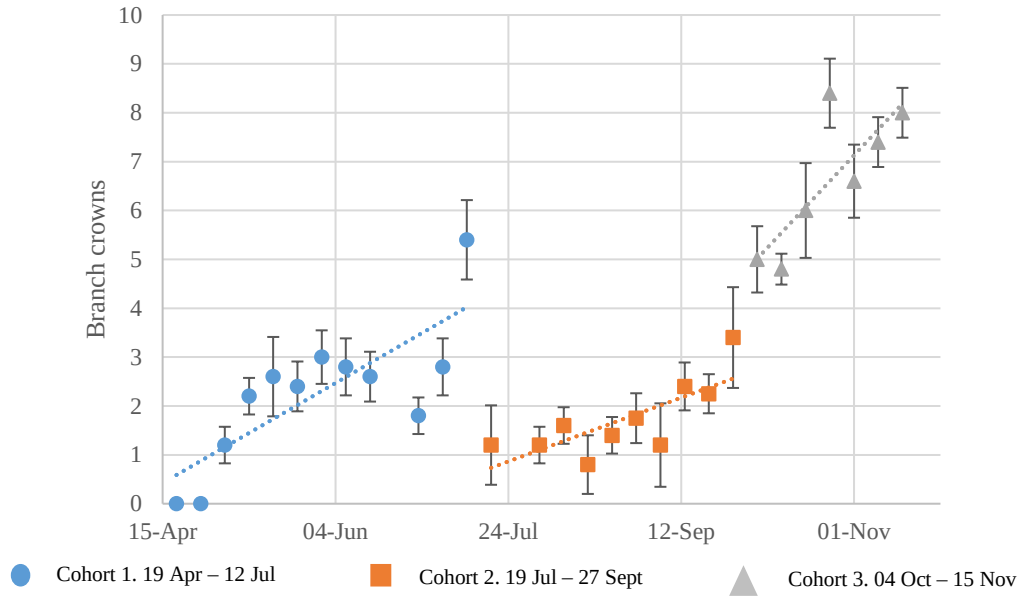


Figure 3.23 Mean number of branch crowns on the main crown during the sampling period. Standard error of the means are shown. The equations fitted by regression analysis were:

Cohort 1 =	0.0409x - 1751.2	$R^2 = 0.62$, d.f. = 11, $p = 0.00$
s.e.	(0.0101) (435)	
Cohort 2 =	0.0261x - 1119.1	$R^2 = 0.58$, d.f. = 9, $p = 0.01$
s.e.	(0.0079) (340)	
Cohort 3 =	0.0755x - 3242.8	$R^2 = 0.65$, d.f. = 6, $p = 0.03$
s.e.	(0.0247) (1062)	

Key interpretation

There was an increase in the average number of runners between April/May and the start of June, after which the number of runners remained relatively constant within cohorts. Branch crown production only increased significantly, due to emergence at both basal and upper nodes, between September and November when there was a corresponding decline in the number of arrested buds. The overall pattern is summarised in Figure 3.24 and emphasises that, as proposed for leaf production rates, developmental shifts were independent of the specific growing environment. There was no significant change in the number of runners, branch crowns or arrested buds in relation to plants being transferred into the field (Figure 3.24).

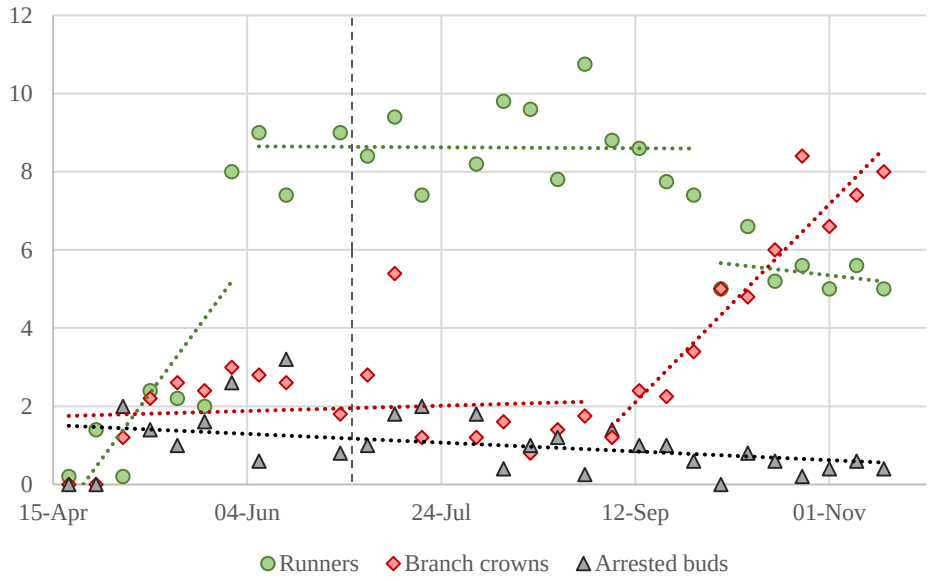


Figure 3.24 Mean change in the number of runners, branch crowns and arrested buds, with regression lines fitted combining cohort data to highlight changes in development. Vertical dashed line represents the time of transfer to the field for cohort one and two plants. The equations fitted by regression analysis were:

	Runners	
April – May =	0.1357x - 5815.1	R^2 = 0.60, d.f. = 6, P = 0.04
s.e.	(0.0497) (2131)	
June – September =	-0.0005x + 30	R^2 = 0.00, d.f. = 14, P = 0.93
s.e.	(0.0077) (331)	
October – November =	-0.0112 + 488	R^2 = 0.09, d.f. = 6, P = 0.53
s.e.	(0.0165) (709)	
	Branch crowns	
April – August =	0.0027x - 114.71	R^2 = 0.01, d.f. = 17, p = 0.71
s.e.	(0.0072) (311)	
September – November =	0.1014x - 4355.5	R^2 = 0.90, d.f. = 10, p = 0.00
s.e.	(0.0110) (472)	
	Arrested buds	
April – November =	-0.0045x + 193.43	R^2 = 0.14, d.f. = 28, p = 0.05
s.e.	(0.0022) (92.5)	

Floral meristems

Floral growth and development was observed from 20/09/2016 and there was a significant increase in the total number of inflorescences over the remainder of the sampling period (F_1 = 150.79; p = 0.00) (Figure 3.25). Inflorescence development was initially observed with the emergence of a single (primary) flower at the shoot apex and the number of inflorescences (typically primary, secondary and tertiary) at the apex of the main crown continued to increase from September-November (Figure 3.26). Inflorescences also developed at the apices of pre-

existing branch crowns at the base and upper sections of the main crown and were distinguished from inflorescences at the apex of the main crown (even though these also had leaf initials). Inflorescences were not observed at existing branch crown apices until 11/10/2016 and then continued over the duration of the sampling period (Figure 3.27); pre-existing branch crowns with developing floral meristems were referred to as branch crown extensions.

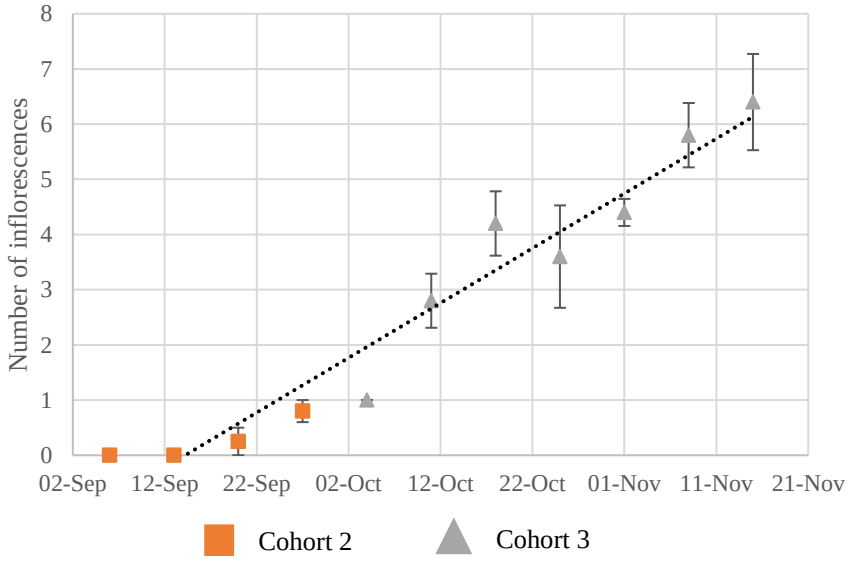


Figure 3.25 Mean number of total inflorescences throughout the plant from September. Standard error of the means are shown. The equation fitted by regression analysis was:

Cohort 2 and 3 = 0.0993x – 4268.5 $R^2 = 0.94$, d.f. = 10, $p = 0.00$

s.e. (0.008) (328)

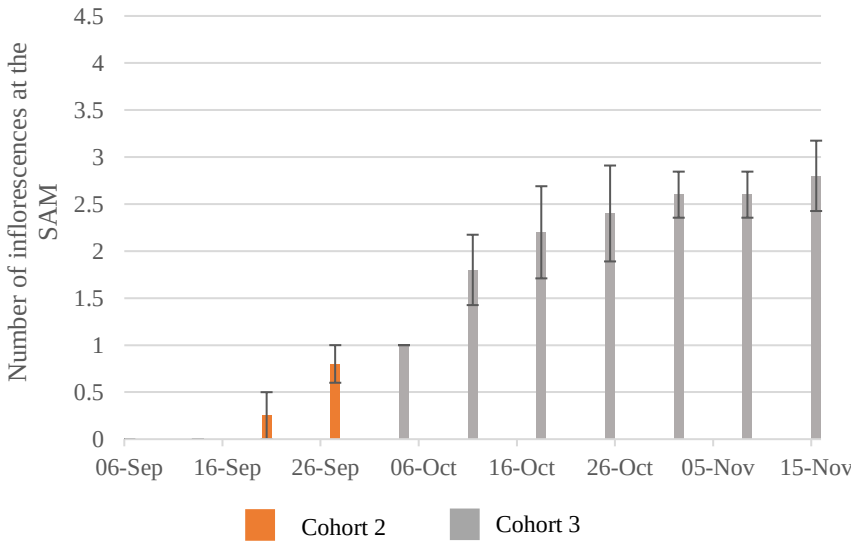


Figure 3.26 Mean number of inflorescences at the terminal SAM, including primary, secondary and tertiary inflorescences. Standard error of the means are shown

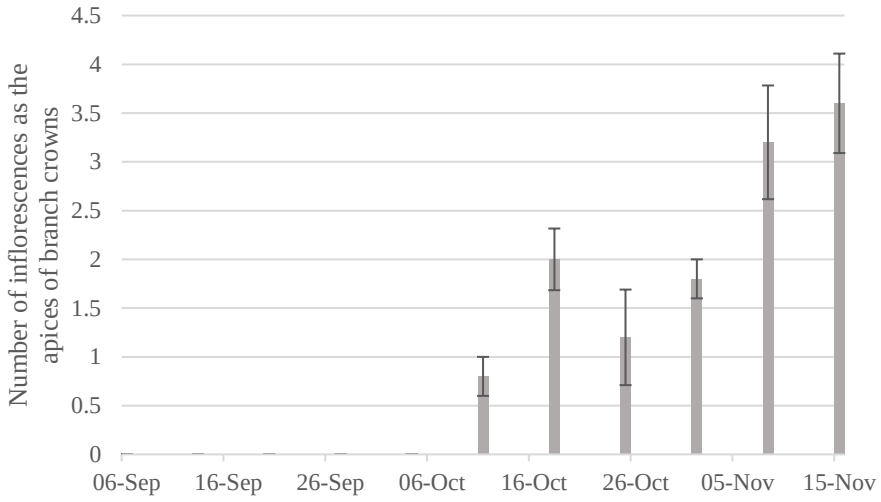


Figure 3.27 Mean number of inflorescences at the apices of existing branch crowns throughout the main crown (basal and upper positions), for the third cohort of plants. Standard error of the means are shown

3.3.3 Floral development

September

The flower developmental stages devised by Hollender *et al*. (2012) were used here. From 27/09/2016 flower initiation was observed as a doming of the terminal SAM as the developing bud reached stage 2/3 (Figure 3.3 and 3.28).

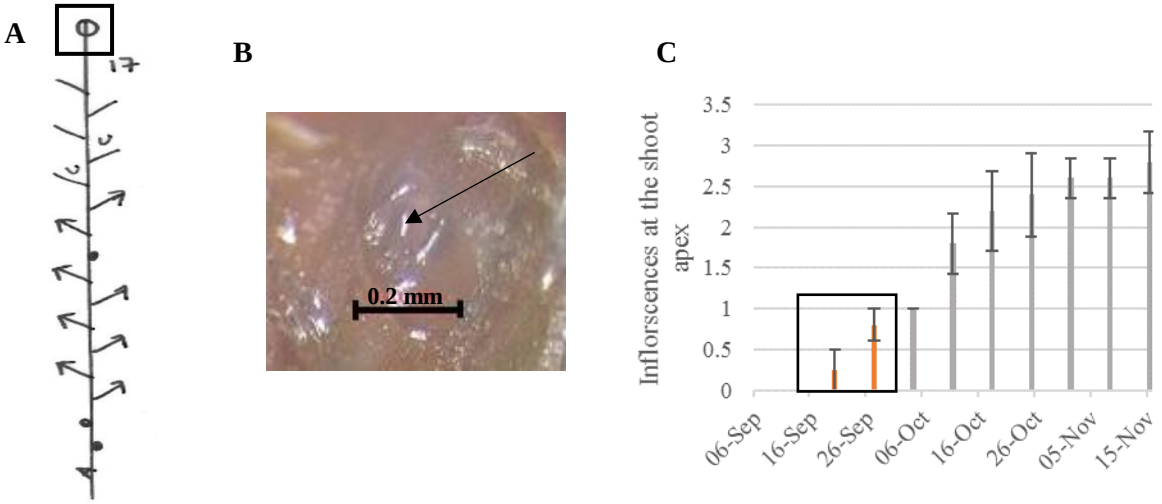


Figure 3.28 Typical state of floral development at the end of September (27/09/2016): A) architecture of the main crown with floral development at the terminal SAM; B) primary flower (arrowed) on the primary inflorescence typically at floral stage 2/3; C) average number of inflorescences at the terminal SAM

October

The primary flower was observed at the terminal SAM of all plants by 04/10/2016 (Figure 3.26). Secondary flowers had formed by the end of October on the primary inflorescence of all plants. By 26/10/2016, the primary flower was typically at floral stage 4/5 and secondary flowers were at stages 3/4 (Figure 3.29; see Hollender *et al.*, 2012). Secondary and tertiary inflorescences at the shoot apex (beneath the terminal inflorescence) were also typically present, with an average of 2.4 ± 0.5 inflorescences at the terminal SAM by the end of October (Figure 3.26). Two-five leaves were typically initiated prior to the initiation of the terminal flower of the secondary and tertiary inflorescences; as a result these inflorescences bore some resemblance to branch crowns, but were distinguished from branch crown extensions to be consistent with the terminology of Guttridge (1985). Primary and one to two secondary flowers were typically present at the apex of these secondary and tertiary inflorescences by the end of October, and were at an earlier stage of development than the equivalent flowers on the primary inflorescence (Figure 3.29). Branch crowns that were present and had initiated leaves/leaf initials prior to floral induction were typically found in the basal or upper sections of the main crown; these are referred to as branch crown extensions once floral (Figure 3.29). Floral development was recorded at the apices of some pre-existing basal branch crowns from 11/10/2016 (Figure 3.27 and 3.29). By 25/10/2016 there was an average of 1.2 ± 0.5 floral branch crown extensions with a primary flower typically at stage 2/3 (Figure 3.29). Branch crown extensions were present in all plants by 01/11/2016.

November

By 15/11/2016, there were 2.8 ± 0.4 inflorescences at the terminal SAM, similar to the number at the end of October. Inflorescence development continued, with secondary, tertiary and in some cases quaternary flowers being initiated during November. In the primary inflorescence, the primary flower had typically reached stage 9 by 15/11/2016, while secondary and tertiary flowers were typically at stages 6/7 and 4/5 respectively (Figure 3.30). Flowers in the secondary and tertiary inflorescences had also developed, with primary flowers at floral development stage 7/8, and secondary flowers typically at stage 5 (Figure 3.30). By this time basal branch crown extensions had initiated stage 2/3 secondary flowers at some apices and the primary flowers had reached stage 5/6 (Figure 3.30). Pre-existing upper branch crowns had also become floral (usually at nodes 11-12), with primary flowers at stage 2/3 (Figure 3.30). The induction of upper branch crown extensions resulted in a continued increase in the total number of inflorescences (Figure 3.25 and 3.27), even though at the SAM inflorescence initiation had plateaued.

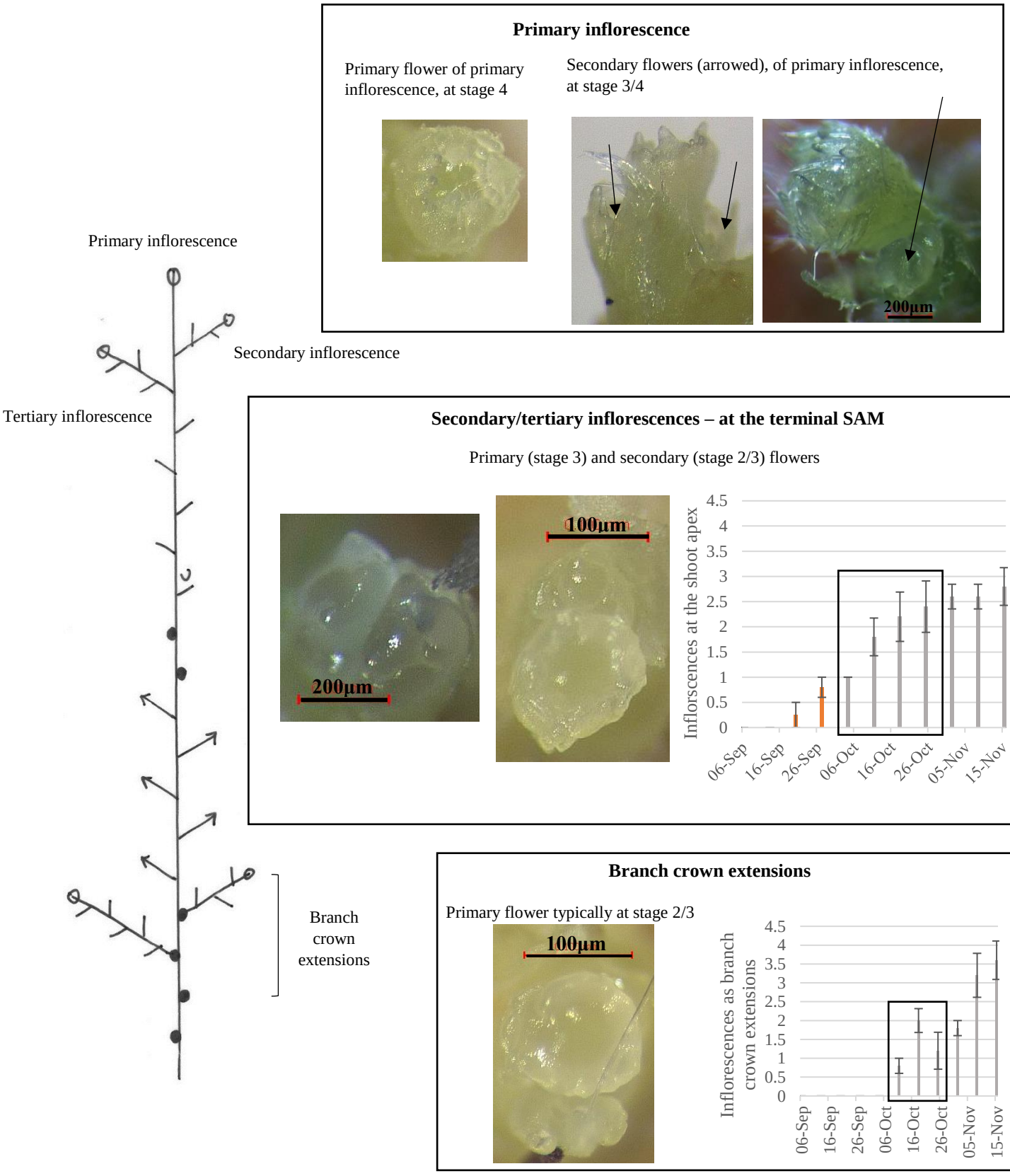


Figure 3.29 Typical plant architecture and state of floral development of inflorescences at the shoot apex and basal branch crown extensions at the end of October (25/10/2016)

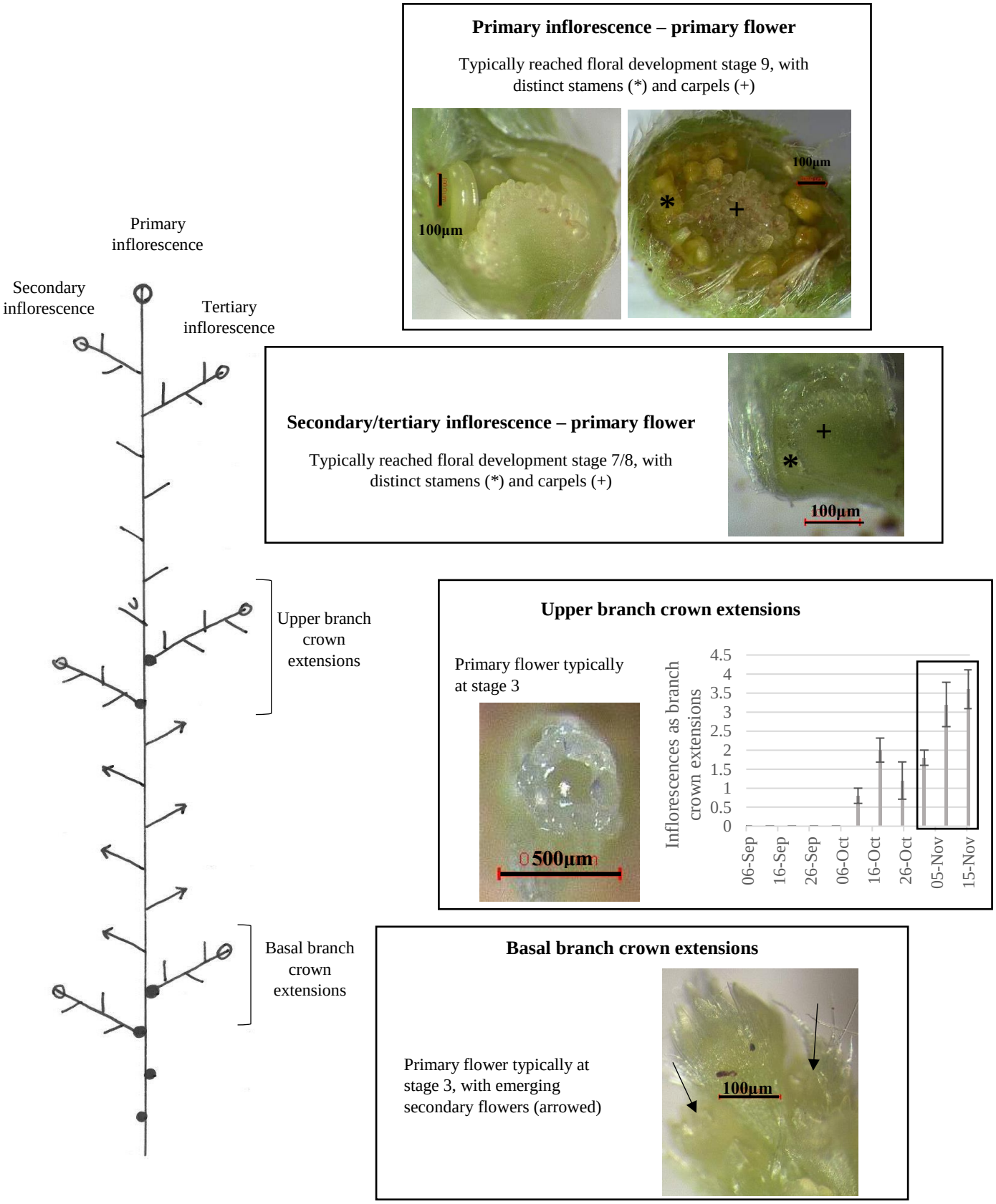


Figure 3.30 Typical plant architecture and state of floral development of inflorescences at the terminal SAM and in branch crown extensions by mid-November (15/11/2016)

3.4 Discussion

The research described in this thesis was designed to address fundamental questions concerning the regulation of growth and development by the environment in *F. vesca*. The main approach was to quantify responses at the morphological level in order to provide a basis for understanding the effects of environment on the annual cycle in this species. As a model species, the responses of *F. vesca* could also be helpful in suggesting the potential implications of climate and climatic change for other perennials, particularly within the Rosaceae. The purpose of this Chapter was specifically to provide a detailed account of the changes in growth and development during the main growing season, to create a foundation for the experimental chapters of the thesis, and to facilitate comparison with other species. This was necessary because although there has been extensive research on the genus *Fragaria*, previous work has tended to focus on particular seasons and aspects of development, often those which are of commercial importance (such as yield), in isolation. Controlled environments have typically been used to provide a better understanding of optimal and threshold responses, rather than natural, fluctuating environments which are probably more pertinent in the context of the overall annual cycle. For example, the interactive effects of temperature and photoperiod on flower initiation have been comprehensively described for *F. vesca* and *F.* x *ananassa*, but studies neglect to provide a description of the response to natural environmental conditions and how changes in such an environment might affect flowering (Le Mière *et al.*, 1996; Heide and Sønsteby, 2007). Other studies provide a detailed description of morphological changes, for example in flower initiation, but without addressing the integrated response to environment (Taylor *et al.*, 1997; Hollender *et al.*, 2012).

In summary, although there is often a good understanding of the conditions regulating specific developmental phases, and the changes in morphology are well documented for some of these, studies have not aimed to provide a comprehensive description of growth and development, particularly with a view to understanding how preceding and subsequent processes relate to and influence each other. Neither has such an integrated view been sought under a natural environment, to enable potential temperature and photoperiod effects to be inferred. This highlighted a need to quantify morphological and developmental changes during the annual cycle, and to relate these to environmental conditions as a basis for the experimental and inferential work in the rest of the thesis.

Sampling began from mid-April and plants showed active growth with an increase in the number of emerged leaves at a rate of 0.8/week until the end of May; this rate of emergence was not, however, maintained over the sampling period, and declined over the summer (June-August).

This implied a fairly restricted period of rapid, active vegetative growth at the SAM for about seven weeks at the start of the sampling period; it is important to note that sampling did not start until 19/04/2016. The rate of leaf initiation/emergence was matched by that of leaf senescence from June and maintained throughout the sampling period, resulting in no net increase in the number of leaves and leaf primordia after June, indicated very restricted SAM activity from June until September when floral initiation took place.

The restricted phase of active leaf production at the SAM was reflected in runner production, with an average increase of 1.1 runners/plant/week from April-May slowing to 0.1 runners/plant/week over the summer (June-August). This is consistent with the fact that the initiation of new leaves is required for the production of new axillary buds (here, runners). Branch crowns on the main crown were observed throughout the sampling period, with the exception of the initial dissections when axillary buds had not become visibly differentiated. These branch crowns were initially confined to the basal nodes but from July were also observed sporadically in upper nodes, and from October were recorded in node positions with the most recently discernible axillary bud character. This resulted in an average increase of 0.7 branch crowns/plant/week from September-November, consistent with previous reports for both *F.* x *ananassa* and *F. vesca* (Guttridge, 1955; Mouhu *et al.,* 2013). The transition to reproductive growth occurred at the terminal SAM from the end of September. Floral meristems were subsequently initiated at the shoot apices of branch crowns, first at the basal nodes of the main crown (from mid-October) and later at upper nodes. The average number of inflorescences per plant throughout the main crown increased at a rate of 0.8/week from 20/09/2016. This sequence of floral initiation has also been shown in *F.* x *ananassa* (Guttridge, 1955; Darrow, 1966).

The exact early events in formation of the terminal flower have received different interpretations. Jahn and Dana (1970a) reported the emergence of inflorescence bracts prior to the observable initiation of the flower; however, in the wider literature, the enlargement of the apex has been reported as the first stage of floral initiation (Guttridge, 1955; Darrow, 1966; Guttridge, 1985). For the *F. vesca* plants described here, the doming of the SAM was the first observable sign of floral development, with bracts typically visible only from stage 4, as found by Hollender *et al.* (2012). During the floral initiation phase the number of runners and leaves on the main crown remained fairly constant, as expected based on the cymose pattern of inflorescence development in *Fragaria* and the conversion of existing axillary meristems to branch crowns with inflorescences.

Flower and runner emergence was observed in April 2017. The time of floral initiation and the process of development was considered to have been similar to that observed by dissections in

the autumn (2016), but it was not possible to determine the time of initiation of the first emerging runners from the dissections. Runners were observed in the axillary buds of branch crowns beneath the terminal inflorescence(s), and it was not clear whether these runners were determined prior to floral induction or under spring conditions following winter chilling. For the latter to be true, these buds would have had to remain undetermined during floral induction, or not been present at that time; some axillary buds within branch crowns were observed to have an undiscernible character. Studies of *F.* x *ananassa* suggest that the first runner to emerge usually develops from the axil of one of the new leaves initiated in the spring (Darrow, 1966; Dana, 1980). The results from this Chapter appear to contradict this statement, with runners emerging from leaf axils that were probably present before the spring, due to the observation of a floral primordium at the shoot apex. Regardless of the time of initiation, this observation is consistent with others made in this thesis: that some axillary buds do not confirm to the character expected based on the conditions to which they had been exposed.

The order of flower and runner emergence under natural spring conditions was similar to that observed in other studies of natural populations (see Chapter 6 for further discussion), with inflorescences typically emerging before runners. However, the time of emergence of these inflorescences and runners was very close, and some plants showed a reversed order of emergence. Flowering potential (number of flower buds) has been reported to influence time of runnering in the spring, plants with no or few flowers buds start producing new leaves and runners before plants with more flower buds (Darrow, 1966). The similarity in timing of flower and runner emergence for plants in April 2017 might have been linked to the flowering potential of these plants, as they were transferred into the field (flower induction conditions) later than the earlier cohorts, so may have initiated fewer flowers/inflorescences. A repeat experiment would be necessary in order to explore the influence of flowering potential on the timing and order of flower and runner emergence.

Plant growth and development during the sampling period was therefore for the most part as expected, and comparable to the annual cycle of seasonal flowering *Fragaria* described by Carew and Battey (2005) (compare Figures 1.6 and 3.31). One difference was the observation of runners in April (2017), while Carew and Battey (2005) suggested later initiation; however, Carew and Battey (2005) described the annual cycle of commercial strawberry (*F.* x *ananassa*). Another unexpected finding of particular importance was the clear developmental shift in June affecting the rates of leaf production (initiation and emergence) and newly initiated runners on the main crown, such that these processes were reduced to a minimum over the summer and autumn.

Leaf initiation			*					
Leaf emergence			*					
Runner initiation	*		*					
Branch crown development								
Flower initiation								
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov

Figure 3.31 Timing of key vegetative and floral processes during the sampling period (shaded); notable changes in development are marked (*)

There appears to be only one previous study in which a comparable, though different, change can be inferred. This was work with *F.* x *ananassa* by Arney (1954), who described seasonal variation in the rate of leaf emergence and expansion over the growing season, with runners maintained on the plants. He reported a rapid initial increase in leaf production and initiation from March-June which was suggested to be associated with the accumulation of leaf primordia at the SAM over the winter (Figure 3.32). It is important to note that this flush in initial growth could not have been observed in the plants dissected in this Chapter over the main sampling period (April-November 2016), as they were vegetatively propagated from newly emerged daughter plants in the spring (2016). Arney (1954) reported that after this initial flush, the number of leaves initiated/month stayed constant over the summer (June-September) at a rate greater than that described for *F. vesca* in this Chapter. It is not clear whether Arney (1954) included senesced leaves in the total leaf count.

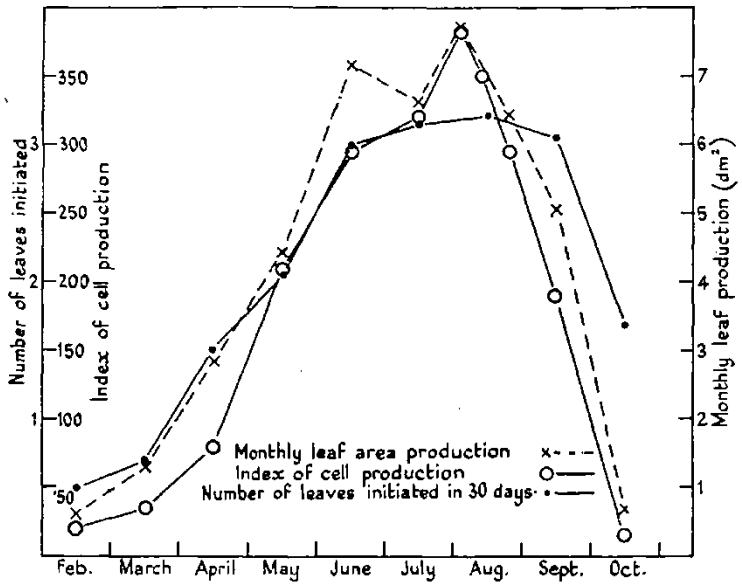


Figure 3.32 Diagrammatic presentation of two leaf growth indices (monthly leaf area production and cell production) and leaf initiation (from Arney, 1954)

The marked slow-down in leaf initiation and runner production by the main crown of *F. vesca* from June is important because it is fundamental to the annual cycle, and the observed changes in character of the plants through the year. It partly accounts for the highly seasonal pattern of runner production. Axillary bud character is predominantly a consequence of environment, with the number and length of runners per plant and number of daughter plants per runner promoted by LD (16h) and high temperatures (21-30°C) in *F.* x *ananassa*; the response is highly cultivar/genotype-dependent in *F.* x *ananassa*, *F. chiloensis* and *F. virginiana* (Darrow, 1936, 1966; Heide, 1977; Serçe and Hancock, 2005; Hytönen *et al*., 2009; Hasan *et al*., 2011). In this Chapter it has been clearly shown that over the summer (June-August), when conditions were most similar to those reported elsewhere as optimal for runner production, the average number of runners per plant did not increase significantly. It appeared that the slow rate of leaf initiation over the summer restricted runner production, rather than environmental regulation of axillary bud character.

What might cause the decline in terminal SAM leaf initiation after June? Temperature has been shown to affect vegetative growth in *Fragaria*, with an increase in relative growth from approximately 18°C to an optimum of 25°C (Hancock, 1999); but temperature continually increased from the end of May until mid-July and this was not reflected by a parallel increase in the rate of leaf initiation. It seems possible that photoperiod could regulate SAM activity, as it increased to a maximum in June, the time that the change in the rate of leaf initiation occurred. An absolute photoperiod effect, or one related to its rate of change could have been responsible. Baker *et al*. (1980) proposed that the response of leaf appearance rate was influenced by an interaction of rate of change of photoperiod and temperature in winter wheat. Rate of change of photoperiod has also been linked to other growth responses, such as flowering time in yam (Ile *et al*., 2007). An important observation, however, is that the observed decline in leaf initiation observed here for the main crown of *F. vesca* over the summer did not occur in the runners themselves, because they continued to grow throughout the summer. Runners of cohort one plants typically grew by 5.8 internodes/week between the end of May and mid-July; active runner growth was also observed by cohort two which increased by 3.5 internodes/week from mid-July to mid-August (see Box 3.1). One explanation may be, therefore, that runners dominated growth, reducing the rate of leaf initiation (and axillary bud initiation) at the terminal SAM.

Box 3.1 Runner growth

Recording of runner growth was not the primary objective of this Chapter, however, a number of parameters (the number of internodes and daughter plants, and the length of the runner) were recorded for each runner on the main crown during the sampling period. Active runner growth was observed over the summer with an increase in the average number of internodes, daughter plants and total length of runners for cohort one (dissected until 12/07/2016) and two (dissected from 19/07/2016) plants (Figure 3.33).

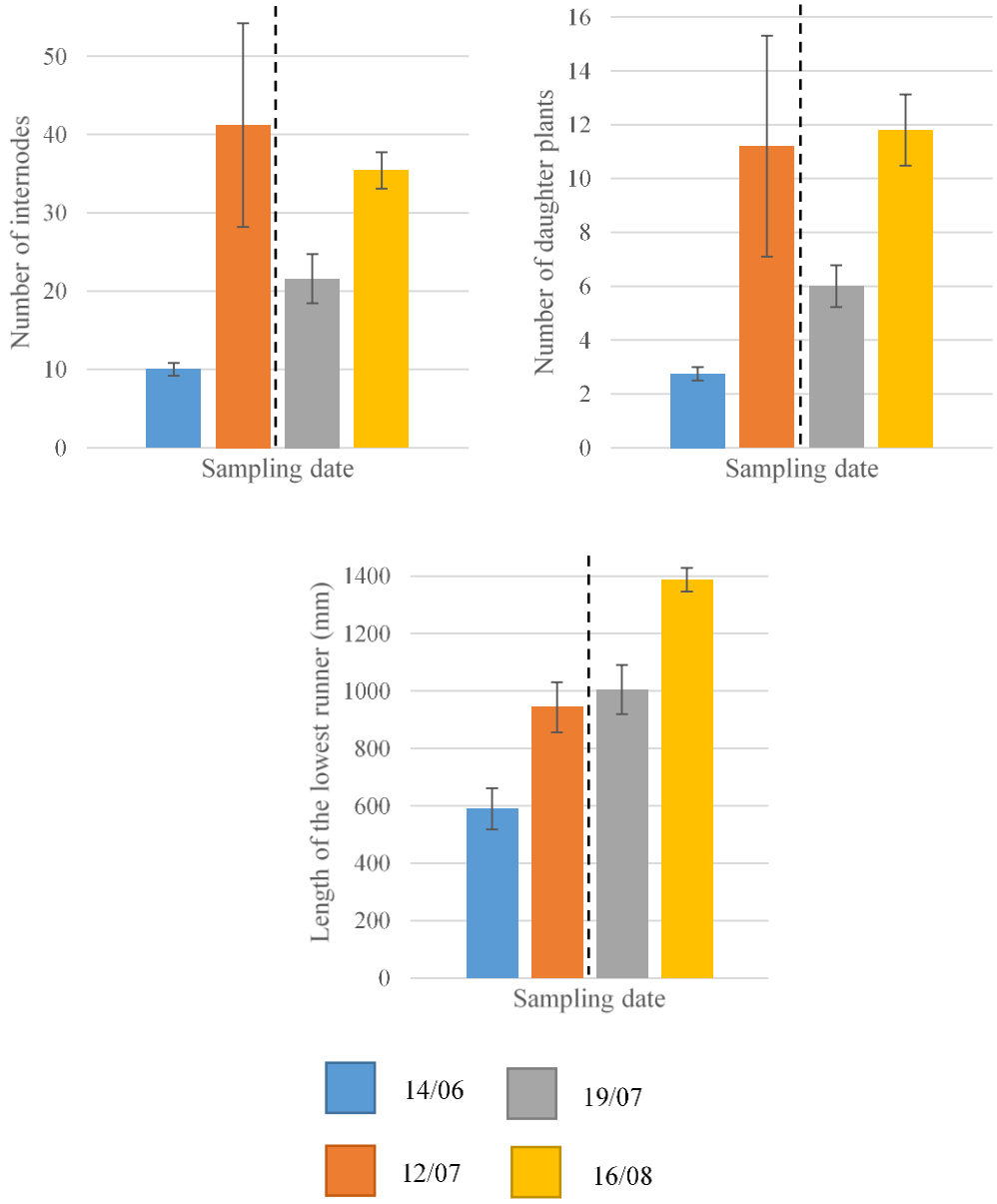


Figure 3.33 Average number of internodes and daughter plants and length of the lowest, intact runner on the main crown. The vertical, dashed line represents the switch from cohort one to cohort two plants

Runners have been reported to compete with apical growth in *F*. x *ananassa* by Darrow (1930): with runner removal (from June) shown to increase total leaf area on the main crown of the mother plant, whereas plants without runner removal had a more constant and in some cases a decline in total leaf area per (mother) plant. The extent of rooting of runners can influence their effect on mother plant growth. Picio *et al*. (2014) showed that unrooted runners reduced shoot dry matter mass for mother plants and acted as a sink to the mother plant, while rooted runners were a reciprocal source to the mother plant and maintained growth of defoliated mother plants. The plants used here were grown on staging and the runners hung freely and unrooted from the plant, so they are likely to have acted as sinks for mother plant resources. Dominance of lateral growth over terminal SAM growth may be an ecological strategy; genotypes of *F. chiloensis* can be ranked according to their potential for resource sharing and in an artificially heterogeneous environment, where connected ramets were grown under different environmental conditions, genotypes with a higher-sharing potential had a significantly greater total dry biomass (Alpert *et al.*, 2003).

The influence of runner growth has also been linked to other responses, with runner removal affecting fruit number, weight and yield (Black, 2004), and yield and fruit number being responsive to the extent of runner growth (Lyu *et al.,* 2014). This implies that continued runner growth activity is important, and it is not simply the presence of runners on the main crown that represses growth. The continual removal of developing runners from plants also influences branch crowning (Staudt, 1926; Hancock, 1999; Black, 2004). Deyton *et al.* (1991) investigated the effect of a chemical runner suppressant (paclobutrazol) on plant growth response and showed an increase in the number and size (total dry weight partitioned into crown) of lateral (branch) crowns with increased application of paclobutrazol. Hytönen *et al.* (2009) also showed runner inhibition with the application of prohexadione-calcium, which induced branch crowning and was correlated with a decline in GA_1 level. An increase in the number and size of branch crowns with reduced runnering (through suppression or removal) suggests active lateral growth even in the absence of runners.

The rapid terminal shoot meristem growth during spring and early summer observed in this Chapter for *F. vesca* appears similar to the reported pattern for apple, where there is a rapid increase in spur leaf biomass following bloom, followed by a decline in growth from mid-June, resulting in a relatively constant rate over the remaining growing season (Neilsen *et al*., 1997). This has also been shown for shoot length and leaf production in apple, with a decline from June onwards (Avery, 1969; Grossman and DeJong, 1995). A comparable response has been observed in a number of *Prunus* species, with a rapid initial increase in shoot length followed by a decline in growth. Species vary in the timing of this growth decline, with a relative plateau in shoot length

observed from May for almond and June for peach and plum (Razouk *et al*., 2013). As previously discussed, rapid spring growth (leaf emergence) has been reported in *F.* x *ananassa* in response to accumulation of leaf initials over the winter (Arney, 1954), but this does not explain the observed rapid spring growth from April-May 2016, as these plants were propagated in the spring and did not overwinter.

The results presented in this Chapter are therefore largely consistent with previous research, but highlight a marked decline in leaf initiation and emergence from June which has consequences for the overall pattern of seasonal growth and development of *F. vesca*. The cause of this change is not clear, but it could be due to competition from runners, an environmental influence (most likely photoperiod), or an endogenous control mechanism (for example, fixed growth duration of the terminal SAM). The transfer of active growth from the terminal SAM to the axillary positions (branch crowns) during the autumn, is an interestingly, possibly analogous process to that described for vegetative growth during the spring and summer. It may be a general feature of *Fragaria* that dominance of the terminal SAM is only exercised weakly and temporarily during development. A repeat of the annual cycle analysis reported here, with the inclusion of a runner removal treatment, is needed to confirm conclusively the regulatory role of runners on the terminal SAM deduced here (see Chapter 7, Box 7.1).

It is important, finally, to note the limitations of the work described here. A single *F. vesca* ecotype of material propagated vegetatively (through runners) was used, and several cohorts were required to maintain plant numbers; observations were made over only a single season. Ecotypes vary in the timing of flower emergence, runner production, flower initiation and dormancy (Heide and Sønsteby, 2007; Hasan *et al*., 2011). They also differ in resource sharing potential (see above). Seed-raised plants, and older vegetatively propagated material, might have different annual cycle characteristics. However, vegetatively propagated material of comparable age to that used here was employed consistently in the research described in this thesis, allowing comparison of results in subsequent experimental Chapters. It would clearly be desirable not only to replicate the observations made in this Chapter over at least one further season; and also, for the ecological significance of the observations to be understood, to replicate them on natural, wild populations (see also Chapter 7, Box 7.1).

Chapter 4: Environmental control of meristem fate

4.1 Introduction

4.1.1 Commitment and determination in plants

The changing appearance of a plant during its life cycle often reflects the developmental decisions in lateral (axillary) structures which arise from the shoot apical meristem (SAM). In the case of *F. vesca*, the annual cycle is characterised by a predictable sequence of changes in developmental emphasis, from runners to branch crowns to flowers, in co-ordination with the external environment (see Chapter 3). This chapter describes experiments designed to establish when axillary structures arise in different environments, how their fate is determined and how the presence of such structures may influence growth of the plant.

In general, structures arising from the SAM (e.g. leaves, runners, flowers) have a form which is characteristic of the species, consistent under varied conditions and, with the exceptions of changes associated, for example, with the juvenile-adult transition, constant during the life of the individual. This observation of consistency suggests final form is usually determined early on during development of lateral structures, and it has generally been found that commitment (or determination) of fate in the organs arising from the SAM does occur early (Smith and Hake, 1992; Lyndon, 1998). For instance, Sachs (1969) found that pea leaf morphology was determined progressively during early stages of leaf development. This surgical study suggested that the overall architecture of the leaf was determined during the first plastochron, but that leaflet morphology was determined later, during the second plastochron. Leaf determination in pea is considered to take approximately two days from the time of initiation (Sachs, 1969; Lyndon, 1990). For axillary buds, the surgical studies on *Epilobium hirsutum* of Snow and Snow (1942) suggested that axillary bud determination following leaf primordium removal can be considered an 'all or nothing' process: if the bud is determined this determination is regular in size, area and timing regardless of leaf presence. They also concluded that in some species, axillary bud determination is dependent upon influences of the subtending leaf. Determination or commitment of the SAM to a floral fate has also historically been a major area of research interest (e.g. Bernier *et al.,* 1981). It is typically rapid, with early flowering *Arabidopsis* ecotypes committed within one day of photoinduction (Hempel *et al.*, 1997, 2000). The duration of the commitment period is, however, influenced by plant age and environmental conditions such as the light integral, wavelength of light, and temperature (Hempel *et al.*, 1997; Lyndon, 1998; Adams *et al.,* 2001).

There are, however, exceptions to this general pattern of early determination. *Ranunculus flabellaris* produces aquatic leaves underwater and aerial, less dissected leaves on reaching the water surface; experiments have shown this change to be determined relatively late in development and that photoperiod is the dominant mechanism controlling determination (Cook, 1969; Smith and Hake, 1992). Late leaf determination has been established in other species, such as maize, with leaf primordia considered to remain undetermined until the leaf is 3mm in length (Orkwiszewski and Poethig, 2000), which is much larger than pea leaves at the time of determination (approximately 100µm; Sachs, 1969). Importantly for the work to be described here, the identity of some axillary buds has also been observed to show changeable fate. *Agropyron repens* axillary buds can form rhizomes or aerial shoots, with studies showing that individual shoots can switch between these two fates (Palmer, 1958). A rhizome could change to an aerial shoot and during the transitional state aerial shoots could be forced to revert to forming rhizomes by altering bud position. In comparable experiments with *Sorghum halepense*, Gizmawy *et al.* (1985) showed that orientation of the parent shoot controls whether the axillary structure develops as rhizome or tiller.

Floral commitment may also be late or even absent, leading to floral reversion (Battey and Lyndon, 1990). In *Impatiens balsamina* floral meristems are initiated under SD, but retain the ability to revert to leaf initiation if plants are transferred to vegetative conditions (LD); however, this reverted apex has been observed to differ from normal vegetative and floral apices, as it has the characteristics of a floral apex but produces leaf initials (Battey and Lyndon, 1986). Battey and Lyndon (1986) showed that *Impatiens balsamina* could also be induced to re-flower, following reversion to leaf initiation, by transfer back to SD conditions. Determination of organ fate in reverted apices is prolonged, with leaf primordia only completely committed when approximately 750µm long; determination also does not occur simultaneously across the organ, with the cells at the tip committed first and those at the base last (Battey and Lyndon, 1988). As a result of this, determination of the leaf as a whole was suggested to occur over 11 days; whilst individual elements of the leaf are likely to become determined in less time (four days or less) (Battey and Lyndon, 1988). In general the reversible behaviour associated with floral reversion suggests that meristems may have functionally significant flexibility in their commitment, allowing a switch from floral to vegetative development and vice versa (Tooke *et al.*, 2005). This contrasts with *Arabidopsis*, which once exposed to sufficient inductive LD is committed to the floral transition even if returned to SD (Torti *et al.*, 2012). It is interesting that in *Arabis alpina* the perennial habit is associated with unstable repression of *PEP1* (the orthologue of *A. thaliana FLC*), and a lack of responsiveness of very young meristems to vernalization (Wang *et al.*, 2009b). This means that all the shoot meristems are not uniformly and irreversibly committed to flowering by an inductive signal (vernalization), so that the plant retains the developmental

flexibility needed for the perennial life cycle. This example may be of particular relevance to the situation in *F. vesca*, where spatial/temporal variation in meristem sensitivity to inductive signals during autumn and winter is likely to be critical for the expression of both floral and vegetative characters in the spring.

The literature on determination in plants therefore suggests that it often, but not always, happens very early in development. Understanding its timing and progress in axillary structures in *F. vesca* is critical for understanding how the plant builds itself during the changing seasons of the year. As well as to floral/vegetative fate determination, this is also relevant to runners and branch crowns, which may be considered as alternative fates for axillary meristems.

4.1.2 The morphology and development of runners and branch crowns

The initial aim of the experiments described in this chapter was, therefore, to ask when determination of branch crowns and runners takes place; to do this it was necessary first to establish the conditions required to cause axillary buds to develop into these structures. There is a long history of study of runners and branch crowns. Guttridge (1955) stated that newly initiated axillary buds could be identified at one and a half to two plastochrons from the inception of the subtending leaf; while fate determination (to either a runner or branch crown) occurred during the formation of the first two internodes of the axillary structure. This corresponded with an earlier finding that photoperiodic induction of runners occurred during the first two plastochrons of axillary bud development, whilst emergence and subsequent elongation was not observed for a further four or more plastochrons (Guttridge, 1953). The delay between apparent determination and visual observation makes understanding the process of actual determination of these structures difficult, with studies typically focusing on the axillary structures once they become visible (for example, Hytönen *et al.*, 2009). For example Kurokura *et al.* (2005b) defined axillary buds larger than 10mm as branch crowns; this type of observation does not address timing of determination, the principal focus of this chapter. Although runners and branch crowns are similar in morphology, branch crowns differ from stolon buds due to the absence of elongated internodes (Guttridge, 1955), and are considered to develop to form axillary leaf rosettes (Koskela *et al.*, 2012), which are usually smaller than the primary crown (Costes *et al.*, 2014); while runners can be considered branch crowns situated on an elongated stem (Heide *et al.*, 2013).

4.1.3 Environmental, positional and hormonal regulation of runner and branch crown production (initiation and later development)

Previous research suggests that LD promote runner production, while under SD axillary buds develop into branch crowns or remain dormant (Hytönen *et al.,* 2004, 2009; Kurokura *et al.,* 2005b; Koskela *et al.,* 2016). Guttridge (1955) established that in young plants the majority of branch crowns are differentiated during the autumn, once stolon differentiation ceases and before dormancy. Kurokura *et al.* (2005b) confirmed that during this period, plants are naturally exposed to shortening daylength and decreasing temperature, the number of branch crowns increases, and runner production decreases. Savini *et al.* (2005) stated that branch crown production is observed from late-summer and occurs under conditions which cause reduced growth of the primary crown. In general, branch crown development is enhanced by environmental conditions favouring floral induction (Koskela *et al.,* 2016).

Correlative factors such as bud position within the main crown also appear to influence axillary bud fate, although it is likely that this may also be linked to environment. For example, young vegetative plants develop two to four dormant buds at the base of the main crown (Neri *et al.,* 2003; Savini *et al.,* 2005); those with only one leaf primordium apparently retain the potential to develop into either branch crowns or stolons, depending on the environmental conditions at the time of development (Kurokura *et al.,* 2005b). Neri *et al*. (2003) also speculated that dormant buds have the ability to form stolons or branch crowns, depending on node position, environmental conditions and state of apical dominance. Dormant basal buds were generally observed to develop to form branch crowns and stolon formation in these node positions was not considered likely (Neri *et al.,* 2003); axillary buds below the inflorescence have also been considered to have their fate predetermined, forming branch crowns irrespective of day-length (Kurokura *et al.,* 2005b). This implies that other factors must be responsible for the fate of these buds; Kurokura *et al.,* (2005b) suggested that the transition from the vegetative to the reproductive phase may be the cause of branch crown development in these buds. Flower induction has been reported to have a systemic effect, with plants under inductive conditions ceasing runner production, and differentiating branch crowns in the upper axillary buds (Guttridge, 1955). Guttridge (1985) reviewed the process of flowering in *F*. x *ananassa* and maintained that buds developed into branch crowns during or after flower induction, while stating that there may be variation as a result of cultivar or environmental conditions. This commitment of axillary buds beneath the inflorescence to form branch crowns and subsequently flower, implies some functional link between flowering and branch crown development. This view has also been stated in more recent literature with Kurokura *et al.* (2005a) suggesting that that the initiation of primary inflorescences affects the development of axillary buds just below the

inflorescence. Furthermore, it is not only the branch crowns in the upper axils that are influenced by flowering, as branch crowns throughout the main crown with more than three to four leaf primordia are generally observed to have inflorescence initials by the time the plants become dormant (Guttridge, 1955).

Savini *et al.* (2005) distinguished between branch crowns according to their position: those just below the inflorescence could be considered extension crowns, and to be different from those further down the main crown which should be considered secondary branch crowns. A distinction between upper buds (those immediately below inflorescence) buds and those lower down the crown, was also made by Costes *et al.* (2014) who suggested that extension axes can develop in the uppermost axillary buds below the terminal inflorescence or in the basal parts of the primary crown. However, they also stated that regardless of position, both forms of branch crowns have terminal flowering (Costes *et al.,* 2014), which could suggest that it is not possible to separate branch crowns from flowering. The question of whether there is a real distinction is important, as the term branch crown could be used to describe buds which were determined before or after development of flower initials; for example, Darrow (1966) stated that optimal photoperiodic conditions for branch crown formation were those too short for runners and yet too long for flower buds. Branch crowns have also been reported to be observed in the spring, but it was suggested that this was due to development of previously latent or dormant buds rather than the initiation of new branch crowns (Savini *et al.,* 2005). The first axillary bud to undergo differentiation in the spring has been stated to form a runner (Guttridge, 1955). The general conclusion is, therefore, that branch crowns are never purely vegetative, that they only form under conditions similar to those that promote flower induction, and that they will eventually produce flower initials.

As well as environmental control, hormone regulation influences axillary bud development, with gibberellin (GA) increasing runner production (Guttridge and Thompson, 1964). Hytönen *et al.,* (2009) confirmed earlier reports of the runner promoting influence of GA, and further established the involvement of GA in regulation of axillary bud differentiation into branch crowns or runners. The presence of GA was observed to promote runnering, whilst the application of a GA inhibitor, prohexadione-calcium (Pro-Ca) inhibited runner initiation from newly developing axillary buds after one-two days (Hytönen *et al.,* 2009) and, like SD promoted branch crown formation. These authors also showed that a 14h photoperiod provided more optimal conditions for branch crown production than 10h (Hytönen *et al.,* 2009).

4.1.4 Aims and objectives of the experiments

Observations of *F. vesca* during its annual cycle (see Chapter 3) indicated that in general the behaviour of axillary buds differed depending on position in the main crown axis: arrested buds, with the potential to develop into branch crowns later in the annual cycle were at the base of the main crown, with between one and three leaf primordia; while branch crowns tended to be in the central region, generally containing between three and five leaf primordia, but during active runnering often remained latent. Runners typically occupied central and upper positions on the main crown. The mechanisms that bring about this general pattern are not clearly understood, notwithstanding the known importance of environment, particularly photoperiod and temperature (see earlier and also Chapters 1 and 3). The aim of the first experiment (AXB1) described in this Chapter was therefore to identify the impact of environment on the determination of axillary bud structures, in particular the influence of photoperiod. A description of the time taken for an axillary bud to be determined to have an observable character, as either a branch crown or runner, was envisaged, using photoperiod transfers to establish when determination as runner or branch crown was complete. Two alternative hypotheses were considered: that determination of axillary bud character occurred in response to sufficient time from the moment of inception under inducing conditions (e.g. a week under LD at high temperature); or that there might be a specific window during development, and the conditions during this window directly influenced character determination, regardless of previous or subsequent conditions. The experiment used successive weekly transfers between photoperiod conditions (LD to SD and SD to LD), to establish how this signal influenced the character of the developing axillary buds. Axillary buds in nodes which had developed sufficiently to be classified as runner or branch crown at the time of transfer were observed at the end of the experiment to establish whether transfer could change the character of these buds. For axillary buds in nodes which were not observable, or whose identity was not clear at the time of transfer, the transfers could potentially indicate the time over which photoperiod determined axillary bud development. It was generally assumed that SD conditions would promote the determination of branch crowns, although the time under SD or the window of axillary development that would be vulnerable to conditions was unknown. Equally, LD conditions were assumed to promote runnering, with the necessary time/stage of development unknown.

The second experiment (AXB2) was designed with similar objectives to AXB1, and therefore had a comparable design. The results from the AXB1 experiment did not show the anticipated difference in axillary bud determination between photoperiod treatments, with runners determined in newly emerged and developed axillary buds regardless of photoperiod and transfer. It was hypothesised that the relatively high temperatures during the experiment might have

limited the interaction of photoperiod on axillary bud determination and therefore promoted runner determination regardless of photoperiod. The main focus of AXB2 was therefore to repeat the first experiment at a lower temperature (18°C) to establish whether photoperiod interacts with temperature during axillary bud determination.

The objective of the third experiment (AXB3) differed from that of the two previous experiments; it was designed specifically to establish conditions suitable for branch crown development and whether this could occur independently of flower initiation in newly developing axillary buds. The results from the first two experiments suggested that low temperature might be required for branch crown determination, as temperatures of 18°C and above did not promote branch crowns either in LD or SD. Plants in AXB3 were therefore exposed to low temperature (11°C) and SD (10h) conditions, which have been reported to promote inflorescence initiation, as well as branch crown development (Sønsteby and Heide, 2006). The presumptive difference between determination as branch crown or inflorescence was hypothesised to be the duration of the cool/SD treatment. Two cohorts of plants were included in this experiment, which differed in their duration of exposure to 11°C/SD, but both were exposed for less time than that deduced to be necessary for optimal floral induction. All cohorts were transferred to 11°C/SD at the same time; the first cohort was transferred to forcing conditions (> 18°C/LD) after two weeks and the second after four weeks.

The final experiment (AXB4) in this Chapter built upon the results of the first two experiments (AXB1 and AXB2), in which plants showed limited terminal growth but very active growth of axillary buds. It was not clear from these experiments whether this active axillary growth was a cause or consequence of the limited terminal growth. AXB4 was therefore designed to determine the influence of runner removal on terminal growth. Two cohorts of plants were grown under favourable conditions for vegetative growth (20°C, LD), with weekly runner removal of one cohort. It was hypothesised that terminal growth would be greater as a result of runner removal; if this proved correct, it might open the way for future experiments on axillary bud determination. It would also raise important questions about the influence of experimental procedure (± runner removal) on overall plant growth and development in *Fragaria*.

4.2 Materials and methods

The *F. vesca* ecotype used throughout this Chapter was originally collected from Park Wood, Mapledurham, and is otherwise referred to UK9. All the plants included within this Chapter were vegetatively propagated from runners.

4.2.1 Axillary bud experiment 1 (AXB1) – axillary bud determination at >20°C

Growth conditions and experimental design

Plants were grown in a multi-factorial glasshouse compartment. LD were provided by exposing plants to natural light only from 0630-2000 with supplementary photosynthetic light between 0500-0630 and 2000-2300 (OSRAM SON-T 400W high pressure sodium lamps; 150 μmol m^{-2} sec^{-1}), giving total day length of 18h. Both SD and LD treatments were exposed to natural light from 0800-1800 (400 ± 23.3 μmol m^{-2} sec^{-1}, average light integral based on five separate, evenly spaced measurements at plant height at midday early June 2016); this was the only daylight received by the SD plants, giving a total day length of 10h. SD plants were moved into blackout cupboards from 1800-0800.

Temperature conditions were monitored using data loggers (TinyTag Extra TGX-3020, Gemini Data Loggers) (Figure 4.1, Table 4.1). Separate loggers were used for plants under SD and LD conditions, with hourly temperatures used to calculate average day temperature from 0800-1800 and average night temperature from 1800-0800, for both LD and SD plants. The data logger maintained under LD conditions was situated above the LD plants in the multifactorial glasshouse compartment throughout the experiment. The other data logger was maintained under SD conditions and situated above the SD plants in the glasshouse during daylight hours (0800-1800), and moved into the blackout cupboards during night hours (1800-0800) along with the plants.

The experiment ran for six weeks, with control plants maintained under constant LD or SD; in other treatments plants were exposed to LD followed by SD, or SD followed by LD, with transfers undertaken at weekly intervals during the experiment (days 7, 14, 21, 28, 35 and 42).

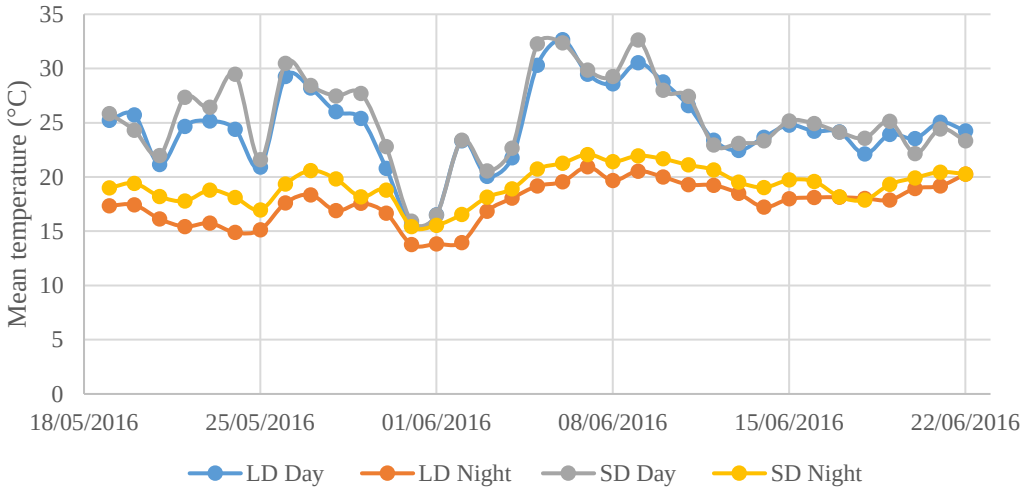


Figure 4.1 Mean diurnal temperature for plants under LD and SD conditions in the multi-factorial compartment

Table 4.1 Weekly and overall mean diurnal temperature (and standard error) under LD and SD conditions in the multi-factorial glasshouse compartment

	Temperature (°C)			
	LD		SD	
Week	Day	Night	Day	Night
2	23.9	16.0	25.3	18.3
3	23.1	16.4	24.2	18.2
4	26.6	18.3	27.2	19.9
5	25.7	19.0	26.1	20.5
6	23.9	18.6	24.0	19.4
Mean	24.6	17.7	25.3	19.3
± s.e.	0.7	0.6	0.6	0.4

Data recording

Weekly data recording was undertaken for plants maintained under constant LD or SD as follows:

By visual observations

- Crown diameter
- Petiole length of the youngest open, emerged leaf in the main crown
- Total number of open leaves

- Total number of emerged leaves
- Number of emerged runners
- Number of observable branch crowns.

Through dissections

- Total number of leaves and leaf primordia in the main crown and branch crowns
- Character of axillary buds in each leaf node – runner, branch crown, arrested or undiscernible
- For branch crowns: whether or not the bud had emerged, the number of leaves, axillary buds and character
- For runners: in week 0, whether or not the bud had emerged; in weeks 1-5, state of emergence and number of established daughter plants along the runner; in week 6, state of emergence, number of internodes and established daughter plants along the runner (see Figure 4.2 for runner internode and daughter plant definition), and length of runner.

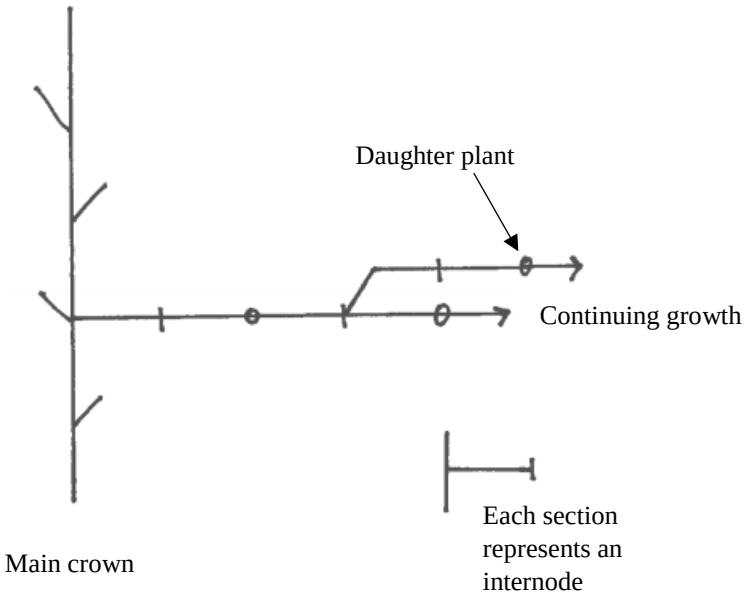


Figure 4.2 Typical runner structure, with internodes separating developing daughter plants; this runner shows three daughter plants, eight internodes and two growing points

Data analysis

When recording axillary bud character, the number of emerged and unemerged axillary buds were combined to calculate the total number of either runners or branch crowns per plant. For runners,

only the runners in the main crown were included; some plants had branch crowns which also showed emerged runners, but these were not counted when calculating total runner number for the main crown.

Similarly to runners, branch crowns were recorded by visual observation of emerged crowns and through dissection of unemerged branch crowns. The total number of branch crowns was summed as the number of branch crowns on the main crown, including the main crown. For example, if the total branch crown number for a plant was recorded as three, then this was a plant which contained two branch crowns on the main crown.

The number of nodes in the main crown was calculated by summing the number of senescent leaves, for which the axillary bud were still present, the emerged leaves and unemerged leaf primordia. Node positions were numbered from the base of the main crown upwards.

To enable comparison between transfers and conditions, individual plant data were used to calculate the modal character of each axillary structure at each leaf node of the control plants and those from each of the transfer treatments. Five plants were transferred and recorded at each dissection. However, for some weeks and transfers there were fewer plants dissected, due principally to plant death; there were no fewer than four plants recorded except on two occasions (three plants for LD7-SD35 and two plants for LD21-SD21). The number of plants dissected at each interval and for each treatment are shown in the Results section (Figures 4.4 and 4.6). Data were tested for normality using the Ryan-Joiner test and shown to be normally distribution. The number of total nodes per plant and the effect of photoperiod were analysed over the sampling period (time as a fixed factor) using a two-way ANOVA in Minitab 17. The response of runner growth was analysed using the same methodology with photoperiod and sampling time as factors.

4.2.2 Axillary bud experiment 2 (AXB2) – axillary bud determination at 18°C

Growth conditions and experimental design

Plants were transferred into controlled environments (Sanyo cabinets, Sanyo Gallenkamp, Leicester) at the start of the experiment (13/07/2016). The overall experimental design was similar to that in AXB1, with two contrasting photoperiod treatments, although plants were exposed to artificial light rather than natural light or a combination of natural and supplementary lighting. SD plants were exposed to a combination of tungsten and fluorescent light from 0800-

1800 (160 μmol m^{-2} sec^{-1}); LD plants were also exposed to this combination from 0800-1800; but, between 0500-0800 and 1800-2300 they additionally received non-photosynthetic light (tungsten only) (3 μmol m^{-2} sec^{-1}). Constant temperature conditions were maintained throughout the experiment, with an average day/night temperature of 18°C.

Transfers were undertaken at intervals during the experiment (days 9, 19, 28, 37, 47 and 55); at each transfer time, a subset of plants was moved from LD to SD and vice versa. Cohorts of control plants were maintained under constant LD or SD.

Data recording

Crown diameter, petiole length (of the youngest open, emerged leaf in the main crown) and the number of emerged leaves, runners and branch crowns were recorded at the beginning of the experiment and at each transfer. Dissections were also carried out and the following parameters recorded:

- Total number of nodes in the main and branch crowns; this included nodes which had emerged leaves, senescent/absent leaves and leaf primordia
- Character of axillary buds in each node – runner, branch crown, arrested or undiscernible
- For branch crowns: whether or not the bud had emerged, the number of leaves, presence of an axillary bud and its character
- For runners: for those on the main crown – state of emergence, number of runner internodes, number of daughter plants along the runner and total length of the runner; for runners within branch crowns, presence and state of emergence. When calculating total runners per plant, only those on the main crown were included.

At the start of the experiment visual observations were made on five plants, which were then dissected. At each time of transfer, visual observations were made on 10 plants from LD and 10 plants from SD; five of these plants were transferred to the alternate photoperiod conditions (LD plants transferred into SD and vice versa), and the remaining five plants were dissected. At the end of the experiment (day 55), visual observations and dissections were carried out for all transferred plants and the remaining control plants.

Data analysis

When analysing total node number and axillary bud character, dissection data for each of the dissected plants (per cohort) were combined and the modal character of the axillary structure at each node was calculated; in instances where modal character could not be simply calculated, the character chosen was that which best represented the general character at this node position. For example, axillary buds at the base of the plant were most commonly either branch crowns or arrested buds, in cases where modal character in these bud positions could not be simply calculated between a runner and branch crown for instance, a branch crown was chosen as the character as it best represented the likely modal character of a wider sample of plants. For some dissections and transfers fewer than five plants but never less than three plants were dissected, due to plant death during the experiment. The number of plants dissected at each interval and for each treatment are shown in the Results (Figures 4.7 and 4.10). Data were tested for normality using the Ryan-Joiner test and shown to be normally distribution. The number of total nodes per plant and the effect of photoperiod were analysed over the sampling period (time as a fixed factor)using a two-way ANOVA in Minitab 17. The responses of runner and branch crown growth were analysed using the same methodology with photoperiod and sampling time as factors.

4.2.3 Axillary bud experiment 3 (AXB3) – axillary bud determination at 11°C

Growth conditions and experimental design

Prior to the experiment, plants were maintained under glass (heated multi-factorial compartment) (>18°C/18h) to ensure they remained in a vegetative state. At the start of the experiment (17/10/2016), plants were transferred into a controlled environment (Sanyo cabinets, Sanyo Gallenkamp, Leicester) and exposed to SD (10h) and cool temperature (11°C). Lighting was provided artificially using a combination of tungsten and fluorescent lights from 0800-1800 (160 μmol m^{-2} sec^{-1}) and an average day/night temperature of 11°C was maintained. The plants were split into two cohorts; the first cohort was maintained under these conditions for two weeks and the second cohort for four weeks. Plants were then transferred to forcing conditions in a multi-factorial greenhouse, with LD (18h) provided by natural daylight with supplementary illumination from 0500-0800 and 1600-2300 from four evenly spaced high-pressure sodium lamps (OSRAM SON-T 400W; 150 μmol m^{-2} sec^{-1}). Temperature remained above 18°C at all times (Figure 4.3).

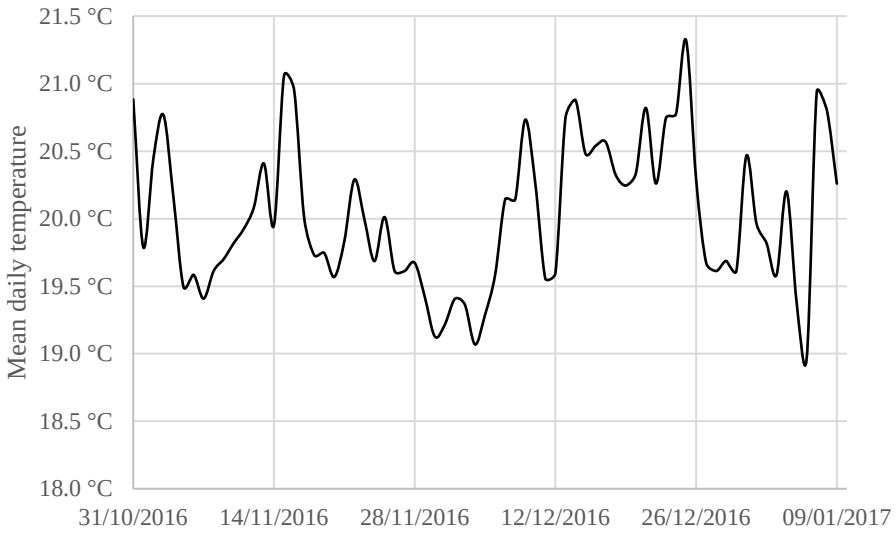


Figure 4.3 Mean daily temperature for plants under forcing conditions (LD) in the multi-factorial compartment

Data recording

Five plants were dissected at the start of the experiment, at the time of transfer of each cohort to the forcing environment, and every four weeks after this; the following parameters were recorded:

- Total number of nodes in the main and branch crowns; this included nodes which had emerged leaves, senescent/absent leaves and leaf primordia
- Character of axillary bud in each node – runner, branch crown, arrested or undiscernible
- For branch crowns: whether or not the bud had emerged and whether the branch crown was vegetative or reproductive.

Additional plants were included in this experiment to account for possible plant death, which was commonly observed in experiments throughout this thesis. Twenty plants were transferred to forcing for each cohort, although only 10 were dissected.

Data analysis

The same method of data analysis was employed as in the previous two experiments (AXB1 and AXB2); when analysing total node number and axillary bud character, dissection data for each of the dissected plants (per cohort) were combined and the modal character of the axillary structure

at each node was calculated. In instances where modal character could not be simply calculated, the character chosen was that which best represented the general character at this node position. Data were tested for normality using the Ryan-Joiner test and shown to be normally distribution. The number of total nodes per plant and the effect of photoperiod were analysed over the sampling period (time as a fixed factor) using a two-way ANOVA in Minitab 17.

4.2.4 Axillary bud experiment 4 (AXB4) – influence of runners on SAM activity

Growth conditions and experimental design

Prior to the experiment, plants were maintained under glass (heated multi-factorial compartment) (>18°C/18h) to ensure they remained vegetative. At the start of the experiment, they were transferred into controlled environment cabinets (Sanyo Gallenkamp, Leicester) and exposed to LD (18h) at an average day/night temperature of 20°C. Lighting was provided by a combination of tungsten and fluorescent lights from 0500-2300 (160 μmol m^{-2} sec^{-1}). There were two treatments: in the first, plants grew as normal under experimental conditions. Plants in the second treatment had all their emerged runners removed at the start of the experiment and every week for the duration of the experiment (10 weeks).

Forty plants were equally split across the two treatments, to allow for dissections and to ensure a sufficient number of plants in the case of plant death. Plants were carefully selected at the start of the experiment, to ensure similarity in plant size; only plants with a single rooted crown were selected.

Data recording

For plants in the runner removal treatment, all emerged runners were removed at the start of the experiment and by hand every week, and the number of runners recorded.

Five plants were dissected at the start and at the end of the experiment to establish the typical state of growth and development prior to and after exposure to experimental conditions. The following parameters were recorded:

- Total number of nodes in the main crown; this included nodes which had emerged leaves, senescent/absent leaves and leaf primordia

- Character of axillary bud in each node – runner, branch crown, arrested or undiscernible.

At the end of the experiment, the plants which had had their runners removed had a number of leaf axils that appeared to have a missing axillary buds. In these instances, these nodes were assumed to have had emerged runners, which had been removed during the course of the experiment, and a runner was recorded as the axillary bud character, even though it was not present.

Data analysis

In order to analyse and interpret dissection data (total node number and axillary bud character), data for each of the dissected plants (per treatment) were combined and the modal character of the axillary structure at each node was calculated. In instances where modal character could not be simply calculated, the character chosen was that which best represented the general character at this node position. Data were tested for normality using the Ryan-Joiner test and shown to be normally distribution. The number of total nodes per plant and the effect of photoperiod were analysed over the sampling period (time as a fixed factor) using a two-way ANOVA in Minitab 17.

4.3 Results

4.3.1 Axillary bud experiment 1 (AXB1) – axillary bud determination at >20°C

Node groupings

See Chapter 3, Figure 3.9 for diagrammatic and descriptive summary of plant general architecture.

Baseline data

A subset of plants was maintained under constant SD or LD conditions to establish how photoperiod interacted with temperature to influence axillary bud determination and development particularly that of newly emerging buds. Plants had a total of 9.6 ± 0.7 nodes at the start of the experiment, which increased to 17.5 ± 0.3 nodes for plants maintained under constant LD and 15.8 ± 0.7 nodes under SD conditions (Figure 4.4 and 4.5). The rate of node initiation and emergence was therefore approximately 1 per week in both LD and SD (Figure 4.5). The ordering of node groupings indicated in Figure 3.9 was maintained over the course of the experiment. The most noticeable change was that node group 2 expanded in size (from three to 10) and contained primarily runners in both photoperiod treatments (Figure 4.4). Photoperiod conditions showed differences in average temperature, with plants under SD conditions receiving slightly higher average day and night temperature (approximately 1°C warmer), than plants under LD (Table 4.1); but this temperature difference not did influence leaf initiation rate.

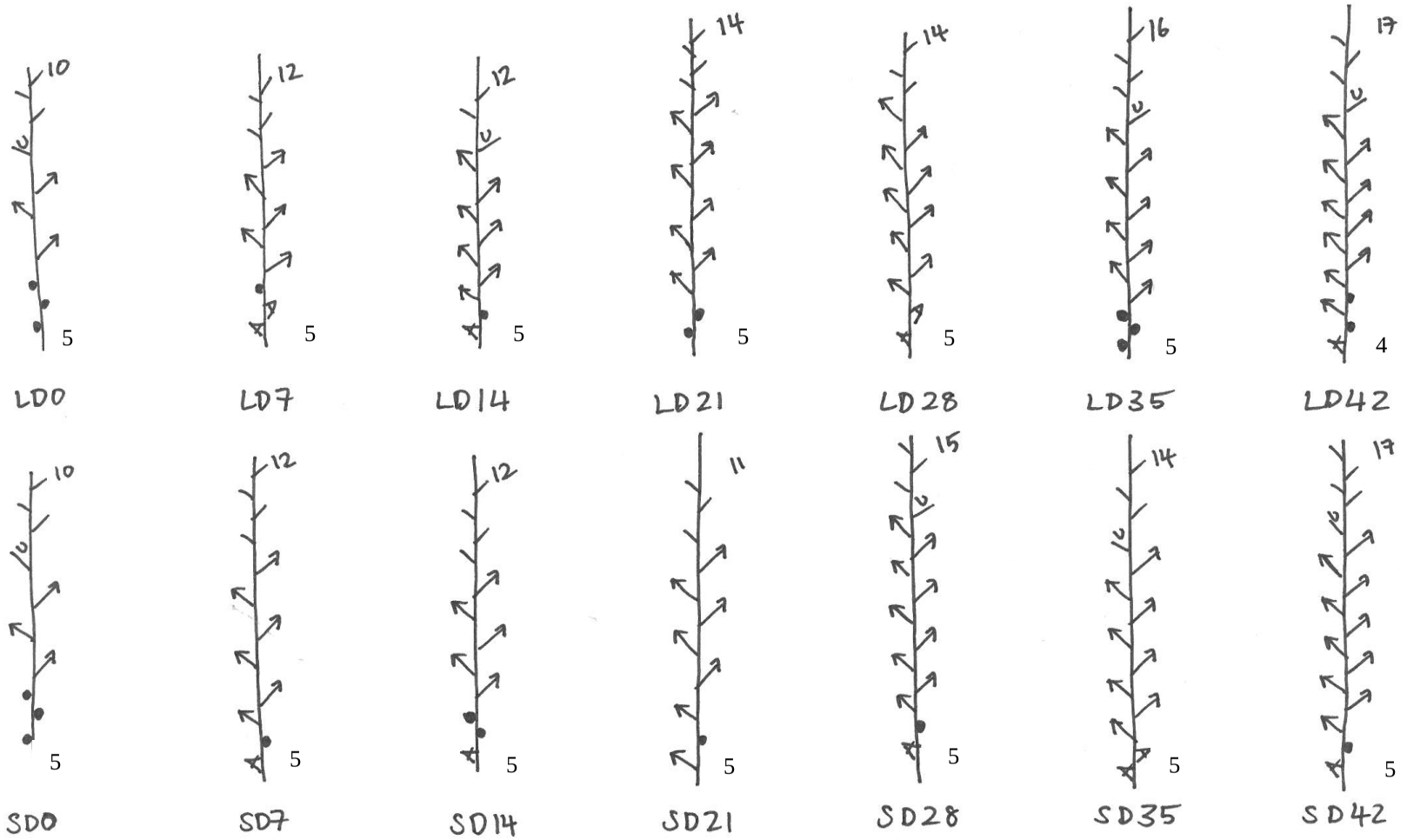


Figure 4.4 Developmental character of control plants maintained under constant LD or SD conditions throughout the experiment (days into the experiment are shown under each diagram e.g. LD7 – seven days under LD). Drawings show the modal fate of individual buds and overall plant structure based on weekly dissections. The number of plants dissected at each interval is shown in the bottom right hand corner of each drawing, and the modal total node number at the top right hand corner

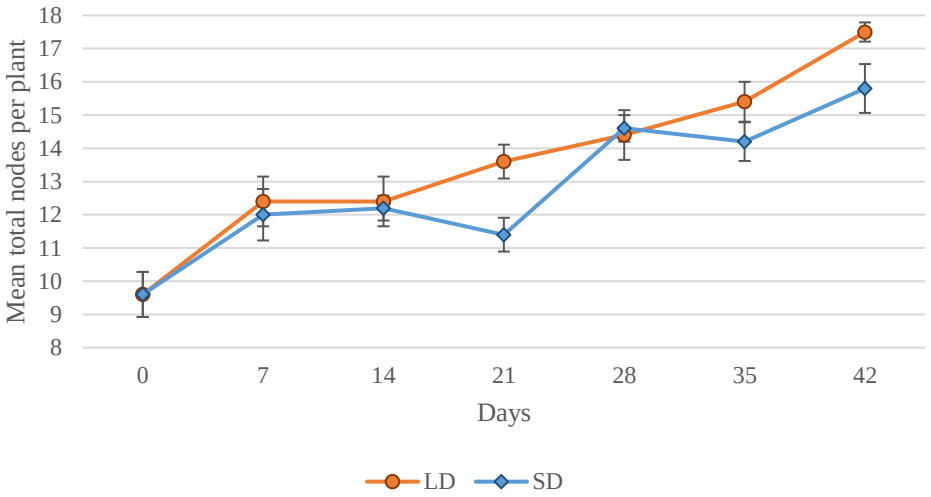


Figure 4.5 Mean total nodes on the main crown per plant for control plants maintained under LD or SD

As well as the effect of experimental conditions on terminal growth, the influence of constant photoperiod on lateral growth was also analysed. Runner development, measured by the number of internodes in each runner, was recorded for plants at the end of the experiment (day 42) and showed significant variation (see Figure 4.2 for runner internode definition). For example, plants under constant LD had 52.0 ±17.0 runner internodes at node 3 (on the main crown) at the end of the experiment (day 42), in comparison to 21.3 ± 6.4 internodes for plants under SD. Photoperiod did not have a significant effect on the number of runners internodes at node 3 at the end of the experiment ($F_5 = 2.85$; $p = 0.17$). A significant difference was however shown between constant photoperiod treatments at node 5 (at the end of the experiment); LD plants had 23.8 ± 6.0 runner internodes, whilst SD plants had 7.0 ± 1.8 internodes ($F_8 = 8.82$; $p = 0.02$). These results imply that in these experimental conditions runner growth and development was very active under both photoperiods, but significantly more under LD.

Transfer data

The similarity in main crown structure between plants under constant LD or SD conditions over the course of the experiment was echoed in the transfer data. Plants were transferred from SD to LD and vice versa every week, and dissected at the end of the experiment (day 42) (Figure 4.6). There were always arrested and/or branch crowns in group 1, and runners in group 2, with no branch crowns at the top of this group towards the apical meristem (Figure 4.6). There was some variation, with plants transferred to SD, especially early in the experiment (LD7-SD35, LD14-SD28 and LD21-SD21) showing a lower total number of nodes at the end of the experiment compared to LD-only plants, but plants grown in constant SD did not show this difference (Figure 4.5 and 4.6).

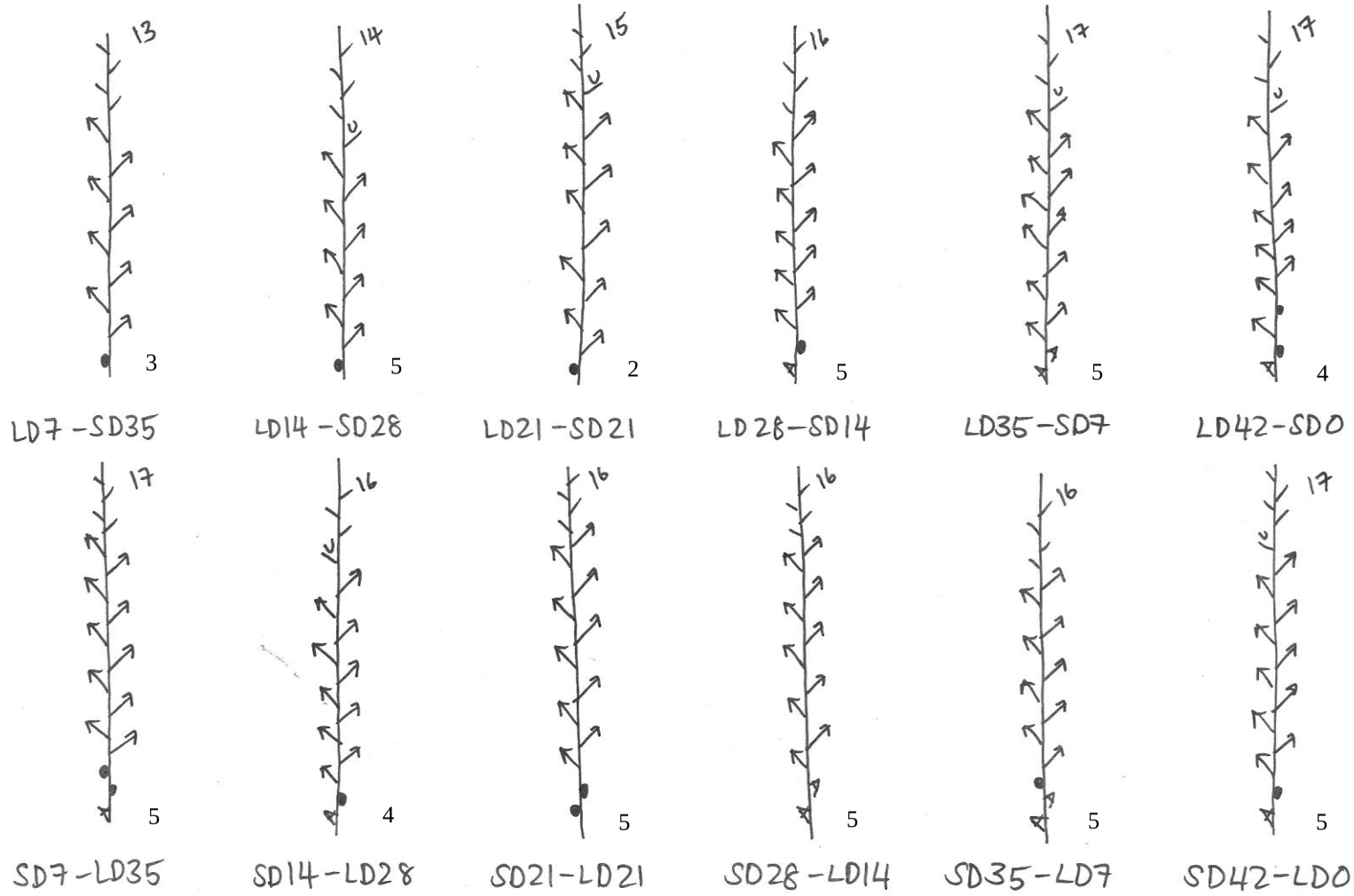


Figure 4.6 Developmental character of plants transferred either LD to SD or SD to LD, as indicated under each diagram. The number of days in each photoperiod treatment is shown (e.g. LD7–SD35 – seven days under LD then transferred to SD for the rest of the experiment (35 days)). Drawings show the modal fate of individual buds and overall plant structure based on weekly dissections. The number of plants dissected at each interval is shown in the bottom right hand corner of each drawing, and the modal total node number at the top right hand corner

4.3.2 Axillary bud experiment 2 (AXB2) – axillary bud determination at 18°C

Node groupings

These were as described for axillary bud experiment 1 (see Chapter 3, Figure 3.9).

Baseline data

As in axillary bud experiment 1, a subset of control plants was maintained under both constant photoperiods (either LD or SD) for the duration of the experiment. Plants were dissected at the start of the experiment (LD0 and SD0) and had 12.8 ± 0.2 nodes: typically there were four nodes in group 1 with arrested buds at the very base of the plant and above this branch crowns, followed by five runners in node group 2 (above) and four nodes in group 3 (the upper buds), where character was undiscernible (Figure 4.7). Subsequent dissections showed that the order of node groups was maintained for the majority of plants; there were, however, some exceptions. One anomaly was the absence of node group 1, with runners recorded from the base of the plant (SD28), whilst in other cases branch crowns interrupted node group 2 (runners) (SD9 and SD37) (Figure 4.7).

By the end of the experiment (day 55), plants under LD had 13.3 ± 1.0 nodes, whilst those under SD had 14.6 ± 0.2 (Figure 4.7). This indicates a key feature of this experiment: growth at the SAM was very limited. Statistical analysis revealed that the difference in mean total node number (leaves plus primordia) during the course of the experiment differed significantly according to photoperiod ($F_1 = 5.04$; $p = 0.03$), but time of dissection did not show a significant difference ($F_6 = 1.90$; $p = 0.10$). As a result the interaction of photoperiod and time of dissection did not significantly affect total node number ($F_6 = 2.02$; $p = 0.08$), which implied that photoperiod did not significantly influence total node number and growth over the course of the experiment.

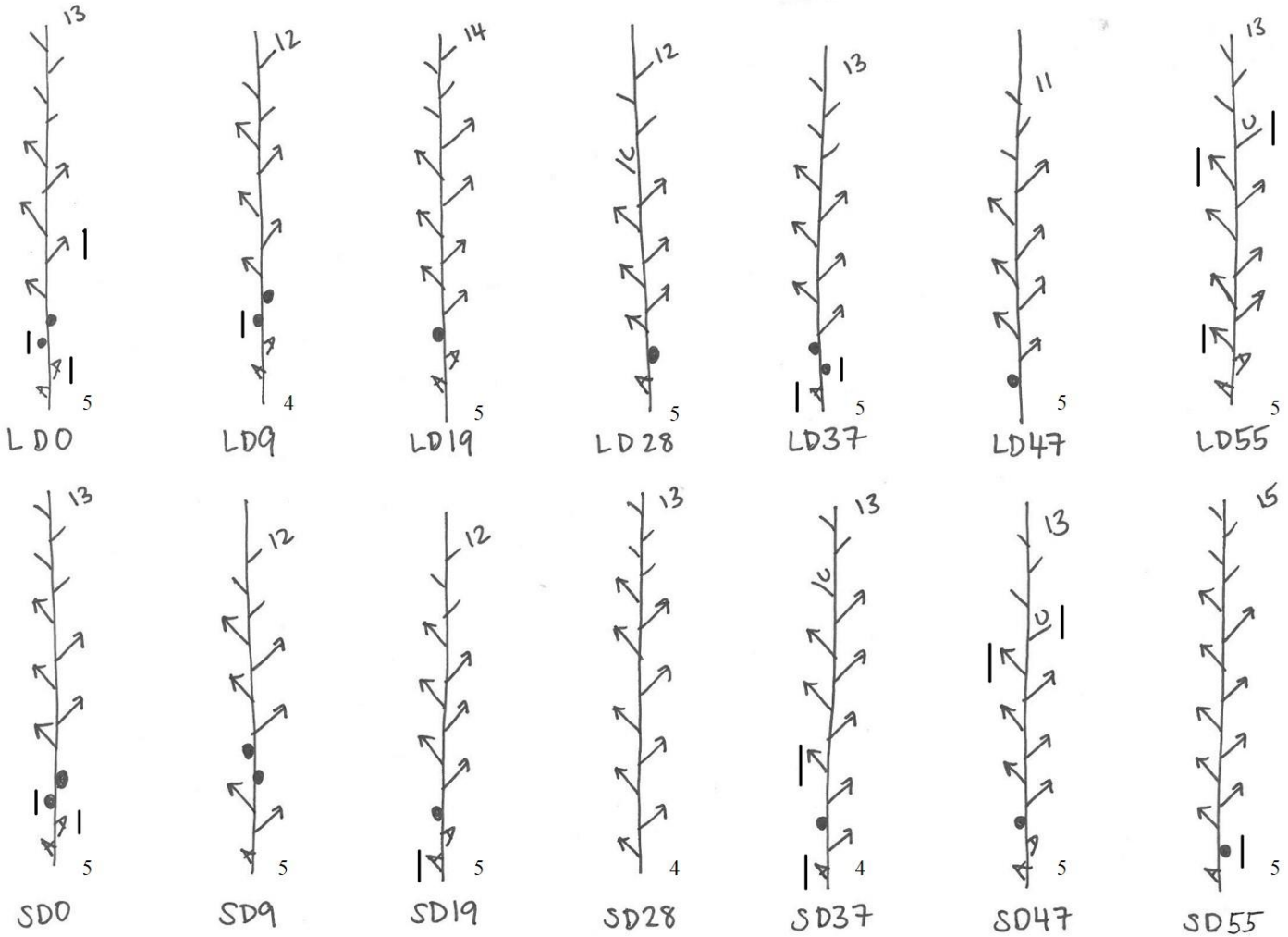


Figure 4.7 Developmental character of axillary buds of control plants maintained under constant LD or SD and dissected at intervals (e.g. LD9 – after nine days in LD). Drawings show the modal character of individual buds; in instances where modal character could not be simply calculated (as highlighted), the character chosen was that which best represented the general character at this node position. The number of plants dissected at each interval is shown in the bottom right hand corner of each drawing, and the modal total node number at the top right hand corner.

Growth of the SAM (regardless of photoperiod) was very slow but did not reflect stasis over the whole plant. The number of internodes and daughter plants on each runner (see Figure 4.2 for definitions) were recorded as indicators of runner development and lateral growth, which was extremely active (Figure 4.8); for example, the runner at node 3 had 14.0 ± 1.0 and 19.3 ± 2.2 internodes under constant LD and SD conditions respectively, by the end of the experiment (day 55). In contrast to AXB1, constant photoperiod did not significantly influence internode number at this node ($F_1 = 3.34$; $p = 0.17$). The effect of photoperiod on runner internode development was also analysed at node 5 and also shown to be non-significant ($F_1 = 0.34$; $p = 0.59$), with 11.8 ± 5.4 internodes under LD and 7.0 ± 1.0 under SD. The number of total runner internodes per plant for those grown under constant photoperiod differed significantly between the beginning and the end of the experiment ($F_6 = 3.42$; $p = 0.00$), but photoperiod did not significantly influence internode. The pronounced development of runners during the experiment suggested that, if runners are maintained on the plant, they have a dominant effect on growth.

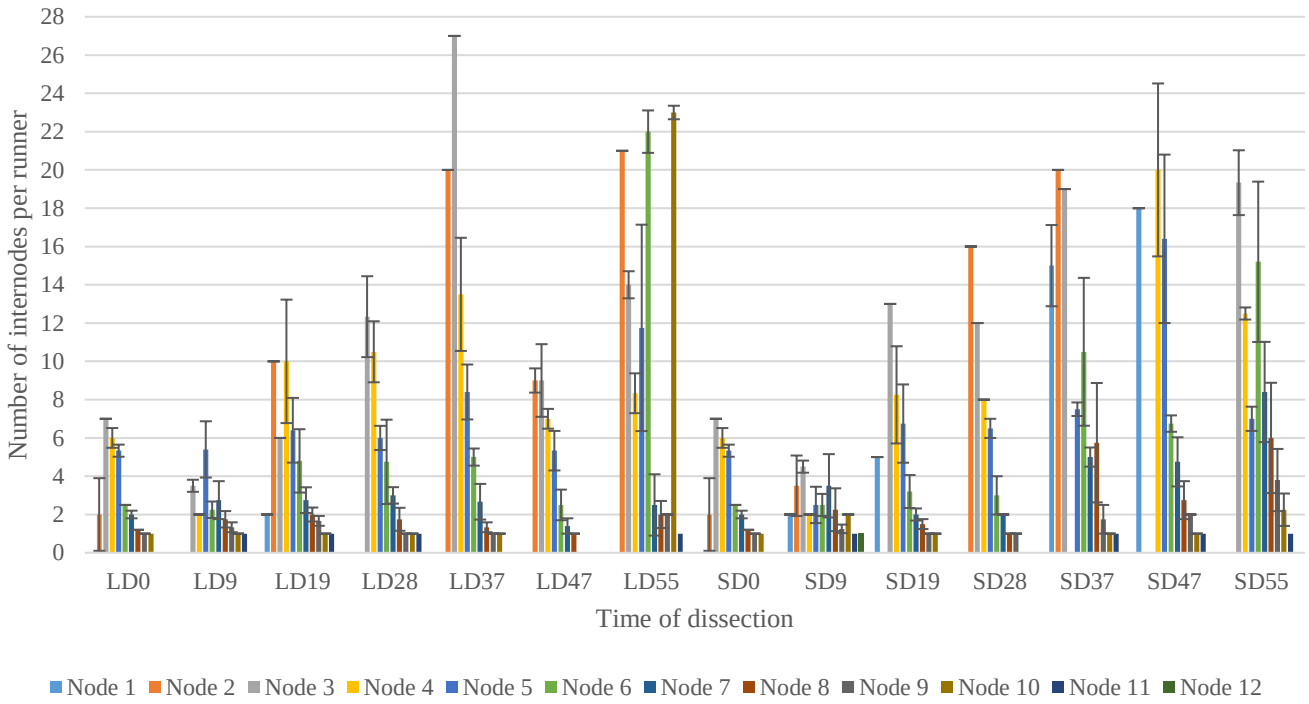


Figure 4.8 Mean number of runner internodes (see Figure 4.2 for internode definition) at each node on the main crown, in instances where a runner was present at this node at time of dissection, in control plants maintained under constant LD or SD conditions. Standard error of the mean are shown

Lateral growth in the form of branch crown development was also observed throughout the experiment, measured by the number of leaves and primordium within branch crowns. Dissections showed that only one axillary bud, and therefore only one lateral structure, is formed in the leaf axils in *F. vesca*, so the average number of branch crowns per node could not be greater

than one. The dissections also showed that branch crowns were predominantly present in the basal nodes on the main crown, so these nodes (1-4) were the main focus of this analysis (Table 4.2). The number of branch crowns present at these basal nodes did not differ significantly between nodes ($F_3 = 1.07$; $p = 0.37$), photoperiod ($F_1 = 0.21$; $p = 0.65$) or with time ($F_6 = 0.67$; $p = 0.67$). This indicates that the number of branch crowns did not change as a result of environmental conditions over the course of the experiment.

Table 4.2 Mean number of branch crowns (± standard error) at nodes 1-4 on the main crown for plants under constant photoperiod treatment (LD or SD) at dissection intervals

	Node 1	Node 2	Node 3	Node 4
LD0	0	0.20 ± 0.20	0.40 ± 0.24	0.60 ± 0.24
SD0	0	0.20 ± 0.20	0.40 ± 0.24	0.60 ± 0.24
LD9	0.20 ± 0.20	0	0.40 ± 0.24	0.80 ± 0.20
SD9	0	0	0	0.40 ± 0.24
LD19	0	0	0.60 ± 0.24	0.20 ± 0.20
SD19	0.20 ± 0.20	0.40 ± 0.24	0.80 ± 0.20	0.20 ± 0.20
LD28	0.40 ± 0.24	0.80 ± 0.20	0.40 ± 0.24	0
SD28	0.25 ± 0.25	0.25 ± 0.25	0	0.25 ± 0.25
LD37	0.40 ± 0.24	0.40 ± 0.24	0.60 ± 0.24	0.20 ± 0.20
SD37	0	0	0.50 ± 0.29	0.50 ± 0.29
LD47	0.80 ± 0.20	0.20 ± 0.20	0	0
SD47	0.20 ± 0.20	0.40 ± 0.24	0.80 ± 0.20	0
LD55	0	0.25 ± 0.25	0	0
SD55	0	0.40 ± 0.24	0	0.20 ± 0.20

Branch crown development at individual nodes on the main crown was also quantified as the total number of leaves and primordia within the branch crowns. Again, only the basal nodes were considered; even though typically node 1 and more often than not node 2 were arrested buds (latent branch crowns), they were still included within this analysis in order to assess branch crown development (Figure 4.9). Visual inspection suggested that at day 55 the information on branch development was anomalous because it suggested a decrease in branch crown development; whereas at all other time points there was no consistent trend in development. For this reason, these data were removed from analysis and comparison of branch development was undertaken between day 0 and day 47. The effect of photoperiod ($F_1 = 0.24$; $p = 0.63$), time of dissection ($F_5 = 0.31$; $p = 0.90$) and node position ($F_3 = 0.57$; $p = 0.64$) were not shown to significantly influence the number of leaves and primordia within branch crowns at basal nodes.

This indicates that branch crowns did not develop significantly over the course of the experiment, which suggests that the conditions used in the experiment were not promotive of branch crown development.

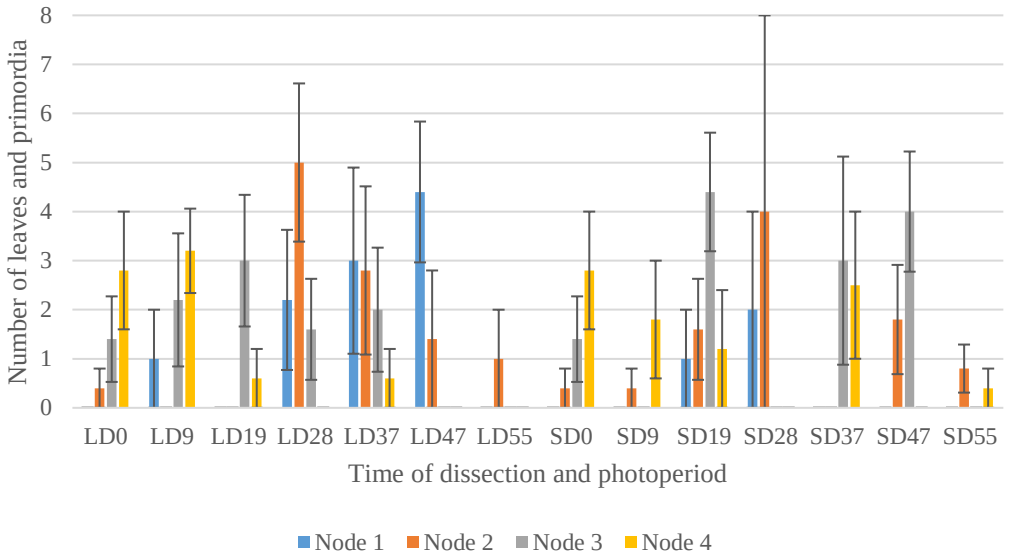


Figure 4.9 Mean number of leaves and primordia within the branch crowns at nodes 1-4 on the main crown. Standard error of the mean are shown

Transfer data

Plants were transferred approximately every nine days from LD to SD conditions and vice versa, and maintained under transferred conditions until the end of the experiment when they were dissected. As with the plants maintained under constant photoperiod, the structure of node groupings was maintained regardless of transfer conditions (Figure 4.10). The lack of growth of the terminal SAM in constant photoperiod was also found in the transfer treatments, with no significant difference in total node number between transfers (F_{11} = 1.67; p = 0.12). This slow growth meant that any information on newly initiated nodes was confined to nodes 10 and 11, which would have just been initiated at the beginning of the experiment. Placing the diagrams in Figures 4.7 and 4.10 alongside each other (Figure 4.11) demonstrates that photoperiod did not cause a consistent change in the fate of existing structures (nodes 1-9) or of those whose character was not discernible on day 0 but was by day 55 (nodes 10-11).

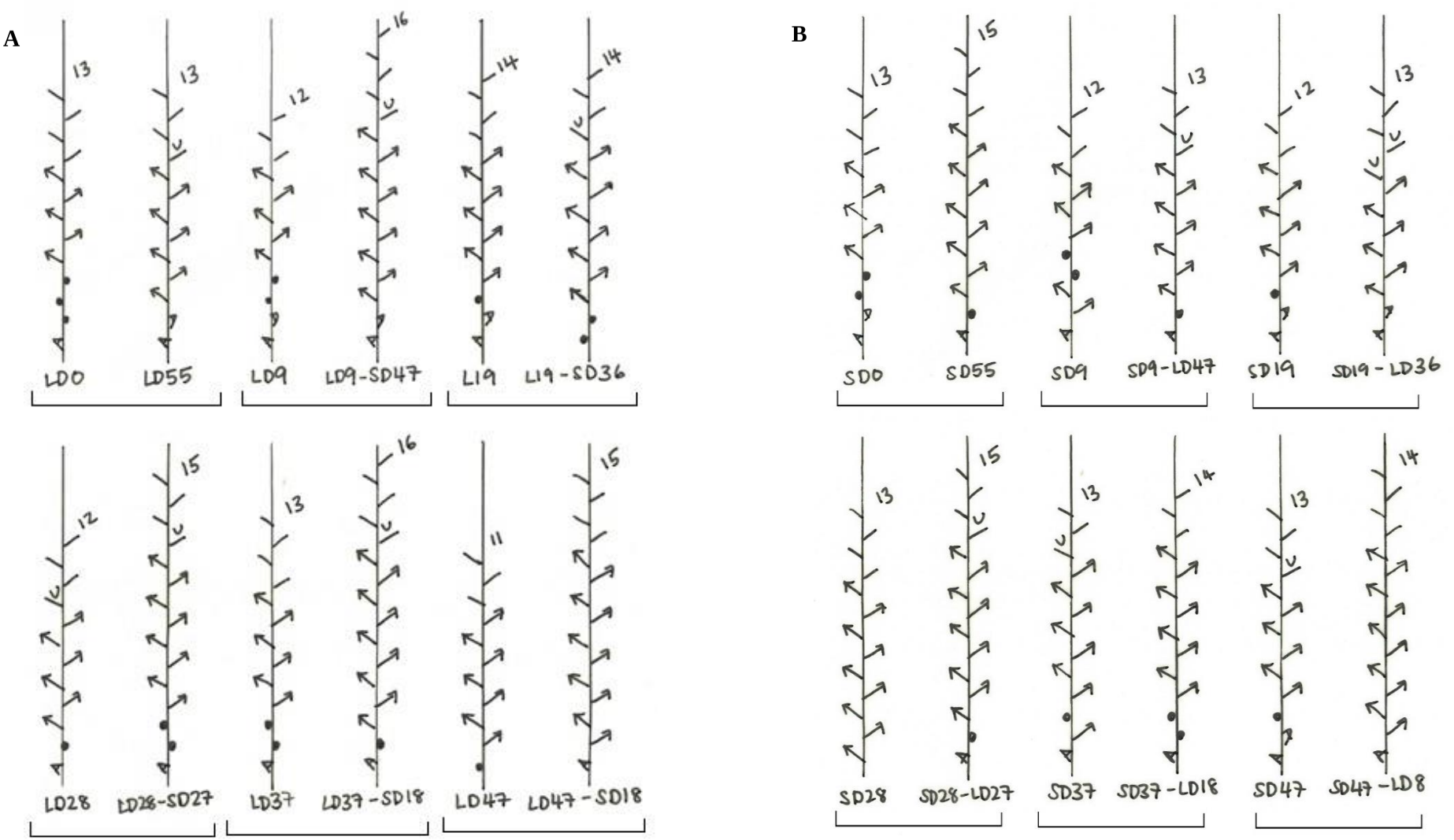


Figure 4.11 Developmental character of axillary buds of plants when dissected at intervals during LD (A) or SD (B) (left of each pair), with the character of buds after transfer from LD to SD (A) and SD to LD (B) until the end of the experiment (right of each pair). Drawings show the modal character of individual buds; in instances where modal character could not be simply calculated (see Figure 4.7 and 4.10), the character chosen was that which best represented the general character at this node position

4.3.3 Axillary bud experiment 3 (AXB3) – axillary bud determination at 11°C

Node groupings were as described for axillary bud experiment 1 and 2 (see Figure 3.9 – Chapter 3). These were observed at the start of the experiment when plants had 12.8 ± 0.4 nodes, and typically had arrested buds and branch crowns (Group 1 buds) at the base of the main crown, with runners in Group 2 node positions (Figure 4.12A). Most plants also had one-two branch crowns within Group 2, while immediately below the SAM (Group 3 nodes), plants typically had one axillary bud with undiscernible character and three leaf primordia that had yet to development visible axillary buds (Figure 4.12A). There was no significant change in plant size after two or four weeks in SD at 11°C ($F_2 = 0.31$; $p = 0.74$). After two weeks in SD at 11°C plants had 13.2 ± 0.5 total nodes. Typical node groupings were maintained and branch crowns were observed at the base of the main crown; most plants showed one-two branch crowns within Group 2 nodes, with runners in the leaf axils above and below (Figure 4.12B). After four weeks in SD at 11°C, plants were similar in their node groupings to the previous dissections (Figure 4.12). Runners were observed where the character of the developed upper axillary buds was discernible; plants typically had one-two visible axillary buds beneath the SAM in which the character was not discernible (Figure 4.12C). The most striking change in plant morphology was the observation of flower initiation in four of the five plants dissected. In these plants, floral primordia were typically observed both at the SAM and in the lowest branch crown (Figure 4.12C).

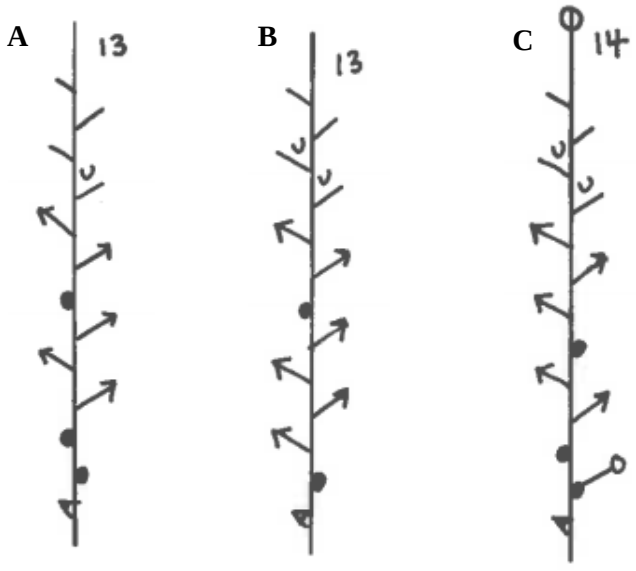


Figure 4.12 Typical developmental character of plants at the start of the experiment (A) and after exposure to SD/11°C for two weeks (B) and four weeks (C), showing the modal number of nodes and character of axillary buds in the main crown

Following exposure to SD/11°C, plants were transferred to forcing conditions (>18°C/LD). The first cohort of plants, which had been exposed to SD/11°C for two weeks, showed no significant increase in total node number during forcing (F_2 = 2.79; p = 0.10) (Figure 4.13). Runners were observed as the discernible character in the upper axillary buds throughout the sampling period (Figure 4.13).

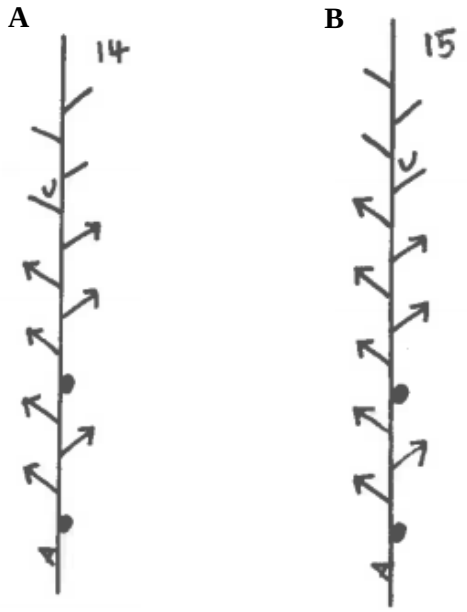


Figure 4.13 Typical developmental character of plants exposed to forcing conditions after four (A) and eight (B) weeks, following exposure to SD/11°C for two weeks. The diagrams show the modal number of nodes and character of axillary buds in the main crown

The second cohort of plants, exposed to SD/11°C for four weeks, had significantly increased in size (total node number) only after eight weeks forcing, in comparison to plants at the time of transfer (F_2 = 4.52; p = 0.03) (Figure 4.14). After four weeks forcing, plants typically had a branch crown in an upper axillary position (Figure 4.14A), and by eight weeks most plants had a runner above this upper branch crown (Figure 4.14B). As well as differences in axillary bud character in the upper nodes, another key characteristic of this second cohort of plants was the emergence of inflorescences; by four weeks forcing, two of the 20 plants showed flower emergence. After eight weeks forcing, inflorescences had emerged in the majority of plants. Three plants of the total twenty plants transferred to forcing did not flower throughout the forcing period and dissections of these individuals showed they had not initiated floral primordia. In the remaining plants, one to three inflorescences were visible, and on dissection at least one leaf primordium could be seen at the shoot apex, parallel to the previously terminal floral primordium (Figure 4.14B).

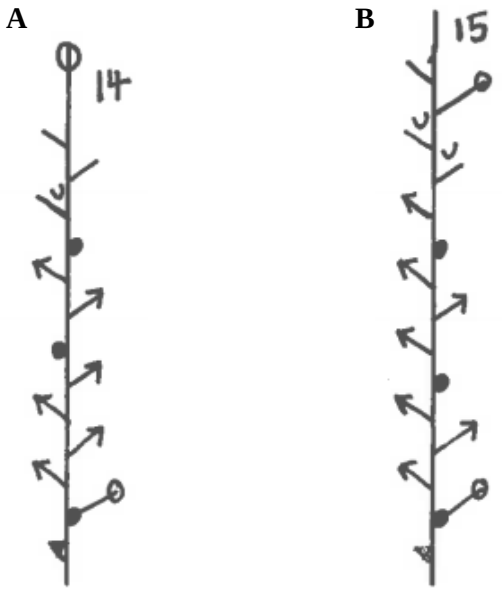


Figure 4.14 Typical developmental character of plants exposed to forcing conditions after four (A) and eight (B) weeks, following exposure to SD/11°C for four weeks. The diagrams show the modal number of nodes and character of axillary buds in the main crown

4.3.4 Axillary bud experiment 4 (AXB4) – influence of runners on SAM activity

At the start of the experiment, plants typically had 11 ± 0.1 nodes, and the node groupings were as described for the previous axillary bud experiments (see Figure 3.9 – Chapter 3). At the base of the main crown, plants had two-three arrested buds/branch crown; above these Group 1 nodes, runners were typically observed in Group 2 nodes, although some plants had a single branch crown at various positions within this node grouping (Figure 4.15A). Towards the top of the plant, there were typically one-two axillary buds with undiscernible character and above these two-three leaf primordia with no visible axillary buds (Figure 4.15A). At the start of the experiment, plants typically had two-three emerged runners (these were removed for plants in the runner removal treatment). After 10 weeks under experimental conditions (20°C/LD) both treatments showed a significant increase in total node number. Plants with runners removed had 19.2 ± 0.4 nodes (F_1 = 280.17; p = 0.00) and those with runners maintained had 12.4 ± 0.4 nodes (F_1 = 7.54; p = 0.03). Runner removal resulted in consistently larger plants (more nodes), than those which maintained their runners.

By the end of the experiment plants in both treatments were similar in their node groupings: runners were predominantly observed in Group 2 nodes, with some plants showing one-two branch crowns in various positions in this node grouping (Figure 4.15 B and C). As expected,

plants with runners removed had produced more runners than those that had maintained their existing runners.

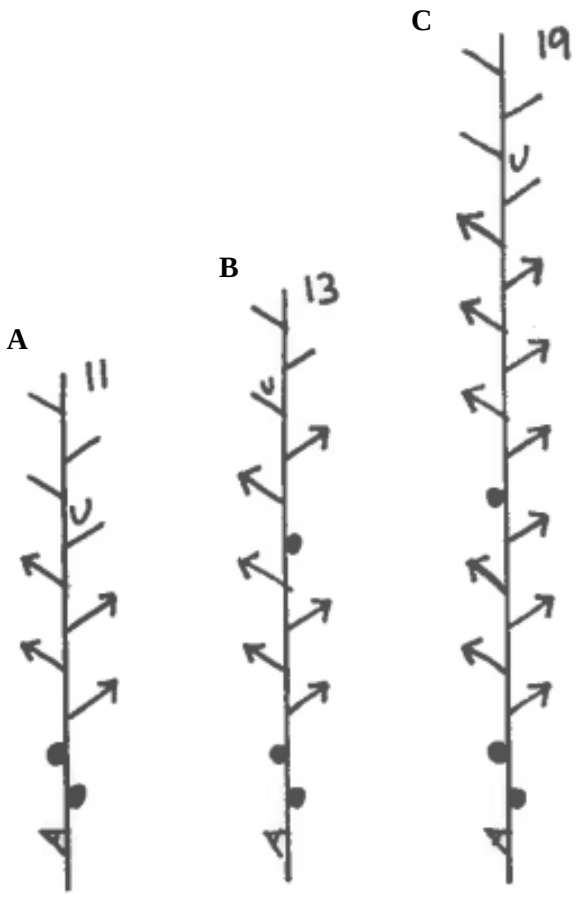


Figure 4.15 Typical developmental character of plants at the start of the experiment (A) and by the end of the experiment for plants which had not had runners removed (B) and those that had emerged runners removed weekly (C), showing the modal number of nodes and character of axillary buds in the main crown

4.4 Discussion

4.4.1 Axillary bud experiments 1 and 2 – axillary bud determination at >20°C and 18°C

The primary objective of the first two experiments described in this chapter was to investigate the timing of axillary character determination in *Fragaria vesca* and to identify the impact of environment on the development of axillary buds, in particular the influence of photoperiod. Photoperiod has been reported as a key regulator of axillary bud character in *F.* x *ananassa,* with SD promoting the development of branch crowns and LD promoting runners (Darrow, 1966; Hytönen *et al.,* 2009). Konsin *et al.* (2001) confirmed that SD promote branch crowns and LD runners, and showed that both types of axillary bud were restricted by their non-promotive photoperiod conditions: runner growth was inhibited under shortening photoperiod, resulting in less runners under SD (12-13.5h) in comparison to LD (18h). Similarly, plants in LD (18h) had less branch crowns than those under SD (12h). This work, like other studies in *Fragaria*, is dependent on observational data (e.g. Hytönen *et al.,* 2004; Bradford *et al.,* 2010) and does not indicate when character determination in developing axillary buds actually occurs. Studies do not record the extent and state of development of axillary buds at the time of environmental treatments; therefore it is not clear whether final character is the result of determination in newly initiated buds or outgrowth of preformed and determined buds. AXB1 and AXB2 showed that runners were initiated and developed from existing axillary buds under both LD and SD, at a temperature of 25°C (AXB1) and 18°C (AXB2). This directly contrasts with research which indicates that 18°C and SD are sufficient for branch crowning in *F.* x *ananassa* (Hytönen *et al.,* 2009).

In another study, focused on molecular effects, Jiang (2013) showed that in LD, 5 weeks at 11°C prevented runner production when plants were returned to 18°C; at the same time branch crown production was accelerated relative to plants grown continuously at 18°C. However, because neither leaf number counts, nor the nodal positions of runners/branch crowns, were reported it is not possible to infer anything from these data about the process of axillary bud determination. However, runner number increased from 1 to 16 after 13 weeks at 18°C; if it is assumed that runner number reflects leaf number, then a leaf initiation rate of about 1/week is implied (although there were 4-5 leaves present at the beginning of the experiment, so some runners would presumably develop from the existing axillary buds of these leaves). This approximate leaf initiation rate is roughly consonant with that in AXB1, but much greater than that in AXB2. Jiang's experiment was carried out during the winter in a heated greenhouse under supplementary illumination of 120 μmol m^{-2} sec^{-1} (+ 10 μmol m^{-2} sec^{-1} for LD extension), which is similar to

that in the controlled environment cabinets used for AXB2. It is therefore interesting that Jiang removed the runners weekly after counting, which may explain the higher (deduced) terminal growth rate compared to AXB2 (see later for further discussion).

Another key factor highlighted by Jiang's (2013) work was that branch crowns emerged as a result of both 11°C and 18°C conditions; the rate of emergence was slower at 18°C, but the overall increase in branch crowns was similar at the two temperatures over the experimental period. However, the temperature conditions did not show a similar effect on flowering, with no flowers observed for plants at 18°C in comparison to those under 11°C, which did flower. This provides evidence for a separation between the environmental conditions regulating branch crowning and those inducing flowering, one of the key areas of interest in the experiments carried out in this Chapter. The plants in AXB2 were also grown at 18°C, and while flowering was repressed under these conditions there was no significant trend in branch crown accumulation (number of branch crowns) or development (number of leaves and primordia within branch crowns), regardless of photoperiod or time. No branch crowns were observed at the upper most axillary buds in either AXB1 or AXB2. One possible explanation for this could have been the duration of the experiment, with Jiang showing the greatest accumulation in branch crowns between seven and thirteen weeks under experimental conditions; whereas the experimental period in AXB1 was six weeks and AXB2 was just under eight weeks in total. However, given that only runners were observed in the upper axillary buds regardless of duration or treatment, it is possible that the difference in branch crown accumulation between Jiang and AXB1/AXB2 was not an effect of time but a difference in plant response.

In summary, the focus of published research regarding axillary bud differentiation has been on the influence of environment on production of axillary buds, rather than the actual process of their determination, which was the principal objective of experiments AXB1 and AXB2. These experiments aimed to establish time to determination, but due to a lack of the alternate character (branch crowns) in newly initiated axillary buds, time to determination could not be established. The influence of conditions on the development of already initiated axillary buds was another key objective of AXB1 and AXB2. As discussed above, LD are generally considered to promote runners and SD branch crowns (Hytönen *et al.,* 2004). In the work described here, however, SD conditions in AXB2 did not promote branch crowns more than LD, with photoperiod having no significant influence on the development of branch crowns under AXB2 conditions ($P < 0.05$). The effect of photoperiod on runnering differed however, between AXB1 and AXB2, with a significant difference in runner growth, as measured by internode number, between LD and SD in AXB1; whereas in AXB2, SD did not have a significant restricting influence on runnering.

This difference is most likely a result of the different total light integral between LD and SD in AXB1, which was deliberately eliminated in AXB2.

AXB1 plants were grown under glass and exposed to natural light levels. SD was provided solely by natural light (400 μmol m^{-2} sec^{-1}), whilst those under LD received daylength extension provided by supplementary lighting (high pressure sodium lamps; 150 μmol m^{-2} sec^{-1}), between 0500-0630 and 2000-2300. This resulted in a difference in the total photosynthetic light received by plants under LD and SD conditions, which could account for the differences in primordium initiation rate in plants in LD (1.3 nodes/week) compared to SD (1 node/week). AXB2 was designed so that plants under LD and SD were exposed to the same duration of photosynthetic light, with plants in both photoperiod treatments exposed to photosynthetic light from 0800-1800 (160 μmol m^{-2} sec^{-1}) and daylength extension for LD plants provided by non-photosynthetic light (3 μmol m^{-2} sec^{-1}). The light integral received by the plants was, however, lower than for those in AXB1, with an average difference of 240 μmol m^{-2} sec^{-1} between AXB1 and AXB2. This difference in light integral may have contributed to the difference in rate of primordium increase between the two experiments.

A striking observation from both AXB1 and AXB2 was the slow rate of primordium initiation of the SAM in both experiments. Under AXB1 conditions, the rate of node initiation was 1/1.3 per week under SD and LD, respectively; whereas plants in AXB2 showed an even slower rate with no consistent increase in node number during the experiment. Temperatures in AXB1 were higher and more varied than in AXB2, with an average day temperature of 24.7°C ± 0.6 for LD and 25.3°C ± 0.7 for SD and an average night temperature of 17.7°C ± 0.3 for LD and 19.3°C ± 0.3 for SD plants, as plants were grown under glass. By comparison, the temperature in AXB2 was cooler (18°C) in both LD and SD. The temperature difference and the variation in day/night temperature may have contributed to the growth rate difference; Chabot (1978) reported that a day/night temperature difference of 10°C caused the greatest total biomass gain in *F. vesca*, suggesting a greater growth rate.

The rate of initiation of new leaf primordia (and its reciprocal, the plastochron) is the resultant of the growth of the apical meristem dome and the amount of tissue allocated to each primordium at initiation (Lyndon, 1998). It varies during development but at any particular phase can, under constant conditions, be taken as one measure of SAM growth. The estimated rate of primordium initiation in these two experiments was about 1/week: in AXB1 the rate was 1-1.3/week depending on photoperiod (SD and LD respectively); whereas in AXB2 it was much slower (0-0.25/week, LD and SD respectively). The higher rate is roughly consistent with that estimated for *F. vesca* from the work of Jiang (2013), and that of not more than 0.9/week (Arney, 1954) or

1.3/week for *F.* x *ananassa* (Arney, 1955; see also Chapter 3). A brief survey of the rate of initiation in other species indicates that this is relatively slow; for example, Williams (1975) reports estimates rising from 1.2/day in flax (*Linum usitatissimum*), and of 0.34/day in tobacco (*Nicotiana tabacum*). In the perennial *Picea abies* the rate in seedlings rose from 1.1/day. In *Impatiens balsamina* the rate was 0.9/day (Battey and Lyndon, 1984), while in *Arabidopsis thaliana* an estimate of the plastochron of 31h implies a leaf initiation rate of 0.77/day (Hempel and Feldman, 1995). It is important to emphasise that these measurements do not necessarily reflect rate of leaf emergence (and its reciprocal the phyllochron) because leaves can accumulate within the apical bud (as, for example, in flax; see also discussion of Arney's work in Chapter 3). Nevertheless, because there was no evidence of accumulation of leaves within the apical bud (which agrees with work published by Arney (1955a) who showed no consistent accumulation of leaves within the apical bud for *F.* x *ananassa* during active vegetative growth), or that the apex increased markedly in size during the experimental period, the data reported here for *F. vesca* strongly suggest that the SAM of this species initiates new leaves and grows at a strikingly slow rate compared to many others. Interestingly, however, in apple (*Malus* x *domestica*) approximately six new leaves were initiated between June and August, over a period of about 10 weeks, suggesting a slow rate, comparable to that of *Fragaria* (Abbott, 1977; Dale, 1982).

It seems possible that woody rosaceous species may generally sustain a relatively low rate of leaf primordium initiation, though a wider, more systematic survey would be required to substantiate this suggestion. More pertinent to the present study is whether and to what extent the slow rate of initiation of leaf primordia in *Fragaria* is a cause or consequence of the very pronounced runner (and/or branch crown) growth generally exhibited by the species, which in physiological terms may be a result of a very weak apical dominance. Although the rate of terminal growth was slow, particularly in AXB2, very active lateral growth (recorded primarily through the growth and development of runners), was observed under SD and LD conditions, with the number of internodes (Figure 4.8) and daughter plants increasing markedly during the experiment. A review of plant architecture in strawberry by Massetani *et al.* (2011) reported that vegetative growth (in the form of runners) was stimulated under high growth rate conditions (high temperature and LD conditions), which was also suggested to result in weak apical dominance. The observation here of runner development in both LD and SD implies that the environmental conditions provided in these experiments were sufficiently promotive for runner growth, which may have resulted in weak apical dominance and a slow terminal growth rate. The interplay between lateral and apical growth, and a probable weak apical dominance in *Fragaria*, may also be associated with the generally short stature of the genus *Fragaria*. Runner removal is undertaken commonly in experimental studies of *Fragaria* (e.g. Hytönen *et al.*, 2004, 2009; Sønsteby and Heide, 2006; Heide and Sønsteby, 2007). It may be important to consider the extent to which this procedure

affects apical growth and therefore the nature of results obtained. The results from AXB2 imply that the pronounced development of runners, if runners are left on the plant, could dominate growth of the plant and perhaps limit growth of the terminal SAM.

4.4.2 Axillary bud experiment 3 – axillary bud determination at 11°C

The aim of this third experiment was to establish a treatment which specifically promoted branch crown development as an alternative to runner production and to investigate whether this could occur in the absence of floral initiation. While branch crowns formed from pre-existing axillary buds after four weeks SD at 11°C, most plants in this cohort had also become induced to flower. This differed from the results reported by Jiang (2013), which suggested that branch crowning could be separated from flower initiation, with branch crown emergence observed for plants under 11°C and 18°C conditions and no floral induction at 18°C. However, no dissections were undertaken in Jiang's study, so it is not possible to conclude where or when vegetative branch crowns were initiated on the main crown at 18°C. Branch crowns have been observed under vegetative conditions (>18°C) in experiments throughout this Chapter and thesis, especially at the base of runner daughter plants and occasionally in node Group 2. It was not clear whether the emerged branch crowns observed by Jiang (2013) at 18°C were from newly initiated branch crowns, or from basal branch crowns, which appear to occur regardless of environmental conditions.

In AXB3, the absence of floral primordia throughout the main crown at available branch crown apical meristems suggests either that floral induction might not have been complete, or that its effects had not yet become visible. After eight weeks of forcing, plants typically showed a runner in the most recently discernible axillary bud position, with a branch crown in the node beneath (see Figure 4.14B). This suggests that vegetative growth might have been re-established, following floral induction which only transitorily promoted branch crowns. Caution is needed here, however, as branch crowns and runners may alternate in successive axillary buds (see e.g. Figure 4.14): for example, in Group 2 nodes, runners may generally be present but one or two branch crowns can also be found. Conversely, the observation of a runner in the most recently developed axillary bud might not necessarily imply floral reversion.

Nevertheless, other observations suggest that floral induction could have been marginal following four weeks exposure to SD/11°C: in this experiment general plant habit had become dwarfed, with darker green leaves, as typically associated with semi-dormancy and the floral state

(Guttridge, 1985). Yet this was also observed in plants grown outside until October (Chapter 5), and was lost when the plants were transferred to forcing in October more quickly than in those transferred to forcing in November. This suggests that such characteristics may not imply that the plant has fully entered semi-dormancy (as also suggested by Sønsteby and Heide, 2006; Heide *et al.*, 2013); similarly flower induction could only be temporary, if marginal. A possible effect of semi-dormancy can also be deduced from Jiang's (2013) work, with five weeks exposure to 11°C preventing runner production under subsequent forcing (at 18°C). By contrast, four weeks exposure to 11°C in this experiment (AXB3) did not appear to restrict runner production, with runners produced following forcing. This further suggests that four weeks exposure to 11°C may not have been sufficient to induce semi-dormancy and/or plants after this duration of exposure retained the ability to resume vegetative growth.

After eight weeks of forcing following four weeks under SD at 11°C, dissections showed a leaf primordium alongside the terminal inflorescence at the shoot apex. This may also have implied marginal floral induction because it differed from the situation in plants after continued induction, such as in Chapter 3, where the primary inflorescence remained in the terminal position at the top of the plant and subsequent floral development occurred laterally, below this primary inflorescence. In contrast, in the second cohort of plants in this experiment the primary inflorescence was displaced laterally during forcing, apparently due to the resumption of vegetative growth (Figure 4.14B). Dissections during the annual cycle (Chapter 3) showed that the upper axillary buds developing during floral initiation typically produced two-five leaf primordia before a new inflorescence. Therefore when plants are transferred to non-floral inducing conditions, as here, it seems possible that growth from this bud may have continued, and effectively a new vegetative SAM formed instead of a new inflorescence.

According to Battey and Lyndon (1990) reversion of flowering can occur as flower reversion (as in *Impatiens*); or inflorescence reversion (where the SAM ceases to initiate bracts with flowers in their axils and instead initiates leaves, as in wallflower); or as a whole plant change from a floral to vegetative state as part of the perennial life cycle, as for example in *Fragaria* in the spring, when vegetative growth resumes. It is not clear whether the phenomenon observed here is a type of inflorescence reversion, an early resumption of spring vegetative growth which would normally occur after a period of winter chilling, or a continuation of floral development which remained vegetative as a result of forcing conditions. This result has an interesting parallel with the 2015-2016 and 2016-2017 experiments described in Chapter 5, where plants were exposed to natural floral inductive conditions before forcing, at monthly intervals. There, UK9 (used in all Chapter 4 experiments) showed active runnering under forcing following transfer in October, at which time flower emergence was also observed. Plants were also transferred to forcing in

November, at which point floral induction was more complete, as all the transferred plants flowered and emergence was earlier than for previous transfers. While runner production was still observed for plants following transfer in November, the number of runners was greatly reduced. This could imply a restraint on floral reversion in the absence of sufficient chilling once plants had become fully induced to flowering; on the other hand, plants with marginal floral induction might be more able to resume/re-establish vegetative growth.

4.4.3 Axillary bud experiment 4 – influence of runners on SAM activity

The primary aim of AXB4 was to determine whether runner removal could influence the growth of the SAM. In AXB2 and AXB1 growth at the SAM was limited: there was no significant increase in the number of nodes at 18°C (AXB2) ($P > 0.05$), regardless of photoperiod, and in AXB1 it was only one node per week, at 25°C, in both LD and SD. In AXB4, at 20°C, runner removal significantly increased the initiation of new leaf nodes compared to plants which maintained their runners ($P < 0.01$). Plants with runners removed initiated 0.8 nodes per week, similar to AXB1, in which runners had remained on the plants. By contrast, the plants which maintained their runners at 20°C initiated only 0.1 nodes per week. This result shows that runner removal has a significant impact on growth (leaf initiation) at the terminal SAM. It offers a potential opportunity in future research for establishing determination time of runners or branch crowns, although the development of the latter at lower temperatures means that comparison of determination times could be difficult.

The effect of runner removal raises concerns over how this method of plant maintenance commonly practiced in *Fragaria* experiments might influence developmental responses. Leaf defoliation has been shown to influence flower initiation (Thompson and Guttridge, 1960), leaf size (Arney, 1954) and growth rate (Casierra-Posada *et al.*, 2012); runner removal has previously been shown to improve yield (provided leaf cover was maintained) (Lyu *et al.*, 2014). Therefore it can be expected that runner removal might also affect other developmental responses, especially as runner production has previously been assumed to interfere with the growth of the parent plant (Darrow, 1930; Arney, 1954). Careful consideration is needed of the impact of runner removal as part of the experimental process; further research is needed to determine its overall influence on growth and development in *Fragaria*.

A further point of interest is the variation in rate of node (leaf) initiation in the experiments discussed in this Chapter and in Chapter 5. Plants in AXB1 with runners left intact, grew at a

similar rate to those in AXB4 with runners removed. This could have been due to higher temperatures (fluctuating, with an average of 25°C *versus* constant 20°C), light (glasshouse environment *versus* growth cabinet), and fluctuating day/night temperatures, or a combination of these factors, in AXB1 compared to AXB4. Other studies have reported differences in responses of plants grown in controlled and natural environments. For example, natural (oscillating) temperatures conditions accumulate chilling in *F.* x *ananassa* more efficiently than under controlled, constant temperatures (Tehranifar, 1997). Although here, vegetative growth and axillary bud determination were the focus of the experiment, it is important to note this observation for future work. However, plants in the experiment to be described in Chapter 5 had runners removed and initiated new leaf nodes at a similar rate to plants with runners removed in AXB4. Because the work in Chapter 5 was conducted under glass, it seems most likely that the difference between AXB1 and AXB4 was due to the temperature difference between the two experiments. An alternative possibility is that the higher rate of leaf node initiation in AXB1 was related to time of year (May-June) when the experiment was conducted. This adds a further factor into the complex web of considerations necessary when designing *F. vesca* experiments: plants may experience variation in growth vigour associated with a circa-annual cycle, and unrelated to treatment (runner removal) and growth conditions. Much more research, however, would be needed to substantiate this idea.

4.4.4 Conclusions

One of the most striking results from the first two experiments in this Chapter was that at temperatures greater than 18°C, photoperiod did not have a direct influence on axillary bud fate. SD have been reported to promote branch crowns (Hytönen *et al*., 2009), but the warm-high temperatures in AXB1 and AXB2 appeared to restrict the possible influence of SD, with runners observed in newly developed leaf axils, under both SD and LD. However, the cool temperature 11°C/SD treatment in AXB3 was sufficient to promote branch crown formation. It was not, however, possible to separate branch crowning from flowering under these conditions, with floral initiation also observed alongside branch crowning.

In AXB3, forced plants which had initiated floral primordia under 11°C/SD appeared to show reversion to vegetative growth, with the presence of a leaf primordium at the SAM alongside the terminal inflorescence, similar to that observed in plants in the spring (April 2017 - see Chapter 3). It appeared that the meristem producing the leaf would continue the vegetative growth of the plant; and that this leaf was from the node immediately beneath the terminal inflorescence, as

described by Guttridge (1985) and shown by spring dissection in Chapter 3. It was not, however, completely clear whether the visible leaf was the leaf at the node beneath the terminal inflorescence, or had been initiated by the axillary meristem associated with this leaf. Regardless of its origin, the presence of a leaf in a terminal position, alongside the inflorescence suggested that eight weeks forcing (>18°C/LD) might have been sufficient to promote floral reversion, by re-activating vegetative growth. One interesting feature that emerged through comparison of AXB3 results with those in Chapter 3, was that the presence of a leaf at the terminal position alongside an inflorescence, was similar to the situation in the spring (April 2017), when vegetative growth naturally resumed, but not comparable with plants in the autumn (2016), during progressive floral initiation. These latter plants did not show the dual presence of a vegetative meristem and a floral structure in the terminal position. One reason for this difference may be that during the autumn the plants develop in such a way as to create a complex, cluttered group of flowers and inflorescences at the terminal SAM and in the upper branch crowns. In the spring, and under forcing in AXB3, the structure of the inflorescences was different and the continuing vegetative meristem, was more clearly visible next to the terminal inflorescence. At least in the spring, the inflorescence became displaced to the side and the vegetative meristem continued, growth, acting as the new terminal SAM. One possible explanation for this displacement of the inflorescence in the spring/under forcing and therefore the appearance of floral reversion, which was not observed during continued initiation, might be that under these spring/forcing conditions vegetative growth is more active so that at the SAM everything appears more elongated and the overall structure is clearer, in comparison to during floral initiation when everything is by comparison more bunched up. Clearly the subject of floral reversion needs further research to determine the precise conditions/duration required.

The experimental design of AXB3 meant that is was not possible to provide a comprehensive understanding of the process of branch crown determination and/or floral reversion. However, the primary objective of this experiment was to discover conditions providing branch crown production and whether branch crowns could occur in the absence of floral initiation, hence the use of treatments thought not to sufficiently promote initiation. Were this experiment to be repeated it would be preferable to include a control under forcing conditions and one at 11°C/SD, as a well as a treatment of plants exposed to 6 weeks at 11°C/SD prior to forcing, and for all treatments to have been forced for a longer duration. The addition of these treatments/factors might enable the following questions to be addressed:

1) How axillary bud character in upper, developing nodes are influenced by flower initiation at the SAM?

2) What is the process of floral reversion/how is vegetative growth re-established following floral initiation?

Runner removal was shown to increase the rate of leaf initiation at the terminal SAM, which may be important in understanding how *F. vesca* growth and development is regulated in a natural context. It is interesting that in this context it might be that runners rapidly come to dominate terminal SAM growth by June; that terminal SAM growth is therefore more-or-less arrested by the time late summer and autumn arrive; and that the key developmental decisions are made in pre-existing but latent or quiescent axillary buds, which pursue a branch crown fate, rather than becoming runners. The critical question then becomes: why do some axillary meristems not develop as runners in warm LD? This question is analogous to one at the heart of flowering and annual growth in other perennials, such as *Populus* and *Arabis*, where it is the maintenance of some meristems in a quiescent state or due to their early stage of development that is crucial for perenniality. This issue is discussed further in Chapter 7; the implication for the original objective of this Chapter is that the determination decision (to make a runner or branch crown) in *F. vesca* is always made locally, within axillary meristems, in a manner unrelated to time of origin at the SAM; the only determination decision associated with the terminal SAM would then be between vegetative or floral. The determination (or not) of an inflorescence character is another topic where understanding remains limited, and may be critical in relation to questions about the possibility of floral reversion.

Chapter 5: Ecotypic variation in flowering and runnering

5.1 Introduction

In *Fragaria*, the induction of flowering by the short days and cooling temperatures of autumn has been extensively characterised (Guttridge, 1985; Heide *et al.*, 2013; Rantanen *et al.*, 2015). This research has indicated that *F.* x *ananassa* and *F. vesca* are broadly similar in their physiological process and requirements (environmental conditions) for induction, although genetic evidence suggest that at the molecular level there may be differences between these two species (Kurokura *et al.*, 2013).

This chapter focuses on the control of flower induction, initiation and emergence in *F. vesca*. The natural transition to flowering occurs through the autumn with activation of flower initiation in response to SD and decreasing temperature (Guttridge, 1985; Mouhu *et al.*, 2013). Studies have also addressed the interaction of temperature and photoperiod in the control of flowering in *F. vesca* and shown that induction occurs across a range of conditions: at low temperatures (9°C) under LD or SD, at intermediate temperatures only under SD, whilst it is prevented by high temperatures, regardless of photoperiod (Heide and Sønsteby, 2007). As well as changes in flowering during this autumn phenophase, changes in vegetative growth are also observed. Runner formation in newly differentiated axillary buds ceases under naturally shortening daylength during the autumn (Konsin *et al.*, 2001) and axillary buds differentiate to form branch crowns (Hytönen *et al.*, 2004). The capacity for leaf formation and petiole elongation is also observed to decrease over the autumn (Sønsteby and Heide, 2011).

The widespread distribution of *F. vesca* raises the question of whether the environmental triggers for flower initiation are consistent species-wide, or if ecotypes have become locally adapted and therefore differ in their responses. An ecotype in the context of this chapter is defined as 'a group of individuals which react essentially alike and come from one climatic region' (Clausen *et al.*, 1940). The ecotype concept is, however, a subject which has received much attention and has been used across an array of biological groups and species. Another definition, building upon Clausen *et al.* (1940), is that an ecotype is a population distinguished by morphological and physiological characters which can reproduce with other ecotypes of the same species which exist in geographical isolation from each other (Turrill, 1946). One of the primary aims of the research described in this chapter was to establish whether genotypes derived from different locations and

maintained by vegetative propagation (here operationally defined as 'ecotype') differed in their requirements for flower induction, and if so, to explore the basis for this variation.

Heide and Sønsteby (2007) studied variation in flowering control in *F. vesca* using ecotypes from a range of latitudinal and altitudinal origins. They showed that although there was a generally similar response to inductive conditions, ecotypes varied; for example most ecotypes showed evidence of flowering following induction under LD at low temperatures but this was not observed across all ecotypes. The number of flowers produced and days to anthesis following induction varied between ecotypes and conditions (Heide and Sønsteby, 2007). Heide and Sønsteby (2007) also aimed to examine the influence of latitude on flowering responses, but although two ecotypes showed differences which suggested some correlation with latitude and critical photoperiod for induction, the majority of ecotypes did not follow this trend. In conclusion, Heide and Sønsteby (2007) stated that there was no clinal relationship between latitude of origin and requirement for floral initiation. This conclusion differs from work on other species, as studies often show a clinal relationship between latitude and response of growth or developmental parameters, for example in poplar (Rohde *et al.,* 2011); *Arabidopsis thaliana* (Stinchcombe *et al.,* 2004); and birch (Myking and Heide, 1995). One explanation that Heide and Sønsteby (2007) provided for this lack of an observable latitudinal cline in *F. vesca* is that the species has had a close interaction with humans, which may have prevented or obscured the otherwise natural occurrence of photoperiodic ecotypes; they also proposed that the clonal nature of this species may have interfered with the development of local adaptation with latitude, as typically observed in other species (Heide and Sønsteby, 2007).

A general method of studying ecotypic variation is common garden or reciprocal transfer experiments (Pelini *et al.,* 2012). These allow comparison of ecotypic responses, and can provide a measure of phenotypic plasticity in the local adaptation of ecotypes (de Villemereuil *et al.,* 2016). The common garden experiment approach involves a selection of ecotypes that are transferred to and grown in a common environment; this enables variation in phenotypic responses to be observed and can indicate the relative importance of genetic and environmental factors (Moloney *et al.,* 2009). This is a long-standing approach to determine and understand ecotypic responses (Bonnier, 1920; Turesson, 1922). For example Stinchcombe *et al.* (2004) considered the effect of latitude on flowering time for 70 *Arabidopsis thaliana* ecotypes from a range of Northern European and Mediterranean origins. They observed differences between ecotypes with regards to bolting, with a significant positive relationship with latitude, ecotypes from more northerly latitudes bolting later than those from more southerly latitudes (Stinchcombe *et al.,* 2004). The use of a common garden indicated that ecotype variation was a result of genetic differences (Stinchcombe *et al.,* 2004).

The experiments described in this Chapter were part of a large-scale collaboration with the University of Helsinki which aimed to:

1. Determine whether a specific photoperiod x temperature interaction enabled timely flower induction of *F. vesca* in different climates;
2. Establish if there are latitudinal clines in the critical temperature limits between ecotypes;
3. Re-address the issue of whether there is a latitudinal cline in the critical photoperiod, at the European level.

To address this series of questions a range of ecotypes of *F. vesca* was included and a number of experiments set up to establish whether there was a latitudinal cline in the critical temperature (2) or photoperiod (3) for flower induction. In order to answer the primary question (1), a preliminary common garden experiment, followed by a series of subsequent common garden and reciprocal transplantation experiments, were planned. This large-scale study aimed to collect and combine data from a number of experimental sites (Finland, Iceland, Spain and UK), to provide a broader understanding relating to the subject of flower induction. These findings could be used for comparison with existing *Fragaria* literature and also with the response of other perennial species, more broadly. At the present time (May 2017) the results of this study are being collated in Helsinki and so will not be included in this thesis.

The experiments described here (referred to as 2015 and 2016), were set up at the University of Reading initially to act as a component of this wider study (2015 results). As a result, the aim of these specific experiments differed slightly from the wider aims, as the focus was on a single common environment and the response of ecotypes to conditions within this environment. The aim of the first experiment (2015) was therefore to determine whether and how ecotypes differed in flowering response when grown in a common environment, and to establish whether these potential differences in flowering response were accompanied by differences in vegetative growth. In order to establish differences in flowering response, flower emergence was recorded under warm, LD (forcing conditions) following transfer of cohorts of plants from the natural environment at intervals over the autumn (August – November). Vegetative response was determined primarily by recording runner emergence, with other vegetative growth responses also recorded (petiole elongation and branch crowning). The aims of the second experiment (2016) were to establish whether the vegetative and floral responses observed in 2015 were consistent in 2016, for plants vegetatively propagated from material maintained in the common garden, and whether inflorescence morphology accounted for differences in flower number.

A third experiment were designed to address whether asexually (from runners) and sexually (from seed) reproduced individuals were similar in their floral response (timing of initiation and emergence), and whether ecotypic differences observed in the 2015 and 2016 common garden experiments were also shown by sexually produced material.

5.2 Materials and methods

A description of propagation and information regarding growing media and equipment are provided in Chapter 2 (General Materials and Methods).

5.2.1: 2015 experiment

Plant material

A range of *F. vesca* ecotypes was included in this experiment, with a total of eight ecotypes, four from Finland and four from the UK (Table 5.1). In June 2015 plant material in the form of rooted runner plantlets was obtained from the University of Helsinki for each of the ecotypes. Plants were separated and potted individually (05/06/2015) and referred to as mother plants. Propagation through runners was carried out to raise sufficient replicates and the runners from these mother plants were rooted in adjacent trays of growing mix 1 and referred to as daughter plants. These daughter plants were individually potted (using growing mix 2) prior to the start of the experiment (18/08/2015).

Table 5.1 Collection information for the *F. vesca* ecotypes used in the 2015 experiment, F ecotypes refers to those collected in Finland and UK ecotypes refers to those collected in the UK. Ecotypes are ordered by latitude, with F7 the most northerly and UK12 the most southerly

Ecotype	Origin	N coordinate	E coordinate	Collection date
F7	Salo, Halikko	60.3706	22.9796	2013
F53	Lohja	60.2076	23.8066	2013
F50	Raasepori, Karjaa	60.1061	23.6782	2013
F6	Hanko, Tyärminne	59.8428	23.2446	2013
UK11	Humble Jumble, Lake District	54.733077	-3.21054	2014
UK2	Thackthwaite, Lake District	54.601389	-3.3196	2014
UK9	Mapledurham, Berkshire	51.484547	-1.026138	2014
UK12	Salcombe, Devon	50.244678	-3.771955	2014

Growth conditions

Plants were initially grown under unheated glass to promote establishment and runner production (05/06 – 29/06/2015). Once plants had become established and begun producing daughter plants, they were transferred into the field onto staging situated in the Experimental Grounds,

Whiteknights Campus, University of Reading (29/06 – 06/07/2015). However, due to a period of high temperatures plants were transferred back under unheated glass, in order to allow careful monitoring and the addition of shade netting (06/07 – 29/07/2015). Once conditions had cooled, plants were re-transferred into the field (29/07/2015). This provided the baseline treatment in the naturally changing environment from summer to late autumn (18/11/2015). Plants were randomly arranged within ecotype groupings on staging and exposed to natural conditions until transfer to forcing conditions. Conditions in the field (temperature and photoperiod) are indicated in Figure 5.1.

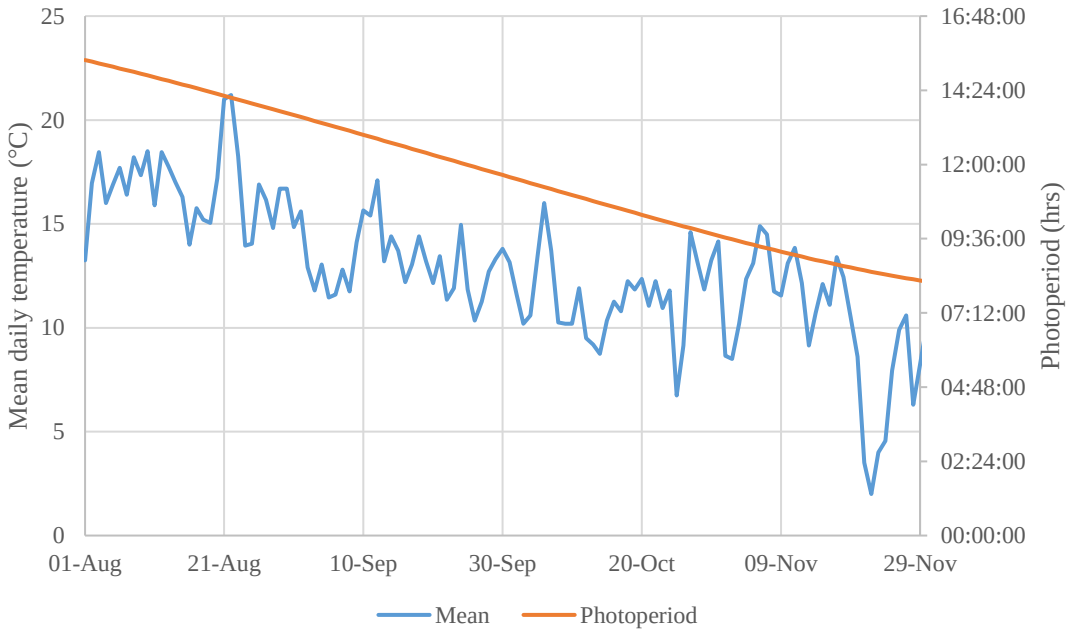


Figure 5.1 Mean daily temperature (°C) from August-November 2015 (University of Reading Meteorological Department (http://www.met.reading.ac.uk/weatherdata/) and photoperiod (hours) for Reading (http://dateandtime.info/citysunrisesunset.php?id=2639577)

Cohorts of plants were transferred every month (Table 5.2) into forcing conditions (18/15°C day/night, 18h photoperiod) provided by a multi-factorial greenhouse compartment, which was designed to prevent flower induction. Each transfer cohort was randomly arranged in the forcing greenhouse, where the 18h photoperiod was provided by supplementary illumination from four evenly spaced high pressure sodium lamps (OSRAM SON-T 400W); the duration of supplementary lighting varied during the experiment according to photoperiod (Table 5.3). The actual greenhouse temperatures are presented in Figure 5.2.

Table 5.2 Date of monthly transfer from natural inductive conditions to controlled forcing conditions

Transfer month	Date of transfer
August	18 August 2015
September	15 September 2015
October	14 October 2015
November	18 November 2015

Table 5.3 Supplementary illumination duration and time periods

Experiment period	Morning (AM) Extension	Evening (PM) Extension
18 August 2015 – 21 October	0500 – 0700	1800 – 2300
22 October – 1 November	0500 – 0800	1700 – 2300
2 November – 10 February 2016	0500 – 0800	1600 – 2300

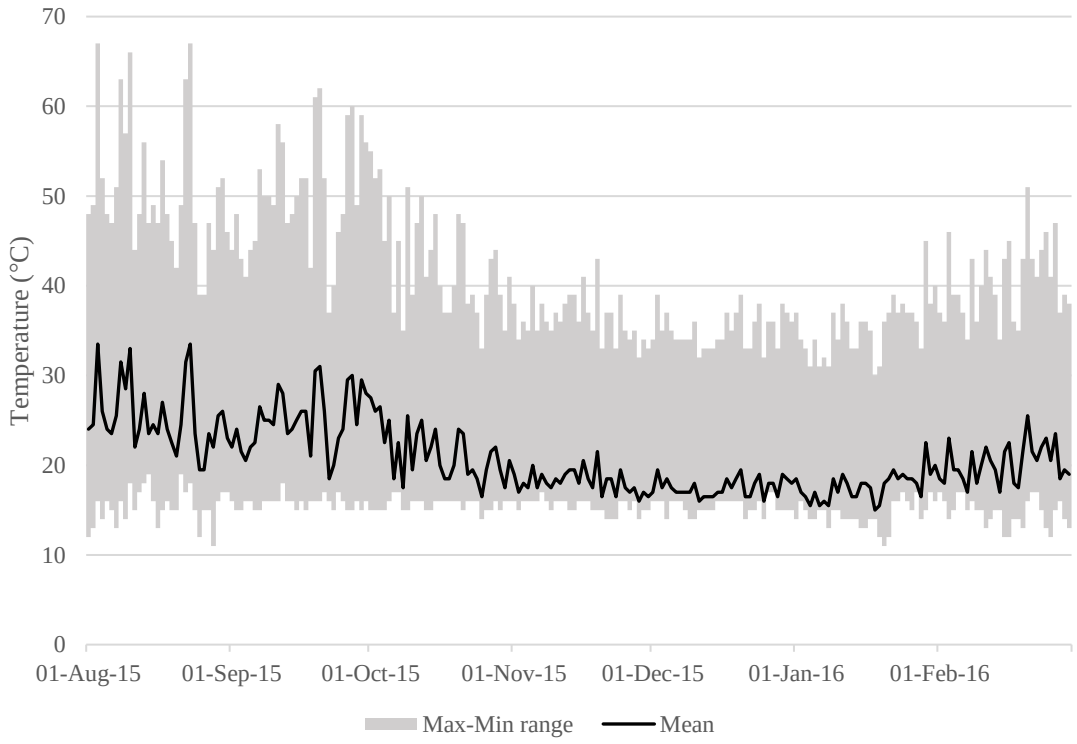


Figure 5.2 Mean daily forcing temperature (°C), with maximum-minimum temperature range shaded in grey

Plant numbers

360 plants were used over the course of this experiment (45 replicates of each of the eight ecotypes). These plants were initially maintained in the natural environment, and at time of transfer a randomly selected cohort of 10 plants was transferred to forcing conditions. Over the experiment 40 plants per ecotype were transferred from field to forcing conditions, with five additional plants maintained in the field in the event of plant death.

Parameters recorded

Prior to the start of the experiment, all runners were removed and subsequently the following growth and development parameters were recorded:

In the field

- All runners were removed and recorded every two weeks

For transferred plants (over the 12 week forcing period)

- Timing of flower emergence (assessed every two-three days once flower emergence was first observed) and number of flowers produced;
- Number of emerged branch crowns at the time of transfer and one month into transfer;
- The youngest emerged leaf was tagged after one and three weeks under forcing conditions and petiole length recorded one month later (at which time it was assumed petiole length growth would have ceased)
- Runners were removed and recorded every two weeks.

Statistical analysis

Data were tested for normality of distribution using the Ryan-Joiner test, and flowering and runnering responses found not to be normally distributed. Kruskal-Wallis tests were therefore performed to analyse these variables and showed comparable levels of significance with equivalent one-way ANOVA. In order to increase the robustness of the analysis and to enable the interaction between factors to be analysed a two-way ANOVA was performed for each of the recorded parameters to establish the statistical significance of each of the factors independently (transfer time and ecotype), as well as interactions between factors. All replicates were included within the ANOVA test, while averages and standard error of the mean were calculated and

presented graphically. In order to establish how ecotypes varied in runner production following the August transfer, a one-way ANOVA and subsequent Tukey Pairwise Comparison was performed.

5.2.2: 2016 experiment

Plant material

Three of the ecotypes from the 2015 experiment were included in the second experiment (2016), to assess whether ecotype response and differences were maintained for plants sustained within a common garden (at the University of Reading). The three ecotypes included were those that showed variation in flowering and runnering response in the 2015 experiment and provided a latitudinal gradient within the UK: UK2, UK9 and UK12. Plants not included within the first experiment were maintained in unheated glasshouses over the winter to protect them for adverse weather conditions and propagated the following year (in June 2016). Propagated daughter plants were individually potted on at the start of July (2016) (growing mix 2) and maintained under unheated glass.

Growth conditions

At the start of the experimental period, plants were transferred into the field onto staging situated in the Experimental Grounds, Whiteknights Campus, University of Reading (15/08/2016) and exposed to natural conditions until transfer to forcing conditions (Figure 5.3). Plants were randomly arranged and evenly spaced on the staging, and re-arranged at the time of each transfer to ensure consistency in spacing.

A cohort of 10 plants of each ecotype was transferred into forcing conditions at intervals throughout the experimental period (Table 5.4). Forcing conditions were provided by a multi-factorial greenhouse compartment, to ensure warm temperatures (mean temperature > 18°C) and a LD (18h) and designed to prevent flower induction and promote active, vegetative growth (including flower emergence). Ecotypes within each transfer cohort was randomly arranged in the forcing greenhouse. The 18h photoperiod was provided by supplementary illumination from four evenly spaced high pressure sodium lamps (OSRAM SON-T 400W); the duration of supplementary lighting varied during the experiment according to photoperiod (Table 5.5). The actual greenhouse temperatures are presented in Figure 5.4.

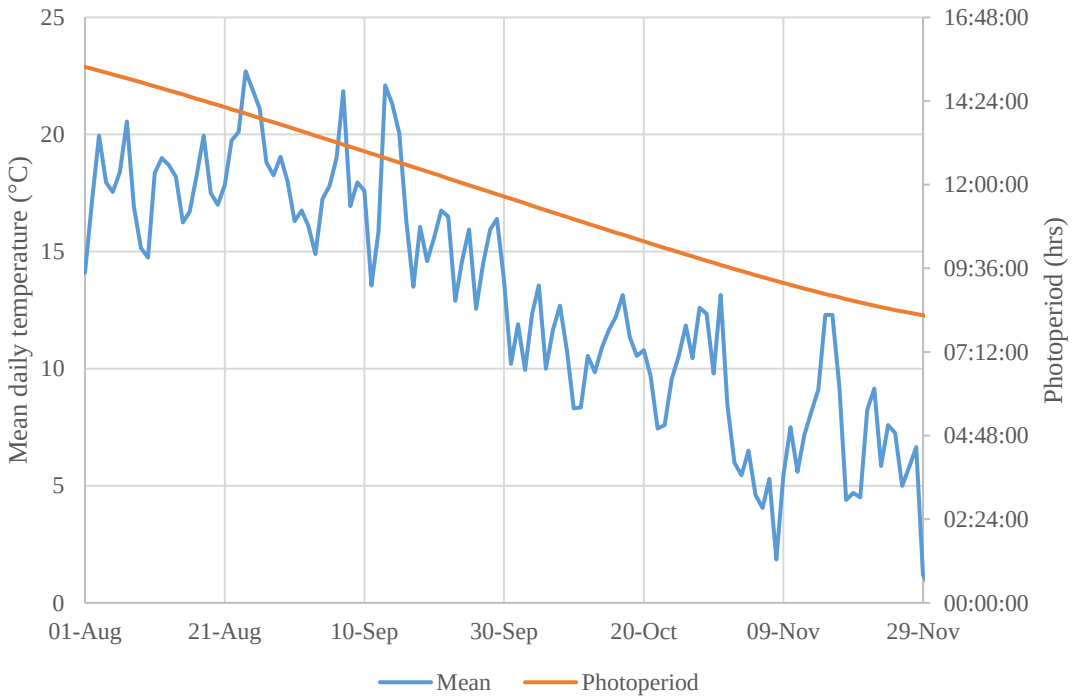


Figure 5.3 Mean daily temperature (°C) from August-November 2016 (University of Reading Meteorological Department (http://www.met.reading.ac.uk/weatherdata/) and photoperiod (hours) for Reading (http://dateandtime.info/citysunrisesunset.php?id=2639577)

Table 5.4 Date of monthly transfer from natural inductive conditions to controlled forcing conditions

Transfer month	Date of transfer
August	31 August 2016
September	14 September 2016
October	12 October 2016
November	16 November 2016

Table 5.5 Supplementary illumination duration and time periods

Experiment period	Morning (AM) Extension	Evening (PM) Extension
31 August 2016 – 21 October	0500 – 0700	1800 – 2300
22 October – 08 February 2017	0500 – 0800	1600 – 2300

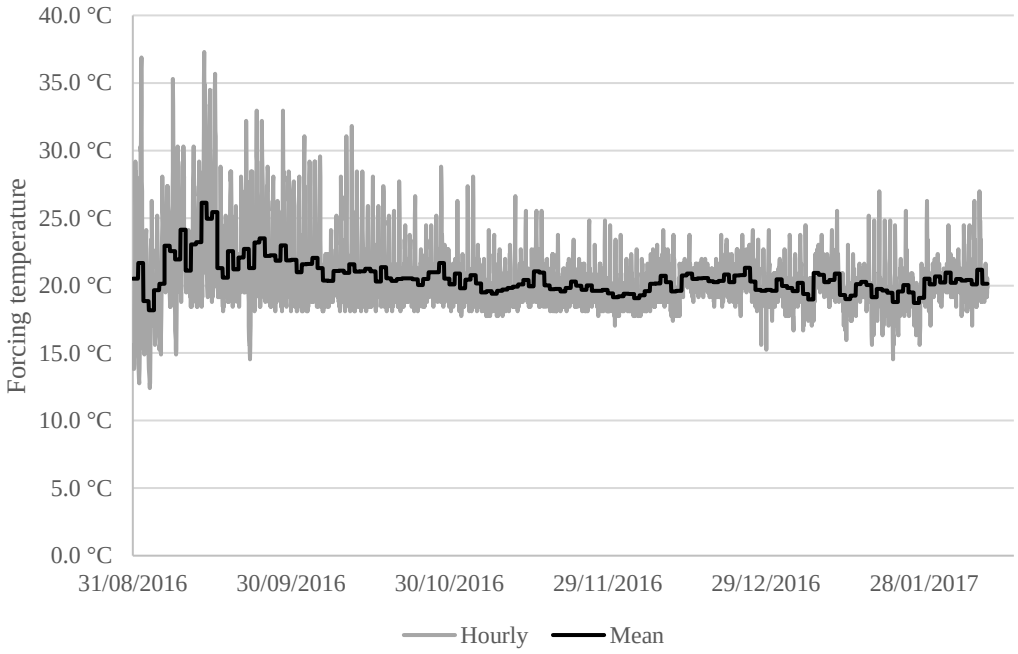


Figure 5.4 Mean daily forcing temperature (°C), with maximum-minimum temperature range shaded in grey

Plant numbers

230 plants were included in this experiment (50 replicates of UK2 and UK12 and 130 replicates of UK9). These plants were initially maintained in the field, and at time of transfer a randomly selected cohort of 10 plants for each ecotype was transferred to forcing conditions; over the experiment 40 plants per ecotype were therefore transferred from field to forcing conditions. More UK9 plants were included because a subset of (10) plants was dissected at the time of transfer to establish the state of floral initiation before transfer to forcing conditions. Additional plants were also included of all ecotypes to allow for plant death in the field.

Parameters recorded

Prior to the start of the experiment, all runners were removed and subsequently the following growth and development parameters were recorded:

In the field

- Runners were removed and recorded every two weeks.

At the time of transfer

- 10 UK9 plants were dissected to establish the state of floral initiation at the SAM and in branch crowns.

For transferred plants (over the 12 week forcing period)

- Timing of flower emergence (assessed every two-three days once flower emergence was first observed)
- Runners were removed and recorded every two weeks.

Inflorescence morphology was recorded at the end of the forcing period (08/02/2017) for November-transferred plants.

The following inflorescence morphological traits were recorded (Figures 5.5 and 5.6):

- Number of inflorescences per plant
- Number of flowers in each inflorescence
- Peduncle length
- Pedicel length to the primary flower
- Total inflorescence length.

Statistical analysis

Data were tested for normality of distribution using the Ryan-Joiner test, and flowering and runnering responses found not to be normally distributed. Kruskal-Wallis tests were therefore performed to analyse these variables and showed comparable levels of significance with equivalent one-way ANOVA. In order to increase the robustness of the analysis and to enable the interaction between factors to be analysed a two-way ANOVA was used to determine the effect and interaction of transfer time and ecotype on runnering and flowering response. Within transfers, a one-way ANOVA was used to determine whether ecotypes differed significantly in these responses. In order to analysis ecotypic differences in inflorescence morphology, each inflorescence parameter was tested for equal variance. Ecotypes varied in sample size due to plant deaths and natural variation in the number of inflorescences between plants and ecotypes. A one-way ANOVA was used to determine whether ecotypes significantly differed for each recorded inflorescence parameter. All replicates were included within the statistical analyses and averages and standard error of the mean were calculated.

Figure 5.5 Parameters of inflorescence structure, including: peduncle length (solid line), peduncle and pedicel length to the primary flower (dashed line) and total inflorescence length (dotted line)

Figure 5.6 Examples of recording inflorescence characters

5.2.3 Seeds versus runner experiment

Plant material

The three ecotypes used in this experiment were included in the 2015 and 2016 experiments; UK2, UK9 and UK12. Plant material was derived through asexual and sexual propagation (runners and seeds, respectively). Runner-derived individuals were collected on 01/08/2016, and vegetatively propagated as described in Chapter 2. Seed-derived individuals were grown from seeds collected from plants in the 2015 experiment (while under forcing conditions).

Growth conditions

Following vegetative propagation, runner-derived individuals were potted on (29/08/2016) (using growing mix 3, into 10cm pots – see Chapter 2 for details) and transferred to a vented and heated multifactorial glasshouse. Temperature was maintained above 18°C to ensure the plants remained vegetative. Plants were exposed to natural photoperiod and in order to maintain LD conditions (to prevent floral induction) supplementary illumination was provided from four evenly spaced high pressure sodium lamps (OSRAM SON-T 400W) to ensure an 18 hour photoperiod. Shade netting was used to prevent scorching as necessary.

Seed-derived individuals were grown from seeds sown in seed trays (09/07/2016) in 2 parts multi-purpose compost: ½ part grit: ½ part sharp sand: 1 part vermiculite. Seed trays were maintained in a propagator with bottom heat for 10 days, after which time they were placed in a conservatory under natural light and temperature. Watering was by hand, as necessary. On 19/08/2016, the seedlings were pricked out into individual cell trays (growing mix 3). They were left in a potting shed with subdued light for approximately one week after which time they were placed in the vented and heated multifactorial glasshouse. Seed-derived plants were potted on 23/09/2016 (in 10cm pots, using growing mix 3) and maintained under glass, alongside the runner-derived individuals.

All the plants were maintained under glass until the start of the experiment (12/01/2017), when plants were transferred to a controlled environment (Sanyo cabinets, Sanyo Gallenkamp, Leicester) to promote floral induction. Under these conditions, plants were exposed to a constant temperature (15°C) and SD (10h). Photoperiod was provided by a combination of tungsten and fluorescent light from 0800-1800 (160 μmol m^{-2} sec^{-1}). Plants were exposed to floral inductive conditions for six weeks, and then transferred to forcing conditions provided by a multi-factorial glasshouse compartment, with high temperature (maintained above 18°C) and LD (18h). The 18h

photoperiod was provided by supplementary illumination from four evenly spaced high pressure sodium lamps (OSRAM SON-T 400W) between 0500-0800 and 1600-2300. Plants were forced for 10 weeks.

Plant numbers

There were six cohorts included in this experiment, with a cohort of runner derived plants and a cohort of seed derived plants of UK2, UK9 and UK12. There were 25 plants in each cohort; five plants were dissected at the start of the experiment (prior to exposure to floral inductive conditions, to ensure they were vegetative) and the remaining plants were transferred to floral inductive conditions and subsequently to forcing conditions.

Parameters recorded

All runners were removed at the start of the experiment and at the end of the floral inductive treatment. Once plants were transferred to forcing conditions, time to inflorescence emergence was recorded, through visual observations every two-three days.

Dissections of seed-derived individuals were undertaken under forcing conditions as the plants did not show any visually observed floral response. Dissections were carried out on a subset of five plants of each of the seed derived cohorts (30/05/2017), with focus at the shoot and branch crown apices in order to whether the plants had transitioned to a floral state.

5.3 Results

5.3.1: 2015 experiment

Flower emergence

The first cohort of plants from each ecotype was transferred on 18/08/2015, and no flowering response was recorded for any ecotype (Figure 5.7). Flower emergence was observed following the September transfer (15/09/2015) in six of the eight ecotypes (F6, F7, F50, F53, UK2 and UK11) (Figure 5.7). However, not all plants in these ecotypes flowered during the 12-week forcing period (Table 5.6). This mixed flowering response was most pronounced for the UK ecotypes: in UK2 and UK11, only 30% and 60% of the plants flowered, respectively (Table 5.6). By contrast, all of the Finnish ecotypes flowered and flower emergence was recorded for at least 70% or more of the plants of each ecotype, with all F53 plants flowering (Table 5.6). Regardless of differences in the proportion of flowering plants, ecotypes showed statistically significant differences in their timing of flower emergence following the September transfer ($F_7 = 10.56$; $P = 0.00$).

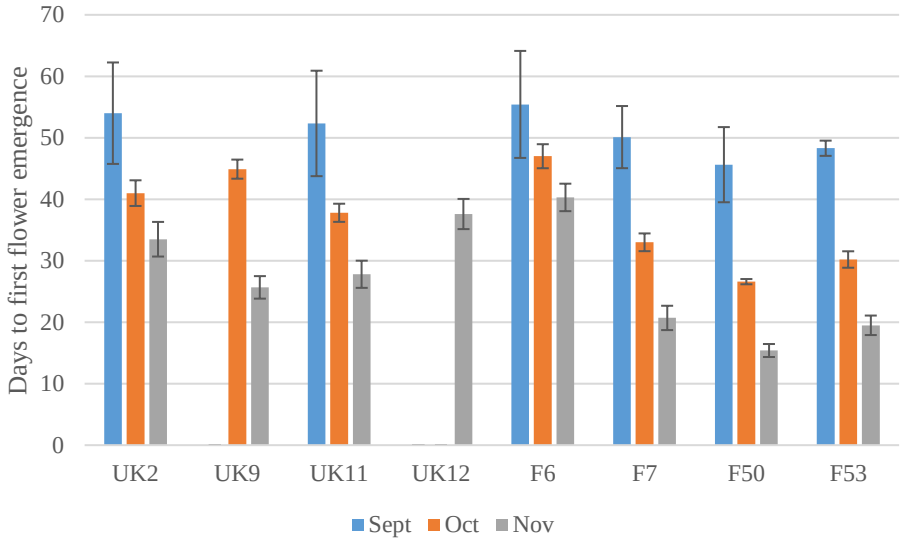


Figure 5.7 Mean days to first flower emergence for *F. vesca* ecotypes in each transfer month. Standard error of the means are shown

Table 5.6 Proportion of plants showing flower emergence, within each transfer month, for all ecotypes. Total plants per transfer = 10

	Transfer month			
Ecotype	Aug	Sept	Oct	Nov
UK2	0	0.3	1	1
UK9	0	0	1	1
UK11	0	0.6	1	1
UK12	0	0	0	1
F6	0	0.7	1	1
F7	0	0.9	1	1
F50	0	0.8	1	1
F53	0	1	1	1

Following the October transfer (14/10/2015), flowers emerged in seven of the eight ecotypes; all of the ecotypes which had flowered following the September transfer flowered, with emergence also recorded for UK9, which had not flowered following the previous transfer (Figure 5.7). Similarly to the September transfer, differences in timing of flower emergence following the October transfer were statistically significant ($F_7 = 106.01$; $p = 0.00$). In contrast to the September transfer, all the plants in each of the seven flowering ecotypes flowered following the October transfer (Table 5.6).

In the final transfer (18/11/2015), all ecotypes and all plants within each ecotype flowered (Figure 5.7; Table 5.6). The differences in flower emergence time between each of the eight ecotypes were statistically significant following the November transfer ($F_7 = 18.41$; $p = 0.00$). Order of flower emergence following transfer of the November cohort was as follows: F50, F53, F7, UK9, UK11, UK2, UK12 and then F6. This order of emergence was similar to that observed following the October transfer, except that UK9 was observed to flower after UK11 and UK2; and UK12 did not flower (Figure 5.7). All of the Finnish ecotypes apart from one (F6) showed more rapid flower emergence on average than the UK ecotypes, this response was observed across transfers.

As well as statistical significance between ecotypes in flowering response following each of the transfers, the timing of flower emergence between transfers, and the interaction between transfers and ecotypes were statistically significant ($F_{21} = 16.21$; $p = 0.00$). In general, the later the transfer the more rapidly flower emergence occurred. Thus, plants of all ecotypes flowered earlier following the November transfer compared with those which flowered following the September transfer (Figure 5.7). As well as differences in the proportion of plants which flowered and the

timing of emergence between transfer months, variation in the flowering response was also observed between ecotypes and transfer months: greater variation in timing of flower emergence was recorded for plants that flowered in the September transfer, compared to those transferred in October and November. Interestingly, plants in the October and November transfer showed similar variation in emergence time, even though November plants flowered earlier (Figure 5.7).

Number of emerged flowers

As well as differences in timing of flower emergence, the number of flowers to emerge on each plant was also recorded. There was a general trend of an increase in the number of emerged flowers with successive transfers, so that for all ecotypes, the plants in the November transfer produced more flowers than those from the September/October transfer by the end of the 12-week forcing period (Figure 5.8). This difference in the total number of emerged flowers between transfers was statistically significant ($F_3 = 426.50$; $p = 0.00$).

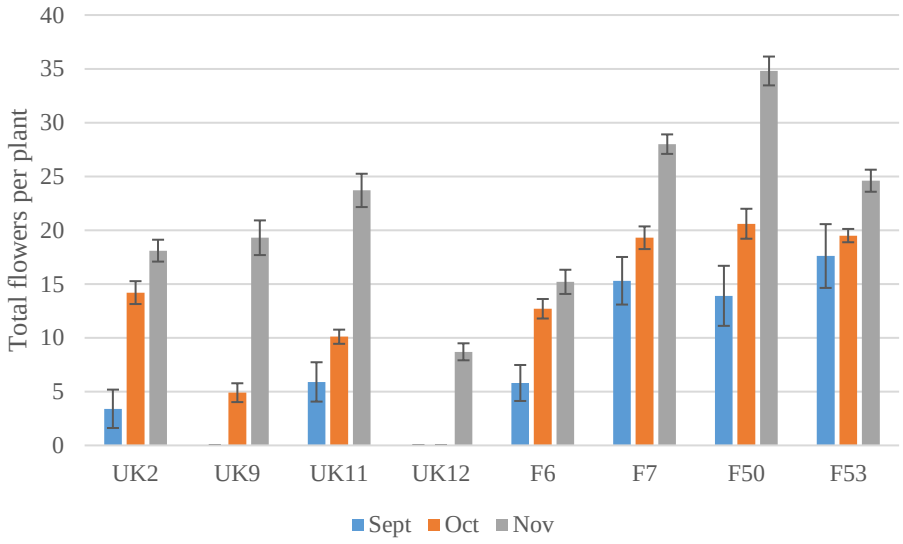


Figure 5.8 Mean total flowers produced per plant for *F. vesca* ecotypes in each transfer month. Standard error of the means are shown

Flower production also significantly differed between ecotypes ($F_7 = 72.31$; $p = 0.00$) and ecotypes showed a varied response in flower production between transfers. For example, ecotype F53 produced the most flowers following the September transfer and there was little difference in the total number of flowers produced in the two following transfers (October and November) (Figure 5.8). On the other hand, ecotype F50, which was similar in flower production to F53 following the September transfer, showed a more pronounced difference in the total number of flowers produced in the subsequent transfer and of all the ecotypes produced the most flowers

following the November transfer (Figure 5.8). There was a significant interaction between transfers and ecotypes on flower production ($F_{21} = 12.47$; $p = 0.00$). In comparison to emergence time, however, within each transfer cohort there was less variation in flower production between Finnish and UK ecotypes.

Runner production

Runner production declined during the experimental period (Figure 5.9). For most ecotypes, plants produced most runners following transfer to forcing conditions in August, with a decline in production in subsequent transfers. However, some ecotypes showed contrasting results: UK9 plants showed similar runnering behaviour following the August, September and October transfers with no evidence of declining production, with the highest number of runners recorded following the September transfer. UK12 also showed contrasting results, with a decrease in runner production following the August and September transfer, whilst plants following the October transfer produced more runners than following any of the other transfers (Figure 5.9). Regardless of specific ecotype differences, runner production differed significantly between ecotypes ($F_7 = 80.29$; $p = 0.00$) and transfers ($F_3 = 329.91$; $p = 0.00$), and the interaction between these two factors also significantly influenced runner production ($F_{21} = 15.81$; $p = 0.00$).

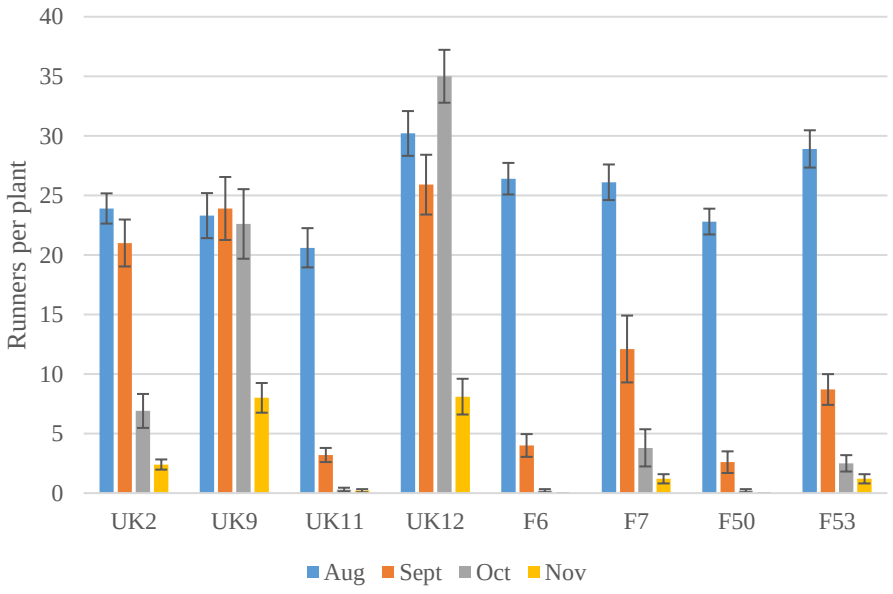


Figure 5.9 Mean total number of runners per plant for *F. vesca* ecotypes accumulated by the end of each successive forcing period. Standard errors of the means are shown

There was a general difference between Finnish and UK ecotypes following the September transfer, with the Finnish plants showing a greater decrease in runner production between August

and September, than was observed for the UK ecotypes apart from UK11. Some ecotypes produced no runners following the later transfers (October and November). This was particularly pronounced for the Finnish ecotypes, which showed greatly reduced runner production following the October transfer and no runner production following the November transfer. All of the UK ecotypes produced runners following the October transfer, although this was greatly reduced for UK11. Very limited runner production was recorded for all UK ecotypes following the November transfers (Figure 5.9). Overall UK11 runner behaviour was more similar to the Finnish ecotypes than the other UK ecotypes, which differed from flower emergence, for which UK2 and UK11 were more similar to the Finnish ecotypes.

Variation in runner production between and within ecotypes was always observed across ecotypes: unlike flower emergence there was no evidence of decreased variation within ecotypes with successive transfers. In order to distinguish whether differences in runner production arose as a result of natural ecotypic variation or in response to inductive conditions, runnering response following the August transfer was analysed, as it showed how ecotypes differed in runner production in the absence of flower emergence (Figure 5.10).

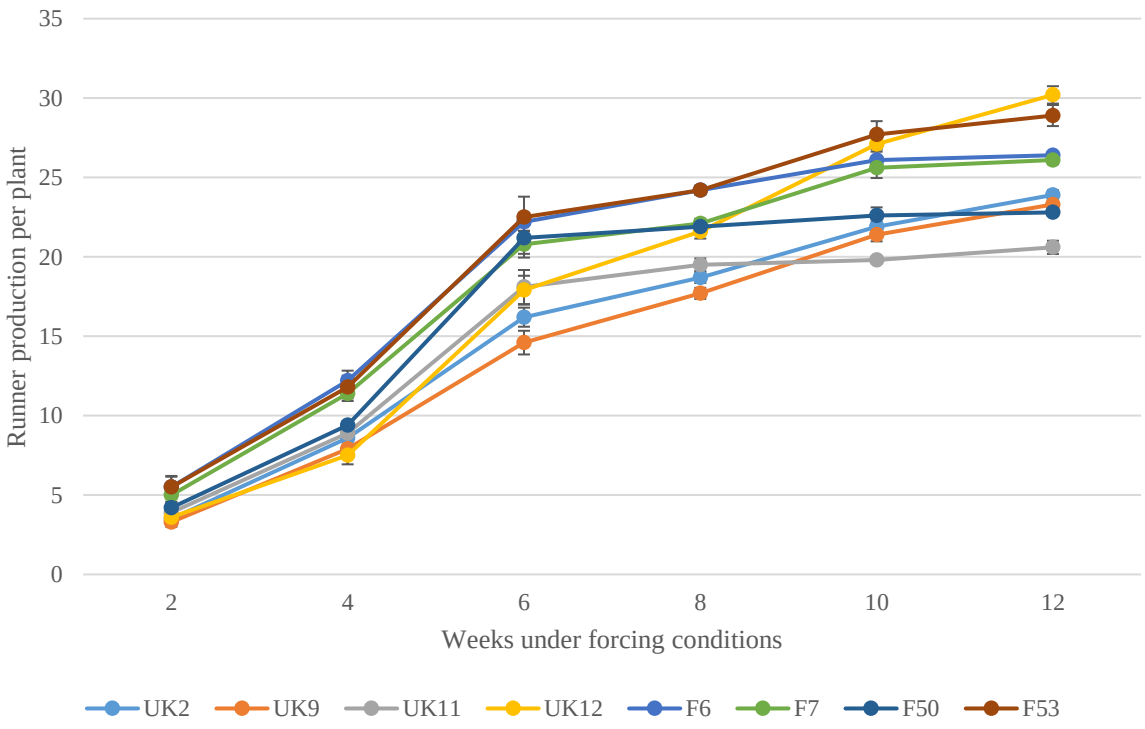


Figure 5.10 Mean accumulated runner production per plant for *F. vesca* ecotypes following the August transfer over the forcing period. Standard error of the means are shown

Plants produced runners over the duration of the forcing period following the August transfer, with all ecotypes showing a general increase in total mean runner production over the 12 weeks

(Figure 5.10). There was evidence of ecotypic variation in runner production over the forcing period (F_7 = 4.31; p = 0.00), and this was statistically significant across all recording weeks (F_5 = 176.63; p = 0.00). There was a general divide between ecotypes across forcing weeks: UK11 and the Finnish ecotypes were not significantly different, whilst the other UK ecotypes (UK2, UK9 and UK11) were significantly different in runner production to the Finnish ecotypes and UK11; this group split was particularly apparent in week 8 and 12. Interestingly, the two ecotypes to show greatest runner production by the end of the forcing period (week 12) following this transfer were UK12 and F53; however, while UK12 remained a prolific runnering ecotype, F53 was not one of the more runnering ecotypes following subsequent transfers (Figure 5.9).

5.3.2: 2016 experiment

The second experiment (2016) used only three ecotypes from the first experiment (2015). These three ecotypes were those used in other experimental chapters, and also represented the greatest latitudinal variation for UK ecotypes. The main objective of this second experiment was to record whether the differences in response observed in 2015 were maintained for plants grown in a common environment (at the University of Reading) over a growing season. Differences in inflorescence structure were also quantified.

Flower emergence

The first cohort of plants was transferred to forcing conditions on 31/08/2016, and no flowering response was observed, regardless of ecotype (Figure 5.11). Flower emergence was observed in 3 out of 10 UK2 and 1 out of 10 UK12 plants following the transfer of the second cohort, in September (14/09/2016) (Table 5.7). Flower emergence occurred first in UK12 (Figure 5.11); however, mean days to first flower emergence did not significantly differ between ecotypes (F_2 = 1.71; p = 0.20).

All three ecotypes flowered following the October transfer (12/10/2016). Flowers emerged earlier for plants transferred in October, in comparison to those in September. Flowering of all plants was only observed in UK2 (Table 5.7), and UK2 was the earliest flowering, followed by UK9 and then UK12 (Figure 5.11). Timing of flower emergence was not significantly different between ecotypes (F_2 = 1.28; p = 0.29).

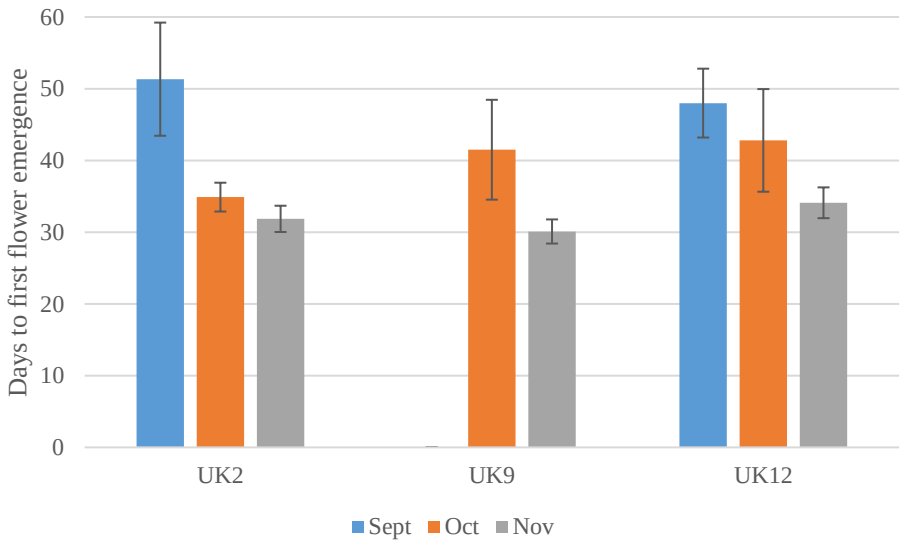


Figure 5.11 Mean days to first flower emergence for *F. vesca* ecotypes in each transfer month. Standard errors of the mean are shown

Table 5.7 Proportion of plants showing flower emergence within each ecotype and transfer month

	Transfer month		
Ecotype	Sept	Oct	Nov
UK2	0.3	1	1
UK9	0	0.7	1
UK12	0.1	0.5	1

As expected, all three ecotypes also flowered when transferred in November (Figure 5.11; Table 5.7). Timing of flower emergence did not significantly differ between ecotypes (F_2 = 1.19; p = 0.32), but the order of emergence differed from October: UK9 first, then UK2, then UK12 (Figure 5.11). Timing of flower emergence significantly differed between transfers (F_2 = 21.08; p = 0.00), and flowers emerged earlier following the November transfer than for any other transfer.

Inflorescence structure

The number of inflorescences per plant was recorded at the end of the forcing period and inflorescences were then removed to allow for further observations of inflorescence morphology. Ecotypes showed no significant difference in the average number of inflorescences per plant (F_2 = 0.50; p = 0.61), with 7.3 ± 0.8, 7.8 ± 0.8 and 8.8 ± 1.3 inflorescences for UK12, UK2 and UK9, respectively, by the end of the forcing period (Figure 5.12A). There was a significant difference between ecotypes in the average number of flowers per inflorescence (F_2 = 5.86; p = 0.00) (Table

5.8), with UK12 having the highest and UK2 the lowest (Figure 5.12B; Table 5.8). Ecotypes varied in the maximum number of flowers per inflorescence, but all had at least one inflorescence with a single flower (Table 5.8). Inflorescences with a single flower were typically those from a branch crown, which was assumed to have been initiated later than the inflorescence at the terminal SAM.

Size of the inflorescences was also recorded. Peduncle length, from the site where the inflorescence joined the main crown to the first branch on the inflorescence (see Figure 5.5) differed significantly between ecotypes (F_2 = 5.43; p = 0.01). UK9 had the greatest average peduncle length, followed by UK12 and UK2 (Figure 5.12C). Pedicel length from the top of the peduncle to the base of the primary flower did not however, differ significantly between ecotypes (F_2 = 0.78; p = 0.46), and in comparison to peduncle length, UK9 had the smallest pedicel length on average, while UK12 had the largest (Figure 5.12D). Total inflorescence length, from the point where the inflorescence joined the main crown to the highest point of the inflorescence was marginally significant between ecotypes (F_2 = 4.69; p = 0.01). Ecotypic variation in this parameter most resembled that for peduncle length: UK9 plants had the greatest total inflorescence length, followed by UK12 and UK2 (Figure 5.12E).

Dissections

In order to establish the accuracy of recording flower emergence under forcing conditions as a measure of timing of floral initiation, dissections of UK9 at the time of transfer were carried out (due to restricted plant numbers, dissections were only possible for this ecotype). There was no evidence of floral initiation in any plants at the time of transfer to forcing in the August or September cohorts (Table 5.9). Dissections at the time of transfer in October (12/10/2016) showed floral initials in six out of 10 plants, and some (more advanced) plants also showed floral initiation in basal branch crowns. Seven out of 10 (UK9) plants flowered following transfer in October cohort (Table 5.9).

All plants dissected in November (16/11/2016) showed floral initiation at the SAM, and floral initials were typically more developed than in October (Table 5.9). Floral initiation was also observed in some branch crowns for all plants. Flower emergence therefore appeared to be a reliable indicator of floral initiation under experimental conditions.

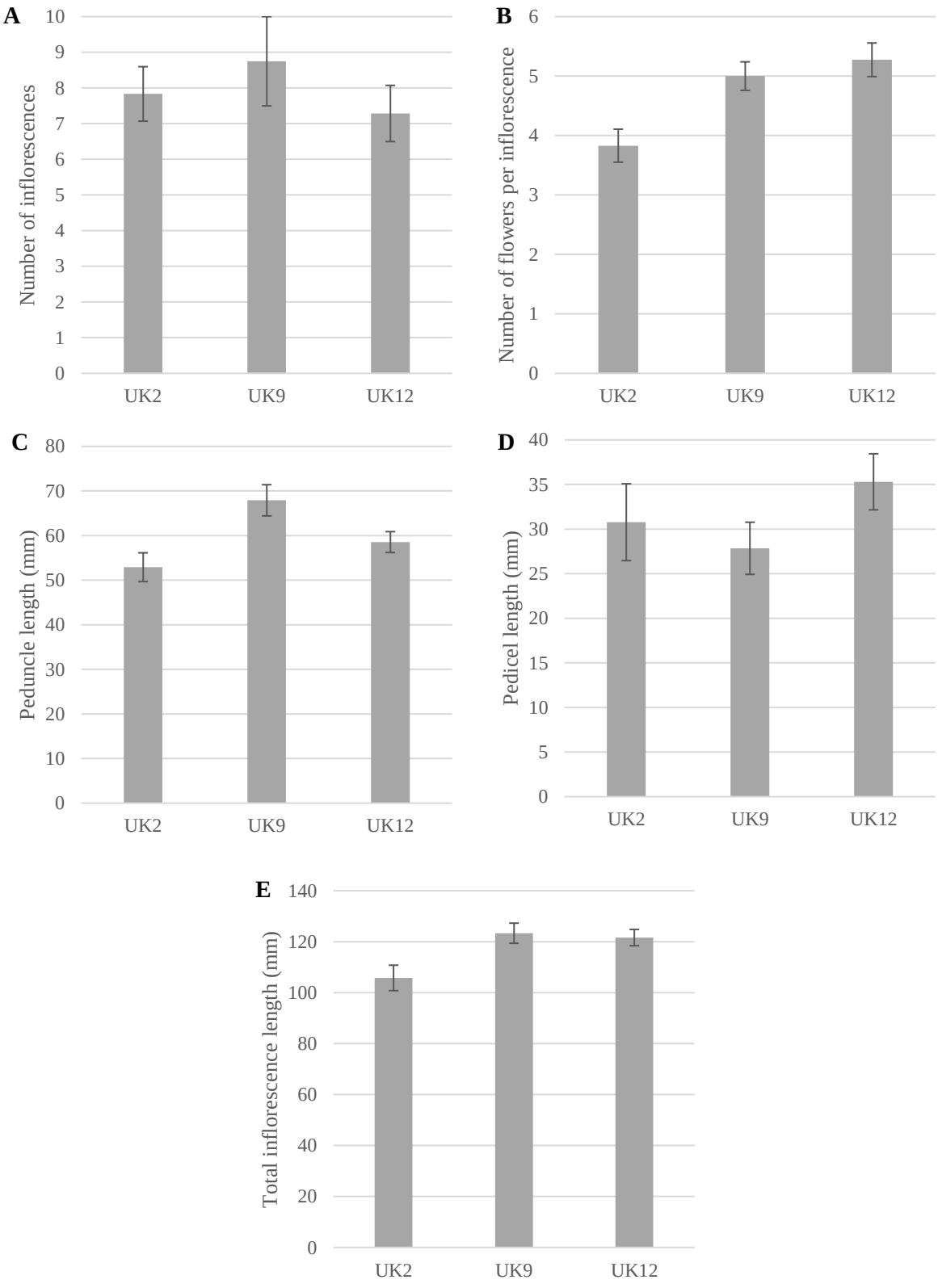


Figure 5.12 Inflorescence morphology in *F. vesca* ecotypes UK2, UK9 and UK12. Means of the following are shown: A, number of inflorescences; B, number of flowers/inflorescence; C, peduncle length; D, pedicel length; E, overall inflorescence length. Parameters were recorded at the end of the forcing period following the November transfer. Standard errors of the mean are shown

Table 5.8 Mean, minimum and maximum number of flowers per inflorescence for each ecotype on 08/02/2017 for plants transferred to forcing conditions in November

	Mean	Minimum	Maximum
UK2	3.8 ± 0.3	1	8
UK9	5 ± 0.2	1	10
UK12	5.3 ± 0.3	1	16

Table 5.9 State of flower initiation, observed through dissections, for UK9 plants prior to each transfer to forcing conditions in 2016

Transfer	**Floral initiation at the SAM**	**Floral initiation in branch crowns throughout the main crown**	**Mean days to flower emergence and the proportion of flowering plants**
August	No floral initiation	No floral initiation	No flowering
September	No floral initiation	No floral initiation	No flowering
October	Some plants showed primary inflorescence – primary flower (typically at stage 2-4), with secondary flowers	Floral initiation in some basal branch crowns	41.5 ± 7.0 days 0.7 plants
November	Primary inflorescence – primary flower (typically at stage 6), with secondary and tertiary flowers	Inflorescences typically observed in basal branch crowns and in some upper branch crowns	30.1 ± 1.7 1.0 plants

Runner production

Runner production varied significantly between ecotypes ($F_2 = 15.86$; $p = 0.00$) and transfers ($F_3 = 82.08$; $p = 0.00$) and was greatest following the September transfer, with more runners produced in September than in August followed by a reduction in runner production after the October and November transfers (Figure 5.13). During successive forcing periods, UK2 plants showed the greatest decline in runner production following the October transfer, whereas in UK9 and UK12 plants it was most pronounced following the November transfer (Figure 5.13). UK12 consistently produced most runners, while UK2 produced more runners than UK9 following the August and

September transfers, but UK9 produced more than UK2 following the October and November transfers (Figure 5.13).

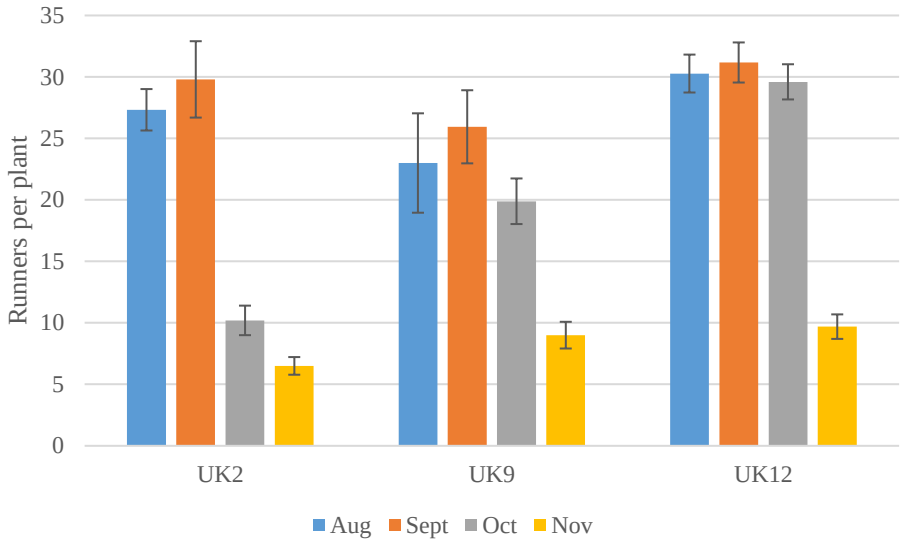


Figure 5.13 Mean total number of runners per plant for *F. vesca* ecotypes accumulated by the end of each successive forcing period. Standard errors of the means are shown

As well as the total number of runners accumulated following each transfer, the pattern of accumulation over the forcing period was also recorded for plants following the August transfer, when no flowering occurred (Figure 5.14). All ecotypes showed an increase in the total number of runners produced over the forcing period (Figure 5.14). UK12 produced the most runners, followed by UK2 and UK9, although the difference in runner accumulation by the end of the forcing period was not significantly different between ecotypes ($F_2 = 2.40$; $p = 0.14$).

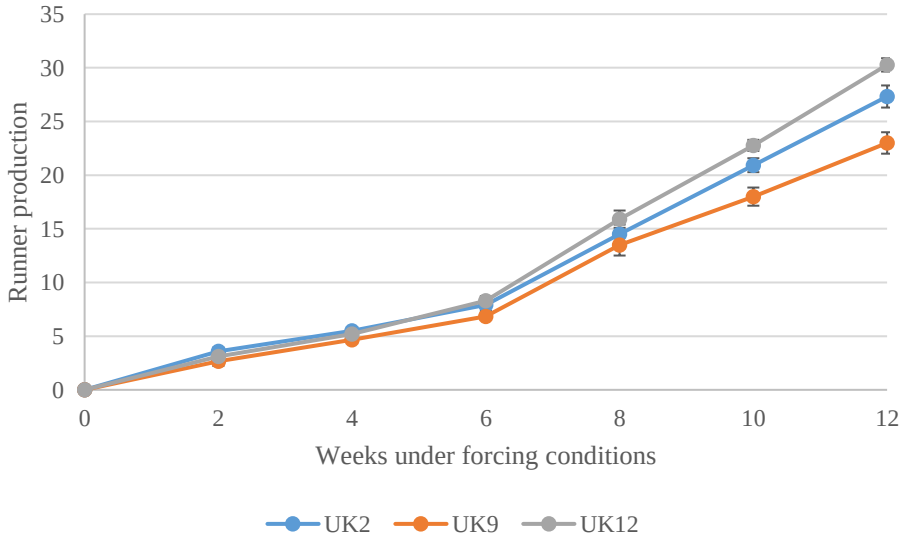


Figure 5.14 Mean accumulated runner production per plant for *F. vesca* ecotypes following the August transfer over the forcing period. Standard error of the means are shown

5.3.3 Summary and comparison of the 2015 and 2016 experiments

Flower emergence and runner production data from both years were compared for UK2, UK9 and UK12, in order to establish ecotypic differences within and between transfers and to assess whether the responses observed in 2015 were maintained in 2016.

Flowering response

Ecotypes had a significant interaction with transfers (F_6 = 12.37; p = 0.00) and years (F_2 = 3.77; p = 0.03) with regards to days to flower emergence, and the mean days to flower emergence was earliest for all ecotypes following transfer to forcing in November in both years. UK2 plants flowered following all three transfers and there was similarity in the time to first flowering between years (Figure 5.15). There was some variation between transfers in the number of plants to flower, but in both years three out of 10 (UK2) plants flowered following transfer to forcing conditions in September and all plants flowered following transfer in October and November (Figure 5.15).

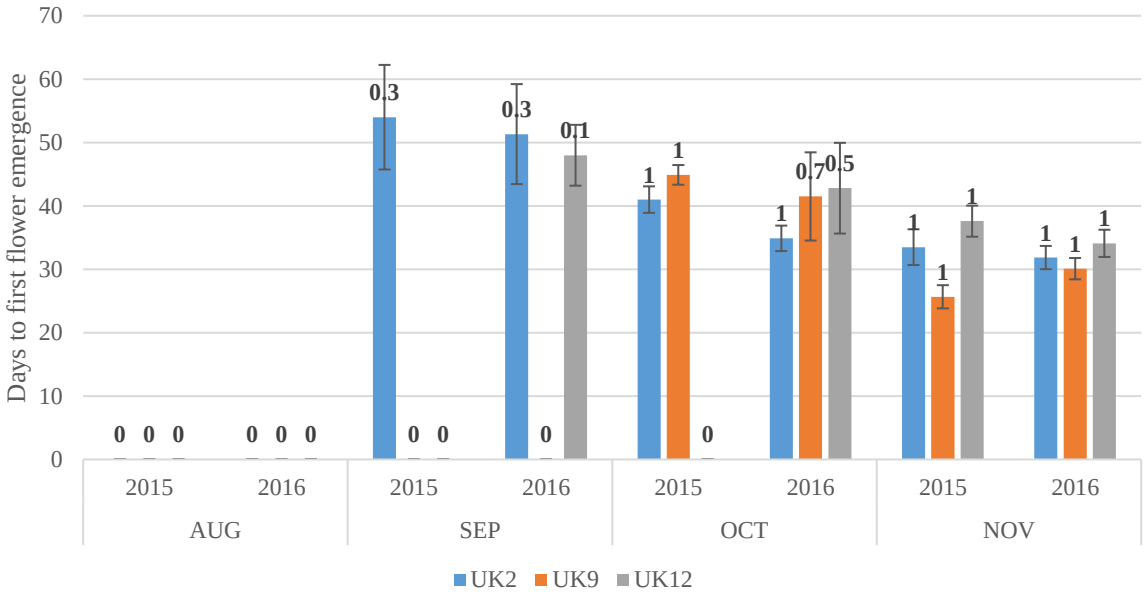


Figure 5.15 Mean days to first flower emergence for *F. vesca* ecotypes included in both experiments, for each transfer month. Standard errors of the mean are shown. The proportion of plants to flower within each transfer cohort is included as a figure above each bar

UK9 plants did not flower until the third transfer (in October) and although this late flowering response (in comparison to UK2) was observed in 2015 and 2016, time to first flowering differed between years, with earlier flower emergence in the second year (2016) (Figure 5.15). The

proportion of flowering plants also differed between years; all UK9 plants flowered following the October transfer in 2015, whereas only seven out of 10 plants flowered in 2016 (Figure 5.15). The final cohort of plants transferred in November showed similarity in their response between years: all plants flowered and this ecotype were the last to flower.

UK12 showed the most pronounced difference in flowering response between years. No plants flowered following the September or October transfer in 2015, but in 2016, one out of 10 plants and five out of 10 plants flowered following the September and October transfers, respectively (Figure 5.15). All UK12 plants flowered following the November transfer in both years and plants showed similarity in their time to first flower emergence between years (Figure 5.15).

Runnering response

Runner production varied between years, transfers and ecotypes, and combined data from both experiments showed a significant interaction between ecotype and transfer ($F_6 = 16.54$; $p = 0.00$), ecotype and year ($F_2 = 4.41$; $p = 0.01$) and transfer and year ($F_3 = 3.08$; $p = 0.03$). Ecotypes varied in their runner production within and between transfers and UK12 produced the greatest number of runners in all transfers, regardless of year (Figure 5.16). UK2 and UK9 both produced fewer runners than UK12 in all transfers, and there was interchange between UK2 and UK9 in which produced the more runners following the August and September transfer.

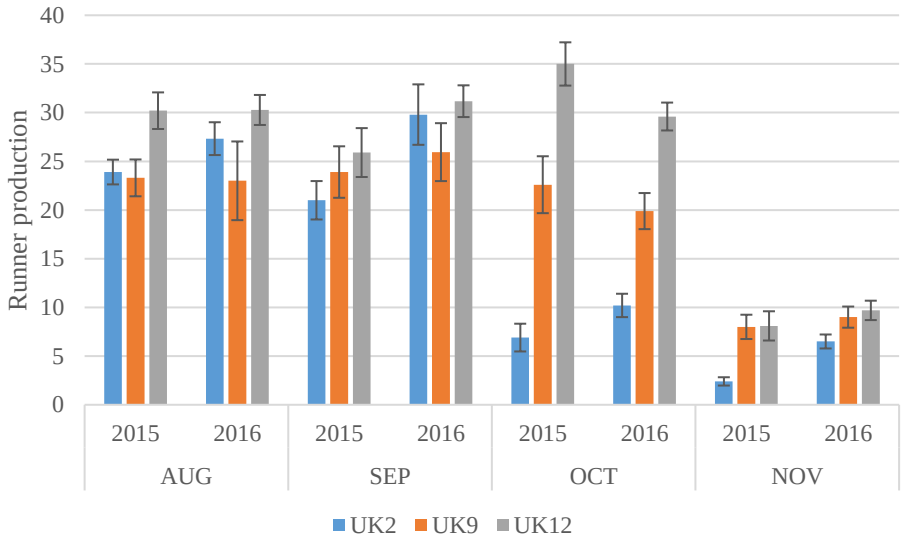


Figure 5.16 Mean total number of runners per plant accumulated by the end of each successive forcing period for *F. vesca* ecotypes included in both experiments. Standard errors of the means are shown

Runner production following transfer in October showed the most pronounced difference between ecotypes in both years, with UK12 maintaining high runner production (similar to that following the August and September transfer), while UK2 showed the most distinct decline in production (Figure 5.16). Runners were produced under forcing conditions for all transfers, although there was a significant difference in the number of runners produced between transfers (F_3 = 127.54; P = 0.00) and plants transferred to forcing conditions in November produced the fewest runners (Figure 5.16).

Runner accumulation during exposure to forcing conditions following transfer in August was illustrated to highlight the pattern of runner production over a singular forcing period (Figure 5.17). Ecotypes differed in the runnering response between years and UK2 showed the most apparent difference in total accumulation. Runner response varied across the forcing treatment between years for each ecotypes, but regardless of this variation, there was a general response of runner production across plants, with an increase in the number of runners accumulated with forcing time, and UK12 produced the greatest number of runners in both years, followed by UK9 and then UK2 (Figure 5.17).

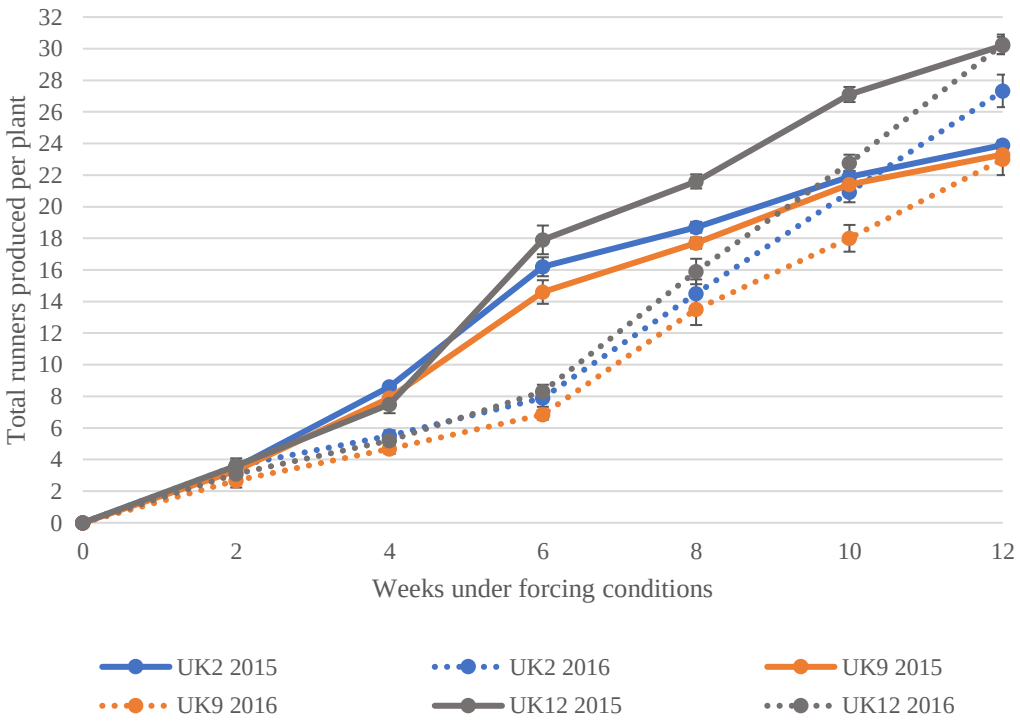


Figure 5.17 Mean accumulated runner production per plant over the forcing period for *F. vesca* ecotypes following the August transfer in both experimental years. Standard error of the means are shown

5.3.4 Seeds versus runners experiment

Dissections at the start of the experiment showed that all cohorts were vegetative. Plants were then subjected to floral induction for six weeks and subsequently transferred to forcing conditions for 10 weeks.

Flower emergence was recorded for the runner-derived individuals of all ecotypes; seed-derived individuals showed no floral response, based on visual observations and dissections. This meant that a comparison of time to flower emergence for seed- and runner-derived individuals was not possible.

5.4 Discussion

In the first experiment (2015), ecotypes of *F. vesca* were collected from a range of locations and grown in a common environment at Reading. The main objective was to establish whether these ecotypes differed in their flowering response to the environment, as measured by successive transfer of cohorts from natural, inductive conditions, into a non-inductive, forcing glasshouse (warm/LD). Between-ecotype variation in the timing of flower emergence in this situation would reflect differences in sensitivity to inductive conditions - shortening photoperiod, declining temperature - and/or differences in the duration of these conditions required for flower induction. An additional aspect of the experiment was to investigate the vegetative response of the plants: did ecotypes differ in their runnering response to the changing environment; were any differences a reflection of differences in flowering (in other words, did the two aspects directly mirror each other); or did runnering and flowering vary independently?

The second experiment (2016) repeated the first experiment with fewer ecotypes (UK2, UK9 and UK12), chosen because of their range of latitudes of origin and because they were early, middle and late in flowering time in the 2015 experiment. More detailed measurements of inflorescence morphology were made to see if this accounted for differences in flower number. Finally, and importantly, the repeat experiment was conducted using material that had been maintained at Reading over the intervening year; this allowed the stability of the flowering time response through vegetative propagation to be tested.

5.4.1: 2015 experiment

In 2015 the ecotypes differed significantly in timing of flower emergence. The more northerly UK (from the Lake District) and Finnish ecotypes (UK2, UK11, F6, F7, F50 and F53) were similar, flowering following the September transfer. The more southerly UK ecotypes (UK9 and UK12, from Berkshire and Devon) did not flower until after the October and November transfers, respectively. There was, therefore, between the two most different ecotypes (F50 and UK12), a three-month difference in timing between the beginnings of flower emergence. As well as variation in timing of flower emergence, differences were also observed in the variability of emergence time. Plants following the September transfers showed greater variation in emergence time than those following the October and November transfers. One potential explanation for this difference in variability between early and later transfers is that flower emergence took longer for the plants following the September transfer and not all plants from each ecotype flowered, which

would have resulted in greater variability than for the subsequent transfers, which showed earlier emergence and complete flowering.

It seems likely that timing of flower emergence, as measured here under forcing conditions, reflected timing of flower initiation, although this required more detailed investigation. Dissections were undertaken at the time of transfer in the following year's experiment (2016) for UK9 plants, to assess the state of initiation at the meristem, prior to transfer for forcing of flower emergence and suggested this was a reasonable assumption (Table 5.9). The implication is that the two most different ecotypes in 2015 (F50 and UK12) either required a very different environment (mean temperature/photoperiod for the month preceding the September transfer, 15.1°C/13 h; for the month preceding the November transfer, 11.9°C/9 h), or very different durations in a generally inductive environment. Presumably the other ecotypes fell somewhere between these two in their requirements for flower induction.

Heide and Sønsteby (2007) showed ecotypic variation in requirements for flower induction; under a controlled environment they suggested a minimum of four weeks exposure to optimal conditions (15°C/16 h or 18°C/14 h) was necessary for induction: all plants exposed to five weeks induction were shown to flower, whilst not all plants exposed to four weeks of inductive conditions flowered, with ecotypic variation in the percentage of flowering plants. The observed differences between the most southerly (UK9 and UK12) and the more northerly ecotypes might reflect a difference in the duration of exposure required to induce flowering and inhibit runner production.

In the 2015 experiment, ecotypes also differed significantly in their vegetative response, recorded by the number of runners produced. UK9 and UK12 were distinct from the other ecotypes in runnering as well as flowering, showing sustained and high runner production throughout the autumn, relative to other ecotypes. Northerly UK and Finnish ecotypes typically showed a decline in runner production following the September or October transfer. UK12 consistently produced the greatest number of runners in all transfers, and UK9 showed higher runner production than the northerly UK and Finnish ecotypes following the September, October and November transfers. Thus it appeared from this experiment that late flower initiation in the two southern UK ecotypes was associated with prolonged runner production. Heide and Sønsteby (2007) also considered ecotypic variation in vegetative response and showed that ecotypes that were "slow-to-respond" to floral induction generally produced more runners. This suggests a possible "choice" between vegetative and reproductive growth within ecotypes, which could reflect differences in reproductive effort of the naturalized populations from which these ecotypes were sampled.

There are many perennial species that reproduce using both sexual and asexual propagules, and how a plant allocates resources between these reproductive means is key for survival and reproductive success. The factors that influence resource allocation include: genetic variation (within or between populations), plant size, plant age, population age, population density and environmental conditions (reviewed by Rauntianinen *et al*., 2004). The trade-off between relative rates of vegetative and sexual reproduction can change across species range, often in response to local ecological and demographic conditions (Eckert, 2001). Fine-scale variation in the reproductive mode (reproductive effort given to asexual and sexual reproduction) of *F. virginiana* ecotypes collected within close proximity have been observed, highlighting that ecotypes under similar macro-environmental conditions (photoperiod and temperature) can significantly vary in their means of preferred reproduction (Wilk *et al.*, 2009). In the 2015 experiment, UK2 and UK11 were similar in their flowering response but differed significantly in their runner response. Both ecotypes were collected within close proximity in the Lake District, suggesting that subtle differences in environment and ecology between their sampling sites could have selected for different balances in the preferred mode of reproduction, which was sustained even under a controlled environment.

In *Fragaria*, population density, plant age and light intensity influence the trade-off between asexual and sexual reproduction. Most of these affect growth *in situ*. In natural populations, plant age is likely to be more variable in comparison to experimental conditions, and in natural populations of *F. vesca* plant age has been shown to affect runnering and flowering response, with some plants producing flowers or runners in one year and then requiring one or more years to accumulate reserves before reproduction is again possible, implying a cyclic pattern of reproduction (Jurik, 1985). All the plants included in this Chapter (and throughout the thesis) were vegetatively propagated and of no more than one year old. As a result, variation in plant age within experiments was low and it is unlikely that natural cyclic variations in reproductive effort could account for ecotypic differences in response, unless such cyclic variations were maintained through vegetative propagation.

Population density is another factor which influences resource allocation between sexual and asexual reproduction in *F. virginiana*, with higher vegetative reproductive efforts in lower density treatments (Holler and Abrahamson, 1977). Under experimental conditions here, plant density was less variable compared to plants grown *in situ*, with plants grown in individual pots with sufficient spacing to prevent shading. As a result, it is unlikely that population density promoted ecotypic differences in reproductive efforts between flowering and runnering responses. Smith (1972), however, dismissed the effect of population density for its influence on reproductive effort and instead suggested that seasonal differences in energy expenditure accounted for

variation in sexual and asexual reproduction. All ecotypes in the 2015 experiment showed seasonal variation in reproductive investment, with a decline in runner production over the experimental period and an increase in flowering. These differences in runnering and flowering response, if reflective of the natural situation, could have long-term impacts on plant survival. For example, resource allocation between runners and flowers and overall reproductive productivity will not only influence dispersal and successful establishment, but also the age of plants within the colony, with mortality linked to reproduction (Cook, 1985).

A critical question highlighted by the results presented here is whether the reproductive process observed for *F. vesca* under experimental conditions is representative of that occurring *in situ*; especially in light of the pronounced cyclic patterns in runner and flower production in natural populations of *F. vesca* and *F. virginiana* (Jurik, 1985). Experimental *Fragaria* studies and reviews do not typically report a cyclic habit of reproduction in *F. vesca* (e.g. Heide *et al.,* 2013), but cyclic flowering has been observed as a reproductive trait in some everbearers (Serçe and Hancock, 2005; Sakin and Hancock, 2007). To answer the question, long-term studies of seasonal growth and development, both under experimental conditions and *in situ* are required, and highlight the importance of the comparison between years (2015 *versus* 2016, see below). It is also important to note that growth and development of *F. vesca* is sensitive to manipulation, with runner removal, plant spacing and environment all influencing growth and mortality; overall, this suggests that plant response and ecotypic differences might not be stable, which confirms that long-term recording is paramount.

5.4.2: 2015 versus 2016

In the second experiment (2016), one of the most striking observations was the change in flowering time of UK12 between 2015 and 2016; in contrast UK2 and UK9 were relatively consistent between years (Figure 5.15). Based on the information in Table 5.9, it can be suggested that UK12 flowers were initiated prior to mid-September in 2016, but not until after mid-October in 2015. Flowering time was measured as days to first flower emergence and the percentage of plants flowering (of 10 replicates) and it is important that this method gave an accurate estimate of flowering time. In *Arabidopsis*, flowering time is commonly recorded by the number of (rosette and cauline) leaves before bolting (Reeves and Coupland, 2001). Leaf number usually correlates well with days to bolting, and a strong correlation has also been found with days to anthesis as a measure of bolting time (Möller-Steinbach *et al.,* 2010). In rosaceous species, first flowering has been recorded as time to first open flower/anthesis in *Prunus, Rubus* and *Fragaria* (Fitter *et al.,* 1995; Le Mière *et al.,* 1998; Sønsteby and Heide, 2006; Miller-Rushing *et al.,* 2007; Heide and

Sønsteby, 2007; Koskela *et al.*, 2012), but it should be noted that frequency of recording is critical to ensure accuracy (Miller-Rushing *et al.*, 2008; Tooke and Battey, 2010). Here, flower emergence was recorded every two-three days, fulfilling this requirement. Peak flowering is another common method of estimating flowering time, either by the percentage of flowers per plant or the percentage of flowering plants (Liebhard *et al.*, 2003; Baret *et al.*, 2004; Miller-Rushing *et al.*, 2007); although more demanding (partly because it is less straightforward), it reduces possible extremities, which may arise through natural population variation in time of first flowering (Miller-Rushing *et al.*, 2008). Population size can influence the variability of flowering time, especially if using first flowering in isolation, so that first and peak flowering can shift independently (Miller-Rushing *et al.*, 2008). Change in timing of first flowering, in some cases, can overestimate the magnitude of changes in peak timing of flowering (CaraDonna *et al.*, 2014).

Time to first open flower, rather than peak flowering date, was used in this Chapter because of its practicality and because of consistent population size between years, so the pronounced change in first flowering date of UK12 between years can be confidently considered to reflect a genuine shift in flowering time. Three hypotheses may explain this change: either the environment differed between 2015 and 2016 in such a way as only to affect UK12; or a carry-over effect existed in 2015 which had been lost by 2016; or a correlative effect occurred in 2015 but not in 2016. Each of these hypotheses will now be considered.

Hypothesis 1: an environmental difference changed the flowering response of UK12. The timing of transfers into the forcing environment was very similar (for transfers which showed flower emergence) in the two years, so variation due to this factor can be discounted (Table 5.10).

Table 5.10 Time of monthly transfer from natural (field) inductive conditions to controlled forcing conditions, for both experiments and whether flower emergence was observed

Transfer	**2015**	**2016**	**Flowering**
August	18/08/2015	31/08/2016	No
September	15/09/2015	14/09/2016	Yes
October	14/10/2015	12/10/2016	Yes
November	18/11/2015	16/11/2016	Yes

In the second experiment (2016), UK12 showed flower emergence (in at least one plant) from the September transfer onwards and average temperature in late August and September 2016 was generally warmer than in the corresponding period in 2015 (Figure 5.18). Mean weekly temperature was consistently higher in 2016 from late August-September, although temperature

only exceeded 20°C for one week at the end of August (Figure 5.18). High temperatures during late August and September would not, however, have been expected to promote flower initiation, because temperatures above 20°C regardless of photoperiod are considered to repress floral induction in all ecotypes of *F. vesca* studied so far (Heide *et al.*, 2013). For the warmer late August in 2016 to account for the UK12 flowering time shift it is therefore necessary to propose a radical change in flowering time regulation, and that instead of being inhibitory, warm temperatures promoted flower initiation. Although this might appear improbable, ecotypes can show pronounced differences; e.g. some ecotypes have been reported to have a vernalization requirement for floral induction (Alta) (Heide and Sønsteby, 2007). Further, high temperatures and LD are typically promotive for floral bud initiation in perpetual-flowering strawberry cultivars (Sønsteby and Heide, 2007) and mutation of the *SEASONAL FLOWERING LOCUS* (*SFL*) changes flowering from seasonal to perpetual in *F. vesca* (Koskela *et al.*, 2012). A mutation of this type is, however, unlikely to be responsible for the change in flowering time of UK12 given that flowering time varied between years and no emergence was observed following transfer to forcing in August, when the perpetual flowering type would have likely flowered.

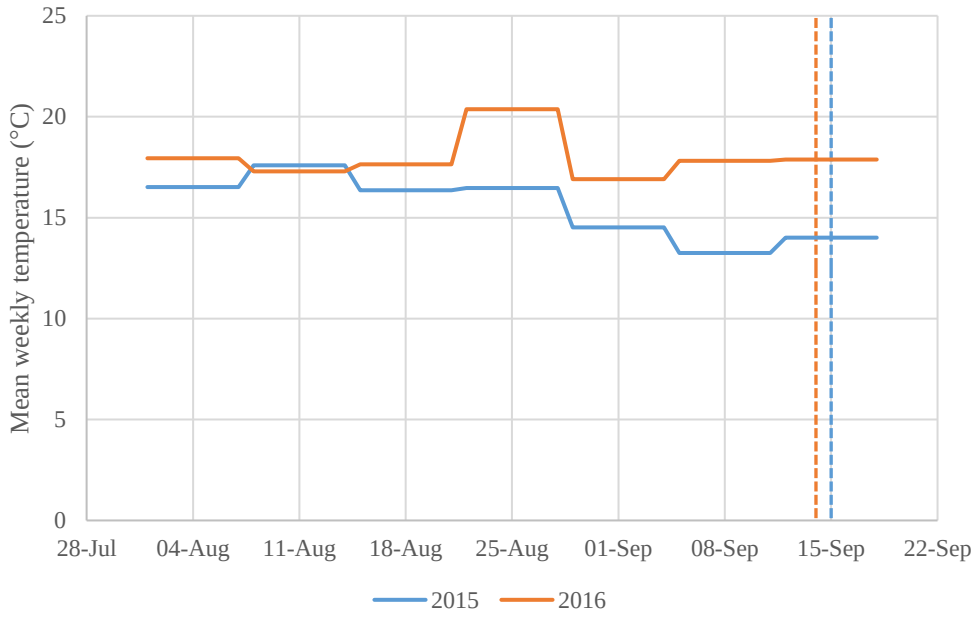


Figure 5.18 Mean weekly temperature in the field during August and September in 2015 and 2016. Time of transfer to forcing conditions for the September transfer is highlighted by dashed vertical lines (blue – 2015 and orange – 2016)

UK12 was collected from Batson Creek, Salcombe, which is situated on the coast. Coastal ecotypes are typically exposed to warmer conditions than those further inland (Herrera, 2005), and as a result flower emergence at coastal sites is typically earlier than for more continental sites (Estiarte *et al.*, 2011), although coastal populations of *Anthyllis vulneraria* have been shown to

exhibit greater fluctuation in the rate of flowering than inland populations grown *in situ* (Sterk, 1975). This might suggest that the flowering response of UK12 observed in 2016 was more similar to the expected response of this coastal ecotype but does not account for the late flowering response in 2015. It is important to note that artificial conditions were used to force flower emergence and natural flowering times have not been recorded for any of the ecotypes under study.

In comparison to both UK9 and UK12, the relative earliness of flowering in UK2 in both years suggested that this ecotype might have been more responsive to floral induction conditions and/or that the duration of induction required might have been shorter. Regardless of these differences, in general the response of UK2 and UK9 appeared to be more similar to other *Fragaria* ecotypes and cultivars, and their differences in flowering time may be attributed to differences in sensitivity to photoperiod. Sønsteby and Heide (2006) demonstrated that some *F.* x *ananassa* have an obligatory SD requirement for flower induction, which could explain the consistent, later flowering response of UK9 compared to UK2.

Ecotypic differences associated with environmental requirement for flower induction have been recorded for a number of other annual and perennial species. Differential sensitivity to photoperiod and temperature has been proposed as an explanation of ecotypic differences in floral initiation (often recorded by flower emergence) in *Arabidopsis thaliana, Medicago polymorpha, Carex* species, *Poa* species and *Trifolium repens* (Thomas, 1981; Heide, 1994, 1997; Del Pozo *et al.,* 2000; Mouradov *et al*., 2002). However, shifts in flowering time year to year, under conditions in which environmental variation is relatively small, have not been reported. Sønsteby and Heide (2006) did, however, show significant differences in dormancy response between years when *F. vesca* plants were exposed to natural conditions.

For the hypothesis of an environmental difference changing the flowering response of UK12, it would have to be accepted that the environmental regulation of floral induction in UK12 was radically different from that of previously described ecotypes.

Hypothesis 2: UK12 may have flowered very late in 2015 because of a carry-over effect resulting from its collection in Devon in 2014 and subsequent maintenance by vegetative means in Finland/Reading. In an ecological context, a carry-over effect can occur in any situation in which an individual's previous history and experience explains its current performance in a given situation (O'Connor *et al.,* 2014). For example, salinity influences timing of flowering in *Iris hexagona* and a strong effect of previous saline conditions has been recorded on flowering time, with this response maintained into a second growth year after transfer to a common garden (Van

Zandt and Mopper, 2002). Drought can also have a carry-over effect on subsequent plant growth, with reduced shoot elongation observed for previously drought-exposed oaks (*Quercus robur*) (Kuster *et al*., 2014); carry-over effects are therefore able, in some cases, to persist for long periods (Billington *et al.*, 1990).

Carry-over effects (through seed) in flower phenology and plasticity have been shown in some species e.g. *Lolium perenne* (Hayward, 1967), *Nicotiana* (Jinks *et al.*, 1972) and *Brassica* (Singh and Murty, 1980) (reviewed in Roach and Wulff, 1987). Carry-over effects have also been observed in species with the means of asexual reproduction, through vegetatively produced modules (e.g. ramets and grass tillers, such as *Holcus lanatus*), where initial phenotypic differences observed in daughter plants correlated with environmental conditions experienced by the mother plant (Bullock *et al.*, 1993). In *Lolium perenne*, the carry-over responses in tiller growth of daughter plants diminished after maintenance in a common environment for a year (Hayward and Koerper, 1973). Turesson (1961) also highlighted loss with time of a dwarfism carry-over effect in vegetatively propagated *Populus tremula*, and the trailing shrub habit in *Sorbus aucuparia*. For *Trifolium repens*, carry-over effects associated with collection site (in particular neighbouring species) were initially observed after four months in a common environment with significant differences in stolon and leaf morphology; these were lost after 27 months (Evans and Turkington, 1988).

Carry-over effects of previous environment on flowering time in vegetative propagated material have, however, not been shown. Epigenetic regulation has been established as a factor that can influence responses both within (epigenetic reprogramming – Kawashima and Berger, 2014) and between generations (transgenerational epigenetics – Quadrana and Colot, 2016). *Arabidopsis* ecotypes can differ in their vernalization flowering pathway requirements within a generation, with this variation epigenetically linked to differences in the duration of cold required between ecotypes (Mouradov *et al*., 2002; Shindo *et al.*, 2006; Bratzel and Turck, 2015). As a result, epigenetic reprogramming mechanisms have been established to regulate flowering time in *Arabidopsis*, plants with epigenetic alleles of *FLOWERING WAGENINGEN* (*FWA*) showing late flowering (Mouradov *et al*., 2002); similar epigenetic mechanisms have also been linked to flowering time in rice (Guo *et al.*, 2015; Shi *et al.*, 2015). Unlike annuals, which typically perish after flowering, perennials are long-lived and therefore the nature of epigenetic effects within these plants is likely to differ from those described for annuals, especially epigenetic reprogramming within a generation. Some studies imply that epigenetic modifications may influence the environmental and developmental plasticity of gene expression relating to flowering time in perennials such as rose (Dong *et al*., 2017), but the molecular basis behind such epigenetic effects is obscure at present. *TFL/FT*-like genes could be proposed as candidate genes for

exploratory work. The transfer of such traits is also important in perennials, such as *F. vesca*, which can produce progeny both sexually and asexually, as it will be necessary to address whether epigenetic effects can be transferred through both means of reproduction.

Hypothesis 3: a correlative effect, resulting from runnering activity of UK12 in 2015, might be responsible for the change in flowering time of UK12. Runner production by UK12 plants in both experiments was highest across all transfers, in comparison to UK2 and UK9. The correlation between runnering and flowering response is summarized in Figure 5.19, in which percentage runner production in relation to the highest mean runner production, and the reciprocal of mean days to first flower emergence, across ecotypes and transfers have been calculated for both years. There was a general decline in time to flowering associated with reduced runner production in 2015 ($R^2 = 0.73$), and no flower emergence was observed with high runner production (> 67%) (Figure 5.19A). This interaction between runnering and flowering response was not, however, maintained in 2016 ($R^2 = 0.44$), with flower emergence observed even with high runner production (Figure 5.19B).

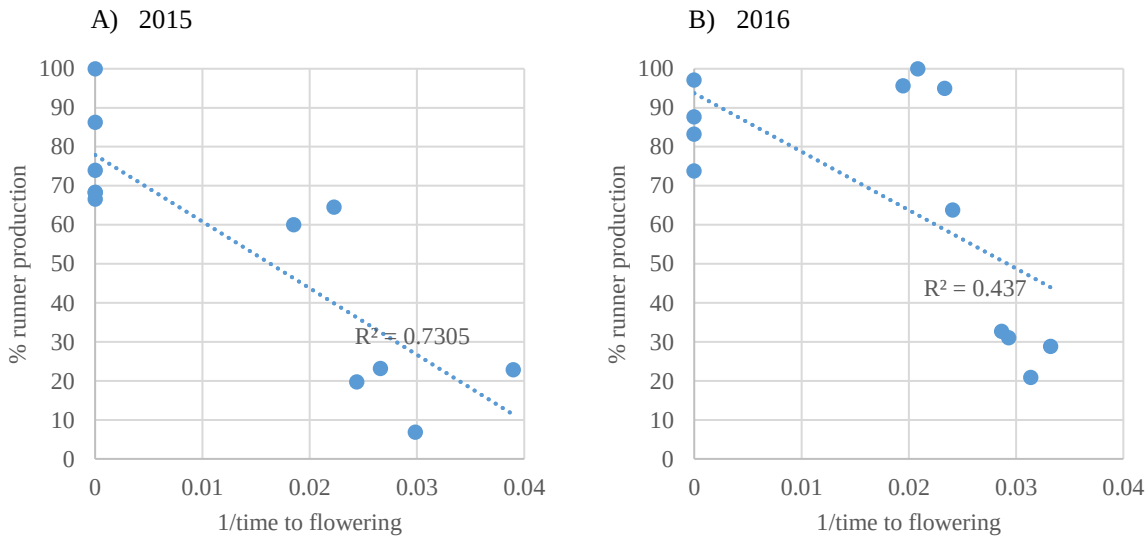


Figure 5.19 Correlation between mean runner production and flowering time following transfer to forcing for all transfers, for UK2, UK9 and UK12 in 2015 (A) and 2016 (B)

In the experiments described here, the proposed correlative effect of reproductive investment was not consistent between years. UK12 showed continued high runner production in both 2015 and 2016, while flower emergence time differed profoundly, with earlier flower emergence of UK12 occurring following transfers in 2016. Nevertheless, UK12 was always the last ecotype to flower within each transfer, suggesting that this ecotype maintained some of its late flowering response. UK12 also maintained its high runner production response between years. The other ecotypes

studied in both years also showed a possible association between runnering and flowering: UK9 flowered later and showed greater runner production than UK2.

Therefore, although there may be a relationship between runnering and flowering, the differences observed here, especially in 2015, implied that the mechanisms controlling runnering and flowering differed. Sønsteby and Heide (2007) found evidence of cultivar variation in runner formation and although LD generally promote runnering; in *F. chiloensis,* Sønsteby and Heide (2009) established there was a temperature-photoperiod interaction associated with ecotypic differences in runnering response. Serçe and Hancock (2005) showed significant differences between *Fragaria* species and genotypes in their flowering and runnering morphology and responses, and suggested that in some genotypes floral bud formation might act as a stronger sink than runner production, preventing active runnering during flowering. This trend was not, however, observed in all cases, with free runnering during flowering observed for some *F. virginiana* ecotypes (Serçe and Hancock, 2005); this could be similar to the response of early flowering and sustained high runner production of UK12 in 2016 but does not explain the change in flowering time between 2015 and 2016.

Other studies have established that although there are some links between flowering and runnering, the mechanisms controlling runnering and flowering response in *F. vesca* are genetically independent (Brown and Wareing, 1965; Battey *et al.*, 1998; Albani, 2002; Heide *et al.,* 2013; Mouhu *et al.,* 2013); hormonal and environmental regulation are thought to alter the balance between vegetative and floral development in strawberry (Perrotte *et al.,* 2016b). Genetic analysis demonstrated that runnering is controlled by a dominant gene *R* (*RUNNERING LOCUS*) (Brown and Wareing, 1965), whereas seasonal flowering in *F. vesca* is controlled by the *SEASONAL FLOWERING LOCUS* (*SFL*) (Brown and Wareing, 1965; Mouhu *et al.,* 2009; Koskela *et al.,* 2012; Kurokura *et al.,* 2013). More recent work has confirmed the independence of the genetic control of runnering (*R* gene) and flowering (*SFL* gene) (Hytönen and Elomaa, 2011). The environmental conditions influencing runnering and flower initiation are similar in species and cultivars/populations of seasonal-flowering *Fragaria* (Jurik, 1983; Yang and Kim, 2016), but the genetic control of runnering and flowering response in *F.* x *ananassa* is not independent, as suggested for *F. vesca.* In *F.* x *ananassa*, *FaPFRU* has been identified as a major QTL influencing both the perpetual-flowering and runnering trait and has an opposing effects on these reproductive traits, positively influencing flowering and negatively affecting runnering (Gaston *et al.,* 2013; Perrotte *et al.,* 2016a). As a result, *FaPFRU* has a direct effect on plant fitness and the balance between sexual and asexual reproduction (Gaston *et al.,* 2013). Although the genetic control of runnering and flowering are independent in *F. vesca*, an association is still

present between traits and plants that show greater flowering i.e. perpetual cultivars, typically runner less (Heide *et al.*, 2013).

As well as large-scale environmental differences, small-scale *in situ* differences e.g. micro-climate, can also influence resource allocation. One such factor is light availability, which can influence reproductive effort in *F. virginiana* and *F. vesca*, with both forms of reproduction decreasing with increased shading, but more so for vegetative reproductive efforts (Jurik, 1983, 1985; Chazdon, 1988). Differences in light availability between sites of *F. vesca* might have had a selective pressure on adaptation and phenotypic differences in reproductive efforts between ecotypes, if these differences were maintained, genetically or through carry-over effects. Nevertheless, such effects do not account for why UK12 showed a distinct shift in flowering time, which was not echoed by a change in runner response or a difference in flowering time for the other ecotypes.

Finally, differences in flowering and runnering response between ecotypes and year could be related to differences in reproductive efforts, as both runner and flower production have a cost to the plant, and ecotypic differences in the balance between these reproductive modes could impact subsequent fitness and reproductive response. Albani (2002) highlighted the severity of the cost of reproduction by growing *Fragaria* plants over a two-year period, and showed that mortality rose to 97% of the original population by the end of second year. There were differences in the rate and severity of mortality depending on flowering (seasonal or perpetual) and runnering (runnering or non-runnering) traits, but regardless mortality was observed across plants (Albani, 2002). The risk of mortality as a result of reproductive efforts stresses the importance of the choice in investment between asexual and sexual reproduction in ensuring longevity of the ecotype; with both forms of propagules contributing in different ways to long-term reproductive success of the species (Winkler and Fischer, 2001). UK12 was the highest runnering ecotype in both experiments (2015 and 2016); therefore the shift to earlier floral initiation in 2016 does not correlate with this runnering response. It does, however, raise an ecological question of whether flowering time in the second year was advanced as a survival strategy to account for the increased likelihood of mortality associated with high runner production.

In conclusion, three potential hypotheses have been explored to account for the shift in flowering time of UK12; further work is necessary to establish with certainty whether any of these hypotheses provide a satisfactory explanation. The most probable scenario, at present, is that the factor delaying flowering of UK12 in 2015 was lost with time and vegetative propagation, enabling plants in 2016 to be more responsive to flower-inducting conditions. The relative

consistency of response between years for UK2 and UK9 suggests that UK12 differs significantly from the other ecotypes, offering an interesting opportunity for future research.

5.4.3 Other considerations

In both years there was a general trend of decreasing variability over the experiment period, with regards to timing of flower emergence, which might be expected as plants are reported to show decreased variability in growth response following optimal, or extended duration of optimal conditions. For example, decreased variability has been reported for the vegetative response of many deciduous perennials following winter chilling, with plants showing earlier budburst with greater chill accumulation (Cannell, 1989). Plants following the October and November transfers would have been exposed to inductive conditions for longer than plants transferred in September, which could explain the decrease in variation of emergence time. All ecotypes showed earlier emergence and less variability in emergence time following the November transfer, in comparison to October, although ecotypes showed some variation in the decline in variability between transfers.

The functional importance of the differences in flowering between ecotypes is unclear: their natural flower emergence time has not yet been established; nor has the impact of timing of autumn flower initiation for other developmental and physiological processes in the annual cycle, particularly dormancy. In 2015, there was a rough, positive correlation between timing of flower emergence and latitude for the UK ecotypes, with the more northerly ecotypes (UK2 and UK11) flowering earlier within and between transfers than the more southerly UK ecotypes (UK9 and UK12); however these groupings in ecotypes were not significant across transfers. It is therefore difficult to argue that the differences in flowering time measured here, using unnatural forcing conditions, are necessarily of ecological significance. While they might reflect population differentiation for functional (adaptive) reasons, they may also be a result of genetic drift.

Ecotypic differences in floral character were noted in the first experiment, through variation in inflorescence morphology (size and length of peduncles and pedicels; number of branches within the inflorescence) and number of inflorescences per crown; these floral characters were quantified in 2016, and confirmed that variation in flower number was a result of differences in inflorescence structure between ecotypes, most significantly inflorescence number (Figure 5.12; Table 5.11). A strong significant positive correlation between flower number and truss number has also been shown when phenotyping *F.* x *ananassa* cultivars (Antanaviciute, 2016).

Table 5.11 Mean number of inflorescences, flowers per inflorescence and flowers per plant (± standard error of the mean) and the significance of ecotypic differences for each flowering character for plants in the 2015 and 2016 experiments, following transfer to forcing in November

	2016			2015
Ecotype	**Inflorescences**	**Flowers/inflorescence**	**Flowers/plant**	**Flowers/plant**
UK2	7.8 ± 0.8	3.8 ± 0.3	30.0 ± 5.8	18.1 ± 1.0
UK9	8.8 ± 1.3	5.0 ± 0.2	43.8 ± 7.5	19.3 ± 1.6
UK12	7.3 ± 0.8	5.3 ± 0.3	38.4 ± 3.7	8.7 ± 0.8
ANOVA	N.S.	$P < 0.01$	N.S.	$P < 0.01$

In both experiments, UK9 had the highest average number of flowers, whereas, UK2 and UK12 showed contrasting results between years: in 2015, UK2 had a similar number of flowers to UK9, while UK12 had more than 50% less than both UK2 and UK9 (Table 5.11). In 2016, there was no significant difference in the average number of flowers per plant between ecotypes, although UK12 plants typically had more flowers than UK2, in contrast to 2015 results. All ecotypes showed greater floral production in 2016 in comparison to 2015 ($P < 0.01$), although this difference in flower number may not truly represent greater flower production in 2016, as the method of data collection differed between years. In 2015, the number of emerged flowers in inflorescences still attached to the plant was recorded during the period of active flower emergence, whereas in 2016, flower number was recorded at the end of the forcing period in inflorescences that had been removed from the plant. Unlike UK9, which showed highest flowering in both years, UK2 and UK12 showed contrasting results. In 2015, UK2 had more flowers than UK12, but in 2016 this pattern of production was reversed (Table 5.11). This suggests a difference in flowering response for UK12 between years that was not observed in UK2 or UK9, which correlates with the observation of earlier flowering for UK12 in 2016.

Within species differences in inflorescence morphology have been considered across a range of species, and one key ecological driver is plant interaction with pollinators (Gómez *et al.*, 2014). This influences a number of floral traits including: flower number (*Phacelia linearis* – Eckhart, 1991), floral morphology (*Sinapis arvenis* – Kuppler *et al.*, 2016), flower colour (*Gentiana lutea* – Sobral *et al.*, 2015) and peduncle length (*Primula farinosa* – Vanhoenacker *et al.*, 2006). In *Fragaria*, it is not known whether selective pressure from pollinators has led to variation in inflorescence morphology between ecotypes. An alternative possibility is that genetic drift has led to the observed differences, and that they are not of selective importance. A study of heritability of flower-related traits showed a high heritability coefficient for flower number within

cultivars (Antanaviciute, 2016), suggesting that differences would persist once established in natural populations.

Finally, the comparison of inflorescence morphology following forced flower emergence may not accurately represent ecotypic differences in inflorescence morphology. The plants used to quantify inflorescence morphology had not been exposed to prolonged autumn initiation, chilling or natural floral development in the spring. A deep understanding of variation in inflorescence morphology between *Fragaria* ecotypes/cultivars is important, as such variation ultimately influences yield (Hancock, 1999; Sargent *et al*., 2004; Sønsteby and Heide, 2008; Bestfleisch *et al*., 2014; Antanaviciute, 2016), which is key given the commercial nature of this crop.

5.4.4 Seeds versus runners experiment

The lack of floral response observed in seed-derived individuals was assumed to be related to juvenility. Many perennials, especially those in the Rosaceae, have a period of juvenility in order to restrict flowering until the plant has sufficiently grown to support flowering and fruiting (Thomas and Vince-Prue, 1984). The extent of the juvenile period varies between species; for example the juvenile period typically lasts for several years in rosaceous fruit trees, whereas strawberry can reach competence to flower in its first season (Kurokura *et al.*, 2013). Some variation in the extent of juvenility has been reported between seasonal and perpetual flowering strawberry varieties; with seasonal flowering types having months of juvenility, in comparison to perpetual flowering types which can flower in the first growing season (Savini *et al.*, 2005; Sønsteby and Heide, 2007; Kurokura *et al.*, 2013).

In the seeds *versus* runner experiment described in this Chapter, seed-derived individuals were not sown until 09/07/2016, and did not germinate and emerge as seedlings until 19/08/2016. They were exposed to vegetative conditions for six months prior to induction under artificial conditions. The lack of floral induction suggests that the juvenile period of these plants (seasonal-flowering types), was longer than six months. Alternatively, six weeks floral induction might not have been sufficient to promote flowering in these plants. It is not possible from these results to determine whether this latter possibility can be rejected.

5.4.5 Conclusions

There are four principal conclusions from the research described in this Chapter:

1. Populations of *F. vesca* from different locations vary in timing of flowering following forcing in a constant environment, most likely due to differences in the duration of conditions needed to induce flowering. There was also variation in runnering characteristics (timing and extent).

2. In 2015, there was some suggestion of a latitudinal divide between ecotypes with regards to timing of flower emergence, but in 2016 the change in flowering time of the most southerly ecotype (UK12) complicated this interpretation. This change was not correlated with a change in runnering pattern. The change in flowering time of UK12 emphases how critical it is to measure traits over more than one year in perennials species. The mechanistic basis for the carry-over effect potentially responsible for this change will be an important topic of future research.

3. Ecotypic variation in runnering response corresponded to variation in flowering time in 2015, but this trend was not so clear in 2016 because of the change in flowering time of UK12. Active runner production occurred during August-September in both years, and appeared to include the generation of new runners by the SAM. This seems likely to have been associated with the removal of existing runners (every two weeks) as part of the experimental procedure. It contrasts with the lack of new runner production after June in the work described in Chapters 3 and 4, where existing runners were not removed. The runnering behaviour of plants in the experiments described here thus provides indirect, corroborative support for the idea that established runners dominate the terminal SAM, preventing its growth.

4. Six weeks exposure to floral inductive conditions (15°C/SD) was not sufficient to induce flowering in seed-derived individuals. It was not clear from the results whether this was due to juvenility of the plants, or differences in inductive response between seed-derived and runner-derived plants.

Chapter 6: The influence of chill accumulation and forcing on spring growth in *F. vesca*

6.1 Introduction

6.1.1 Dormancy and chilling in perennials

Winter dormancy in perennials is typically overcome by cold temperatures. As a result, buds burst as temperatures increase in spring. Much of the research on this topic has focused on perennial species which show true dormancy, a deep dormancy in which no growth occurs during winter, and cannot occur without fulfilment of the chilling requirement. The main aims of the experiments described in this Chapter were to explore the chilling requirement of *F. vesca*, a perennial species which shows semi-dormancy, defined as a state in which some growth is possible during winter.

For a plant to be considered a perennial, the apical meristem of at least one of its shoot axes must remain indeterminate beyond the first growth season (Thomas *et al.*, 2000). Unlike annuals, perennial plants typically repeat the cycle of vegetative and reproductive growth every year, by responding to various environmental signals (Kurokura *et al.*, 2013). Plant developmental processes and the environmental factors which regulate them are much less well understood in perennials than in annuals which have been more intensively researched (Battey, 2000). However, perennials are of great global importance, both in natural ecosystems and as part of present and future agricultural systems (Glover, 2003; FAO, 2014). They are commonly categorised into two types: woody or herbaceous. Woody plants are trees, shrubs, and vines whose above-ground parts persist during winter, and resume growth in the spring. Herbaceous perennials tend to die back to the ground each autumn/winter, with only the roots or rootstocks of these plants surviving, from which the plants re-sprout in the spring (Anderson, 1999). The processes and mechanisms controlling annual events are characteristic for a range of perennial species; the example of apple is shown in Figure 6.1. The developmental cycle occurs over two years, with flower initiation occurring in the summer/autumn of year one, while flower emergence occurs in the spring of year two, following winter dormancy. This separation of flower initiation and emergence is found in many temperate perennials. As a result, a clear understanding of the dormancy stage separating flower emergence from flower initiation is crucial (Battey, 2000).

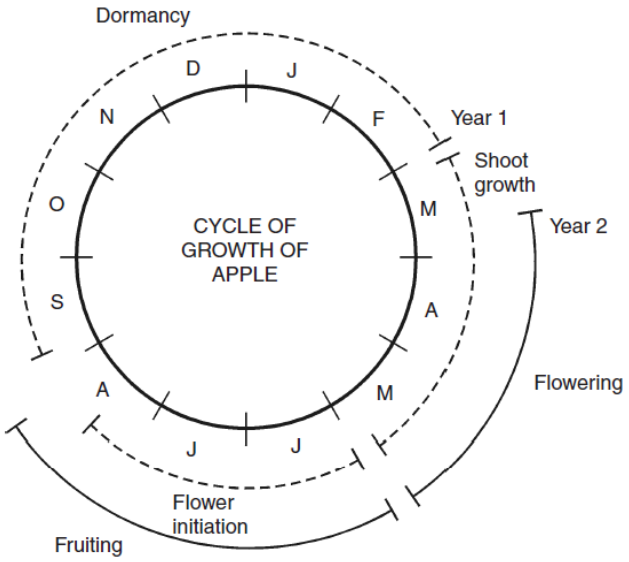


Figure 6.1 The cycle of growth of the apple (Carew and Battey, 2005)

The physiological processes involved in dormancy are complex, and there are different types of dormancy. Lang *et al.* (1987) provided one of the most cited descriptions, summarised in Horvath (2009) (Figure 6.2). The first type of dormancy is paradormancy, associated with apical dominance; here buds are prevented from growth due to signals produced in distal parts of the plant. Another type of dormancy is endodormancy, typically induced in temperate climates by autumn conditions. During this state, bud growth rate is inhibited and, crucially, growth cannot occur even under favourable condition (see Figure 6.2). Ecodormancy is induced by adverse environmental conditions, such as drought, cold or short day length. Unlike endodormant buds, which often require chilling to break dormancy, under growth-conducive conditions, ecodormant buds will resume full growth immediately.

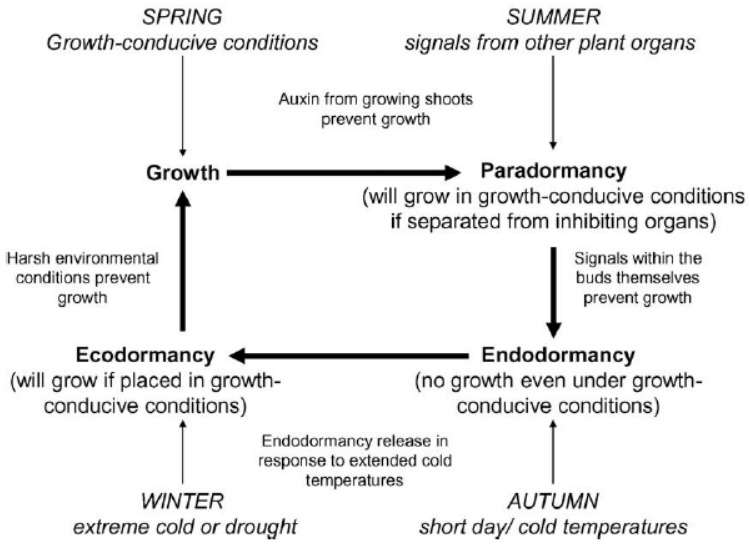


Figure 6.2 Schematic diagram showing common seasonal transitions of dormancy and growth (Horvath, 2009)

The fulfilment of chilling to enable endodormancy release in the spring, and promoting vegetative and reproductive growth, has been a major subject of research. Early work provided evidence that temperature was one of the primary mechanisms controlling both dormancy release and the range of subsequent temperatures to which plants could respond in the spring (Vegis, 1964). Photoperiod has also been shown to play an important role in this process, although in many species photoperiod may only become a critical influence if the chilling requirement has yet to be satisfied by temperature (Wareing, 1956; Battey, 2000).

Campbell (1978) built on this research when considering the regulation of budburst timing by temperature and photoperiod during dormancy for the perennial Douglas fir (*Pseudotsuga menziesii*). He found that chilling had an effect on the ability of buds to respond to flushing temperatures: the longer buds were chilled, the steeper the temperature response curve, as measured by days to budburst. The change in the temperature response induced by chilling was influenced by chilling temperatures, as well as by duration. Furthermore, the time when chilling was experienced influenced the changes induced by chilling, although this was a complex relationship. The results from this work also suggested that temperature is the primary mechanism influencing bud development, whilst photoperiod plays an important role in acting as a modifier.

Campbell and Sugano (1979) expanded on this initial research, by considering more systematically the influence of genetic variation (provenance) on the dormancy response to chilling. Their results were similar to Campbell (1978), indicating a higher flushing rate and earlier flushing date, with warmer flushing temperatures and longer chilling (Figure 6.3). Flushing rates were as fast at 10°C after 77 days of chilling as at 15°C after 11 days of chilling.

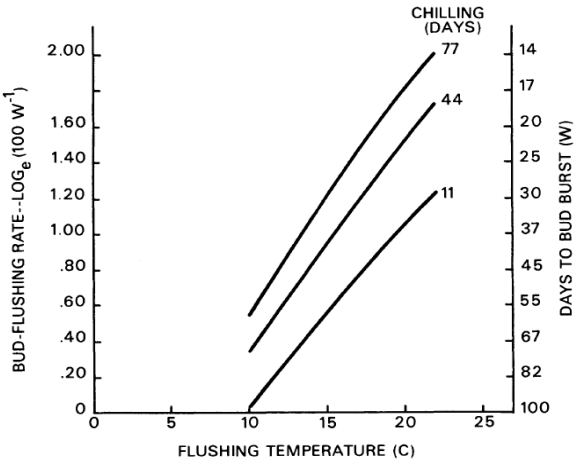


Figure 6.3 Budburst response to flushing temperature and chilling period based on population average calculated from the regression equation for the climatic model. W = days to budburst from the time the plants entered the flushing chamber (Campbell and Sugano, 1979)

Cannell and Smith (1983) reviewed published data concerning the relationship between chill days and thermal time to budburst. They found that a decreasing exponential relationship characterised the response of a number of perennial species, including: *Picea* spp. (*P. abies, P. glauca*), *Malus* spp, *Populus deltoides*, *Tsuga heterophylla* and *Pseudotsuga menziesii* (Figure 6.4).

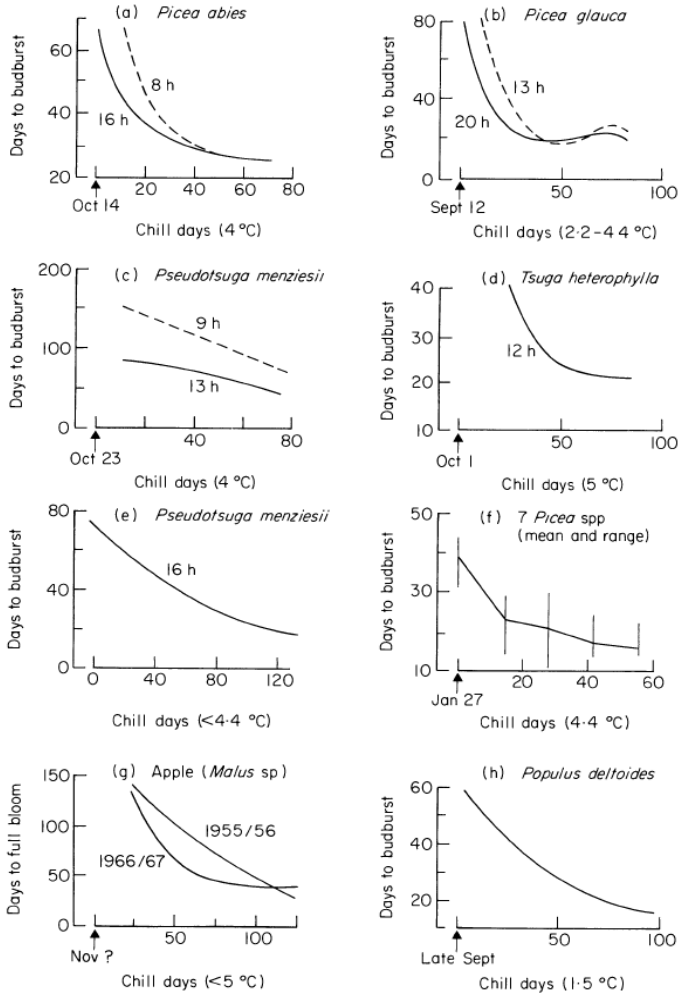


Figure 6.4 Relationships for different tree species between days to budburst or full bloom in warm temperatures and the duration of previous exposure to chill temperatures (adapted from Cannell and Smith, 1983)

These studies helped form the basis of Cannell's approach to quantifying the relationship between thermal time and chilling requirement, and led him to propose that thermal time and chilling requirements are interrelated: the more chilling is fulfilled, the wider the range of spring temperatures to which these plants are able to respond. This suggests that the process of chilling must in some way change the plant's ability to respond to spring temperature. Recent studies have suggested mechanisms controlling the change in buds during this phase; for example in Japanese pear (*Pyrus pyrifolia*), there are several prerequisites of sprouting (emergence), such as the

enlargement of flower buds at the meristem (possibly related to *PpCYCD3s*) and the induction of *PpEXPA2* expression (Saito *et al.*, 2015). Primordium development prior to emergence coincides with the increase of free water content in *Pyrus* (Yamamoto *et al.*, 2010; Saito *et al.*, 2015), and free water content has also been observed to increase prior to emergence in peach (Yooyongwech *et al.*, 2008), suggesting that water uptake potential at the cellular level may be critical for the spring growth response. Although Cannell (1989) did not consider the influence of chilling at the molecular level, it was suggested that as chilling progresses, the potential rate of development for dormant buds increases; whilst the thermal time required to reach spring phenophases, such as budburst and full bloom, should progressively decrease (Figure 6.5).

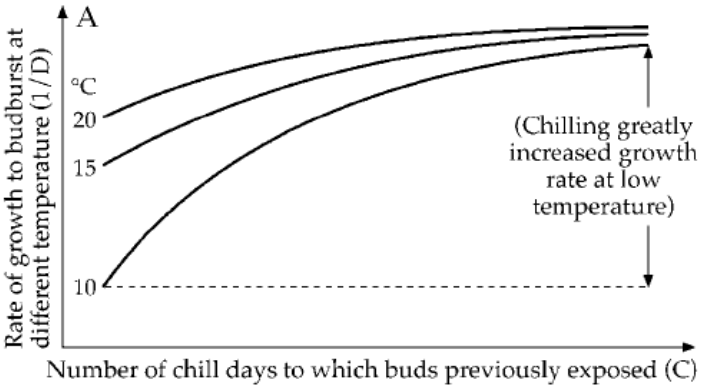


Figure 6.5 Relationships between the days to budburst (or flowering) in constant 'warm' temperatures (D) and the period of previous chilling (C). The relationships are derived from data presented by Cannell and Smith (1983) and Campbell (1978) (see Cannell, 1989)

Much of the existing dormancy work has been carried out on perennial trees (e.g. Figure 6.4), including the work reviewed by Cannell (1989). However, there are a number of issues that arise with experimentation on these species. For example, it is difficult to use mature trees in experiments, especially those involving the use of growth chambers/controlled environments. For this type of research, either bud sticks or juveniles/saplings are often used and there may be issues associated with the use of these mature tree substitutes, including timing of phenology events and experiment longevity (as discussed in Laube *et al.*, 2014a).

6.1.2 Dormancy and chilling in Fragaria vesca

In response to the issues associated with experimentation on perennial tree species, the research discussed here aimed to address the issue of dormancy and chilling, using the herbaceous perennial *F. vesca*. *F. vesca* grows as a rosette and has been suggested as a model species for

perennials (Battey *et al.*, 1998; Hollender *et al.*, 2012); its growth habit is to produce a scaffold fundamentally similar to those of a deciduous fruit tree. However, there are some crucial differences in morphology between *F. vesca* and woody perennials, such as the lack of true buds as discussed in this Chapter (see Chapter 6, Section 6.4.7); therefore, contrary to previous suggestion (Battey *et al.*, 1998) *F. vesca* might not be a good model for trees. Regardless of morphological differences, the environmental cues driving physiological responses in *Fragaria* are thought to be similar to those of other perennials (Galleta and Bringhurst, 1990). The annual cycle of *Fragaria* (Figure 6.6) also shows similarities to that of a woody perennial (Figure 6.1). Both show distinct seasonality, and separation between flower initiation and emergence, during which dormancy occurs. The nature of winter dormancy is one area where the similarity (or otherwise) of *Fragaria* and other perennials needs to be investigated.

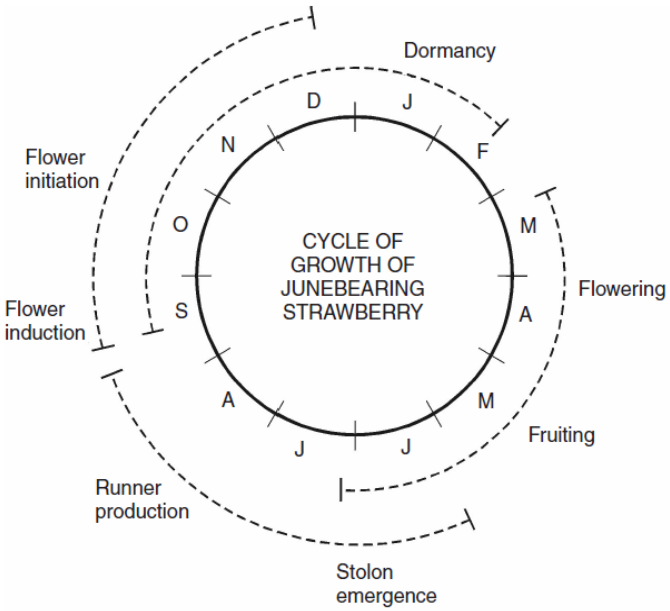


Figure 6.6 The cycle of growth of Junebearing strawberry (Carew and Battey, 2005)

In most rosaceous species, plants enter dormancy in the autumn, in response to low temperature and changing photoperiod; as a result growth is suppressed (Kurokura *et al.*, 2013). In contrast, the state of dormancy in *F. vesca* is considered to be quantitative, and therefore it has been described as semi-dormancy (Guttridge, 1985; Sønsteby and Heide, 2006; Kurokura *et al*., 2013; see also Chapter 1). The same environmental cues are thought to induce semi-dormancy in *F. vesca* as those which induce true dormancy in the tree species discussed above. Semi-dormancy is induced by prolonged exposure to SD and cool temperatures, which both inhibit growth and restore normal growth vigour in the spring (Sønsteby and Heide, 2006, 2011; Kurokura *et al*., 2013). In *F. vesca* the dormancy period is a necessary annual process, in order for floral initiation

to be repressed and winter chilling to be fulfilled, which enables spring growth to occur once the chilling requirement has been satisfied (Battey, 2000).

F. vesca is not the only perennial to exhibit semi-dormancy; other *Fragaria* species are also considered to be semi-dormant. Much of the existing work on semi-dormancy has been undertaken using commercial varieties of *F.* x *ananassa* (Guttridge, 1985; Sønsteby and Heide, 2006; Yamasaki, 2013). This research shows that by not entering true dormancy, even under prolonged SD conditions (over autumn and winter), *Fragaria* retains the capability for growth, albeit at a reduced rate (Sønsteby and Heide, 2006). Guttridge (1985) described any vegetative growth during this semi-dormant state as much restrained: emerging leaves are small with short petioles, no stolons are formed, and the plant exhibits a stunted habit. Semi-dormancy also means that strawberries are able to re-initiate growth at any time once exposed to warming and LD, although this growth will be constrained relative to plants which have been chilled (Jonkers, 1965; Guttridge, 1985).

The previous research on chilling and spring response in perennial species (Cannell, 1989), and the evidence for 'semi-dormancy' in *F. vesca*, prompted the experiments described in this Chapter. The first experiment was designed to test whether Cannell's method for describing the chilling and spring response of perennials showing true dormancy was applicable to a semi-dormant perennial. The influence of conditions during the chill period was explored further in the second experiment, in order to establish how varying autumn conditions could influence spring growth and development. As a result of this, the chill treatments of the second experiment had less chill units accumulated and included warm, potentially chill negating temperatures to determine the effect of these on spring growth. There is a long history of quantifying the effect of chilling on plants, with a range of models used in order to predict spring responses to autumn and winter conditions. These models differ in their approach to quantification, with early attempts (referred to as cumulative chill models) summing chill units equally below a given threshold temperature (Chandler, 1942; Weinberger, 1950). There have been a number of subsequent developments which include: weighted effect of temperatures (Erez and Lavee, 1971; Lantin, 1973; Richardson *et al.,* 1974); a disregard for sub-zero temperatures (see Melke, 2015); and accounting for the influence of warm temperatures as forcing spring growth and/or negating previous chilling (Fishman *et al.,* 1987a and b; Luedeling *et al.*, 2013).

The effect of warm temperatures on both spring growth and winter chilling has been a particular focus of recent research on chill accumulation aimed at predicting the influence of climate change. For example, in apple Legave *et al.* (2015) showed that spring warming advanced flowering time more than winter warming across Western Europe and Brazil. One of the

quantification methods which has received renewed attention as a result of understanding the influence of warm temperatures is the Dynamic Model. This was originally developed by Fishman *et al.* (1987a and b) and incorporates the negating effect of warm/high temperatures on previously accumulated chill (Luedeling *et al.*, 2013). Many of the modern studies which have considered accumulation approaches for chilling and warming, require the use of long-term phenological datasets in order to model the interaction between temperature and spring response (Luedeling, 2012; Legave *et al*., 2015; Darbyshire *et al.*, 2016).

One crucial element not to be overlooked, regardless of the method of accumulation, is the need to determine the importance of temperature at various stages during dormancy and spring growth; and to determine when chill and heat accumulation potentially overlap. Darbyshire *et al*. (2014) suggested that dormancy and growth phases are likely to be blurred, especially under future climate conditions, so that current chill and heat accumulation models often fail accurately to predict timing of physiological processes. Earlier work on long-term harvest data for apples highlighted the importance of cool temperature in late winter/early spring, as high temperatures in February-April are negatively associated with yield, although they can advance bloom time (Beattie and Folley, 1977, 1978; Jackson *et al.*, 1980, 1983). Studies of this nature emphasise the need to understand the specific interaction of temperature on spring response. Modern studies which have aimed to determine the transition between winter chill accumulation and spring heat accumulation phases have often used a combination of modelling forms: the Dynamic Model (for chill), growing degree hours (for heat), and partial least squares regression analysis (Luedeling *et al.*, 2013; Guo *et al.*, 2015). These models are not only complex but also require long-term datasets.

6.1.3 Specific objectives

The first experiment (2014-2015) described in this Chapter was designed to test whether the spring response proposed by Cannell for perennials with true dormancy was also observed for a semi-dormant species. A series of chill treatments was given, to address how extended chilling influenced early spring growth and development, as measured by runner production and flower emergence. Following chill accumulation, a range of forcing temperatures was provided to investigate how extended chilling influenced the release of growth restraint, especially at low temperatures; this would show whether greater chilling increased the responsiveness of *F. vesca* to low temperatures as proposed by Vegis (1964).

The second experiment (2015-2016) built upon these initial objectives, to determine whether the response recorded in the 2014-2015 experiment was confirmed and maintained when lower levels of chilling were provided using controlled environments and treatments with warm breaks. The use of controlled environments for chill accumulation enabled the inclusion of potentially chill negating temperatures (over 14°C) within the chill treatments, to address how warm temperatures during chilling influence spring response. One of the most striking results of the 2014-2015 experiment was the observation of runnering prior to flower emergence. In order to establish whether photoperiod influenced this growth response, LD and SD conditions were included within the forcing treatments in the 2015-2016 experiment to investigate how photoperiod affected timing and/or pattern of flower emergence and runner production.

6.2 Materials and Methods

6.2.1: 2014-2015 experiment

Plant material

The ecotype of *F. vesca* used in this experiment was originally collected from natural populations at Park Wood, Mapledurham (see Chapter 2 for more details), otherwise referred to as UK9. In September 2014, runners were sampled from established plants at the University of Reading (maintained under unheated glass). The daughter plants collected from these runners were rooted and established in 9 cm pots (using growing mix 1 – see Chapter 2) and moved outside into cold frames. Plants of a similar size were selected for the experiment: those that were particularly small or large were not included in an attempt to achieve uniformity across the cohort. The plants selected typically had no more than two established crowns (main crown and up to one established branch crown) and a minimum of five emerged leaves. The plants were regularly watered with tap water as required.

Growing conditions and treatments

Plants were naturally chilled outside (in a cold frame) at the University of Reading, with hourly average temperature recorded using a data logger (Tiny Tag Extra TGX-3580, RS Components, Gemini Data Loggers Ltd, UK) (Figure 6.7). Temperature showed a gradual decrease through November, and remained below 15°C from December to February (Figure 6.7). Chill accumulation was calculated using a cumulative chill hours model, with a threshold temperature of 7.2°C (one chill unit (CU) was accumulated for every hour with an average temperature of 7.2°C or less). Chill units naturally increased over the chilling period, with three chilling treatments used in this experiment: treatment A accumulated 870 CU, treatment B 1080 CU and treatment C 1550 CU (Figure 6.8). The time (days) between completion of each chill treatment was unequal, with less time between B and C than between A and B.

Once the chill treatment requirement was fulfilled, plants were re-potted with the addition of slow release fertiliser (Scotts Osmocote Plus controlled release fertiliser, Attgrow Ltd, Esher, UK), before transfer to spring forcing conditions. 50 plants from each of the three chill treatments were split equally across five forcing treatments: 8, 11, 14, 17 and 20°C, with conditions provided in growth chambers (Saxcil cabinets, R.K. Saxton Ltd., Cheshire, UK). During the forcing

treatment, plants received a 16h photoperiod (LD), supplied by a combination of fluorescent and tungsten lights (270-310 μmol m^{-2} sec^{-1}, 0600-2100 h). Plants remained under forcing conditions for 10 weeks (chill treatment A, 09/01 – 20/03/2015; chill treatment B, 22/01 – 01/04/2015; chill treatment C, 16/02 – 27/04/2015).

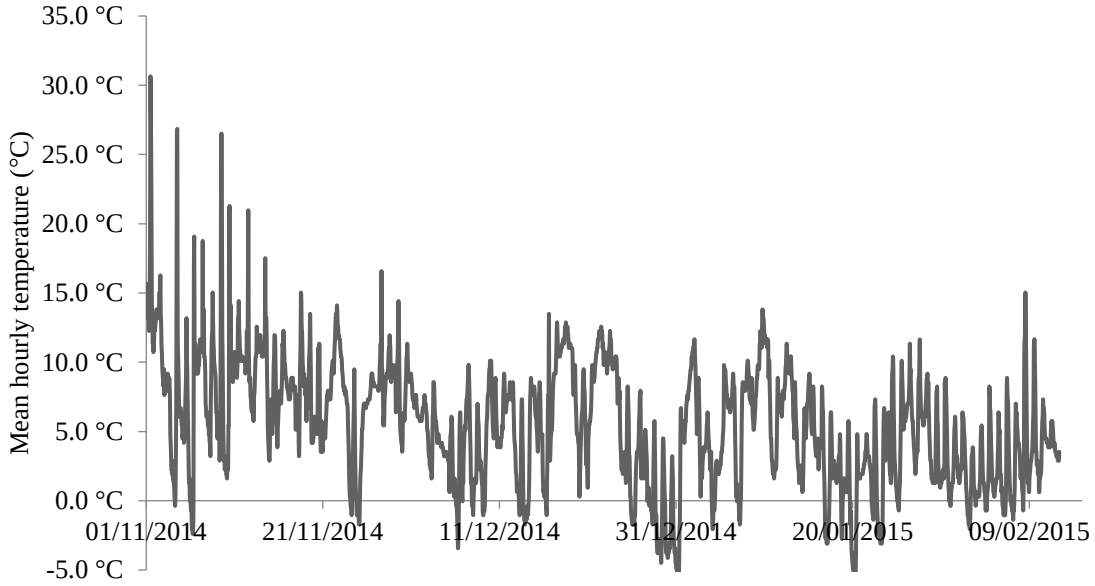


Figure 6.7 Mean hourly temperature, from the start of the chilling period until plants in the last chill treatment were transferred to spring forcing

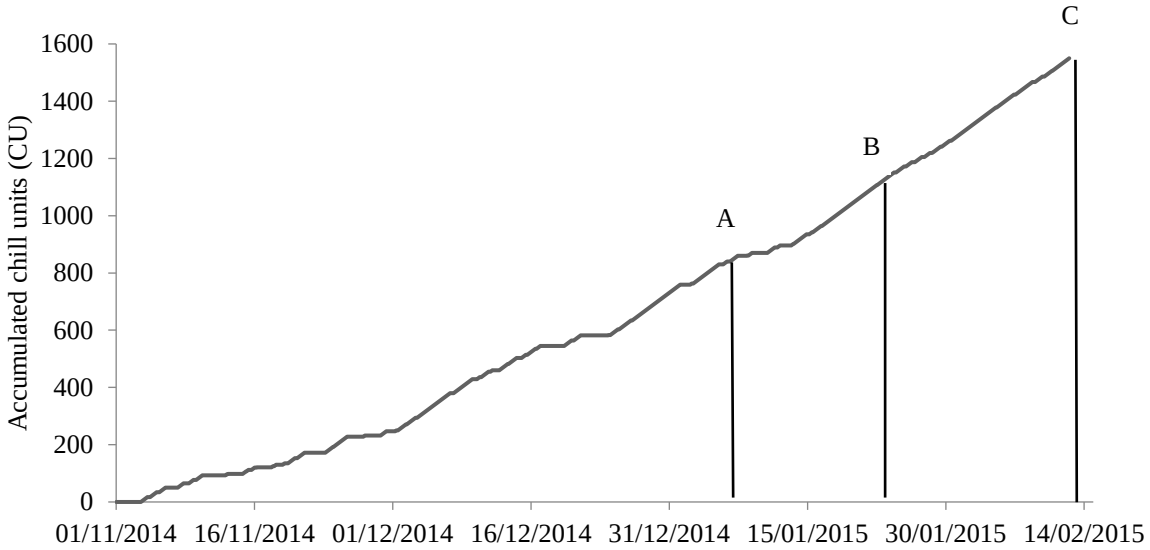


Figure 6.8 Chill units accumulated from the start of the chilling period until the last chill treatment was transferred into the spring treatment. Vertical black lines show the time of completion of the three chill treatments (A, B and C), when plants were transferred from cold frames into growth chambers to begin spring forcing

The experiment employed a randomised block design during the forcing treatment, with 10 plants for each forcing and chilling treatment combination, giving a total of 150 plants. During the forcing treatment, growth and development was monitored by tri-weekly observations. The following parameters were recorded on each plant: emergence and number of leaves, runners, flowers and fruit. Whilst under the forcing treatment no plant material (runners/leaves) was removed. Plants were sprayed three times during the forcing treatment (using Aphox and Calypso – see Chapter 2 for manufacturing details) to control aphids (02/02, 14/02 and 20/03/2015).

Statistical analysis

Data were tested for normal distribution using the Ryan-Joiner test, and data were found not to be normally distributed across chill and spring forcing treatments. Kruskal-Wallis tests were performed for each of the chill and spring forcing treatments and showed comparable results with one-way ANOVA. Generalized Linear Models (GLM) were therefore performed in order to provide a more robust analyse and to establish the significance of the interaction between chill and spring forcing, with chill treatments (chill units) and forcing temperature included as factors. Statistical analysis was undertaken using Minitab 16, averages and standard error of the mean were calculated for each recorded parameter.

6.2.2: 2015-2016 experiment

Plant material

The same ecotype of *F. vesca* was used (UK9, collected from Park Wood, Mapledurham), as in the 2014-2015 experiment. The plants were all propagated from runners, with some variation in origin: some were propagated from plants that had been in the previous (2014-2015) experiment and others were propagated from plants collected in the field (June 2015). They were grown in either 10cm pots or 9cm pots (Optipot, LBS Horticulture, Lancashire, UK) (using growing mix 2 – see Chapter 2).

Growing conditions and treatments

The experiment was carried out solely using controlled environments (Saxcil cabinets) at the University of Reading. Chilling was provided using controlled environments from 08/10/2015 – 04/12/2015. While under chill conditions, plants were exposed to SD (0800-1800), supplied by a combination of fluorescent and tungsten lights (500-600 μmol m^{-2} sec^{-1}). Constant temperatures

were used, which changed weekly depending of chill treatment (Table 6.1). Chill accumulation was quantified using the <7.2°C model, as for the 2014-2015 experiment. Warm breaks were also included within all chill treatments to varying degrees and duration (Table 6.1), which influenced the pattern of chill accumulation between treatments (Figure 6.9).

Table 6.1 Sequence of temperature exposure for chill treatments, provided under control environments

H = 15°C W = 10°C C = 6°C (all cabinets = 8h photoperiod)

Trt	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Total CU
A	H	W	H	W	C	H	W	W	168
B	H	H	W	W	C	H	W	C	360
C	H	H	W	W	C	W	C	W	336
D	H	W	C	H	C	W	C	W	504
E	W	W	C	C	W	W	C	C	696

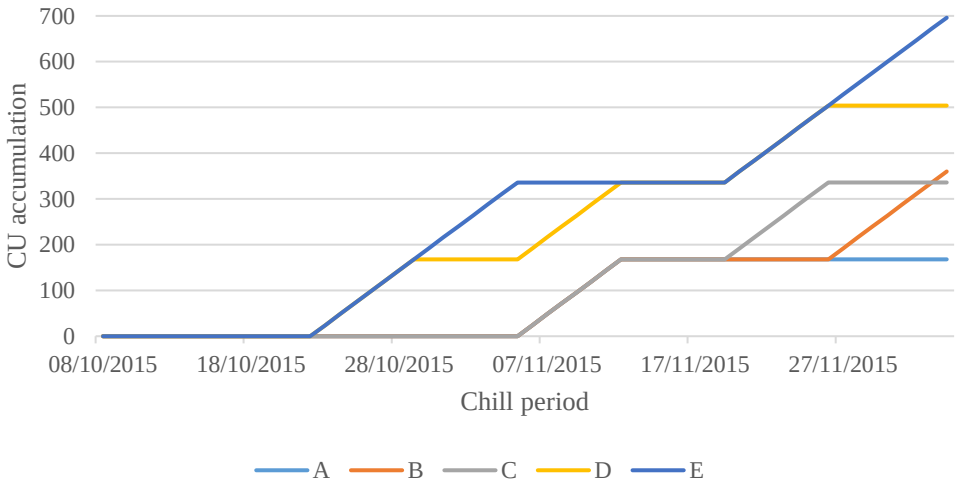


Figure 6.9 Pattern of chill accumulation using the <7.2°C over the chill period for each of the treatments

Following chilling, plants were transferred to a heated glasshouse for a week, during which time the Saxcil cabinets were sterilised. During this time the plants were fed (Vitafeed Standard 1:1:1, Vitax Grower, Vitax Ltd, Leicester, UK) and treated for aphids (Aphox and Calypso – see Chapter 2 for manufacturing details). Plants were then transferred back to controlled environments (Saxcil cabinets) for forcing (11/12/2015 – 07 /03/2016).

During forcing, plants were grown either in LD (0800-2300) or SD (0800-1800), supplied by a combination of fluorescent and tungsten lights (500-600 µmol m^{-2} sec^{-1}) (Table 6.2). As a result of the controlled environment used, chemical treatment for pest and diseases was not possible;

however, multiple PE traps (HORIVER, Koppert, Suffolk) were placed in each of the cabinets to reduce flies. Throughout the forcing period, plants were fed weekly (Vitafeed Standard 1:1:1, Vitax Grower, Vitax Ltd, Leicester, UK). A minimum of 10 plants from each of the chill treatments were randomly allocated to each of the six forcing treatments (Table 6.2), with temperatures chosen to cover a similar range to those included in the 2014-2015 experiment.

Table 6.2 Spring forcing treatments consisting of a number of temperatures photoperiod combinations, with progressively increasing temperature and contrasting photoperiods (LD = 18h; SD = 10h)

Temperature (°C)	**Photoperiod**
8	LD
13	LD and SD
18	LD
20	LD and SD

Parameters recorded

Similarly to the 2014-2015 experiment, growth and development was observed but the range of parameters was reduced, with the following recorded for each plant: the time to emergence of runners and flowers, as well as the quantity of runners.

Statistical analysis

Data were tested for normal distribution using the Ryan-Joiner test, and data were found not to be normally distributed across chill and spring forcing treatments. Kruskal-Wallis tests were performed for each of the chill and spring forcing treatments and showed comparable outputs to those from equivalent one-way ANOVA (except for chill treatments). It was decided, however, to maintain the parametric form of analysis in order to enable comparison between experiments and the same method of statistical analysis was used as in 2014-2015 experiment to determine the significance of the effect of chilling and forcing on spring response. GLMs were performing using Minitab 17, with chill treatments (chill units) and forcing temperature included as factors. The overriding issue of this experiment was that the data were not clear and the trends of significant do not appear consistent with either chilling or forcing; therefore the levels of significance are not taken to highlight the influence of treatments, rather the complexity of the response. Averages and standard error of the mean were calculated for each recorded parameter.

6.3 Results

6.3.1: 2014-2015 experiment

As an initial description of the overall response of *F. vesca*, plant growth and development parameters were recorded once plants were transferred into the spring treatments. Some replicates died (Table 6.3), which affected the choice of statistical analysis and may have obscured some of the recorded traits. However, sufficient data were recorded to provide a description of the response of plants to spring temperature following chilling, with statistical analysis of the data undertaken where appropriate.

Table 6.3 Number of plants that survived the experiment in each treatment; initial replication was ten plants per treatment

	Chill treatment		
Spring temperature (°C)	**A**	**B**	**C**
8	7	6	7
11	10	9	10
14	10	10	9
17	10	4	9
20	10	5	10

The rate of leaf production was slowest in the coolest spring temperature and highest in the warmest spring temperature (Figure 6.10). This trend was consistent across chill treatments, although there was some variation: in chill treatment B the rate of leaf production was less at 17°C than at 14°C after day 20. Overall the responses to spring temperature of plants subjected to chill treatments A and C were quite similar, although the difference between leaf production at 14°C and 17°C was less in treatment A than in treatment C.

Similar to rate of leaf production, rate of runnering increased with spring temperature, with the greatest rate of runner production observed at the warmest spring temperature (20°C) and the lowest at the coolest temperature (8°C) in all chill treatments (Figure 6.11). In chill treatment B production of runners was generally lower than in treatments A and C, especially in the warmer spring temperatures. Time to runner emergence also varied between chill treatments.

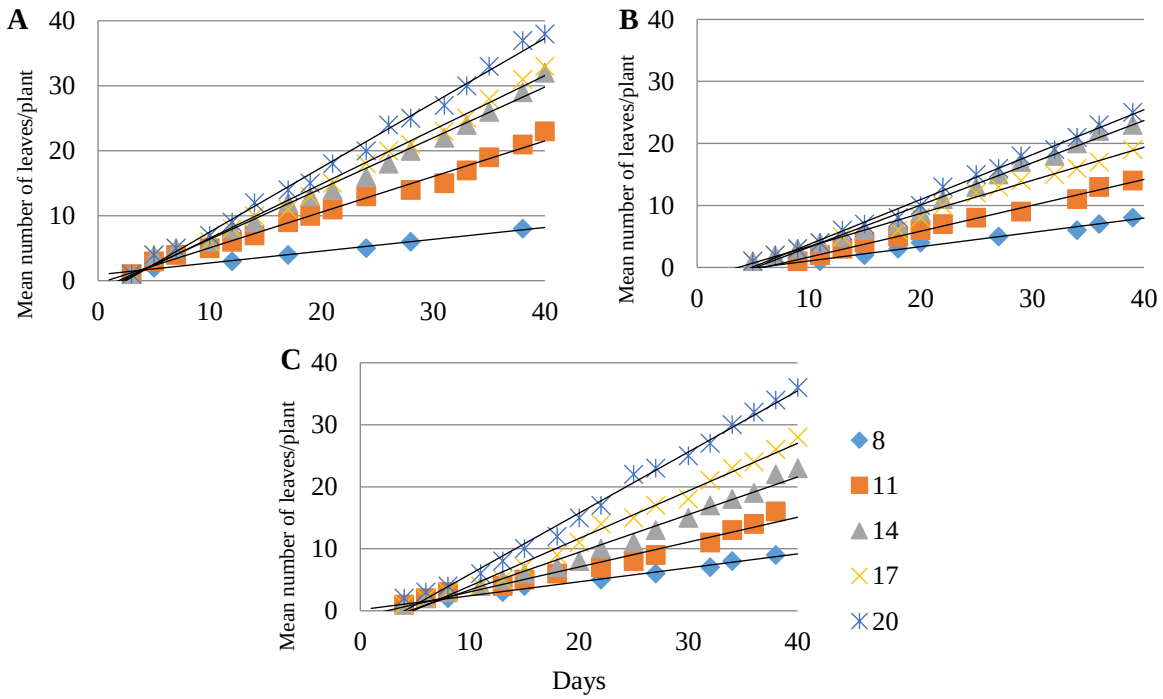


Figure 6.10 Mean initial leaf production per plant across chill and spring treatment combinations; the letter in the top left corner represents the chill treatment. Linear lines of best fit have been included to aid comparison of leaf production between spring temperatures

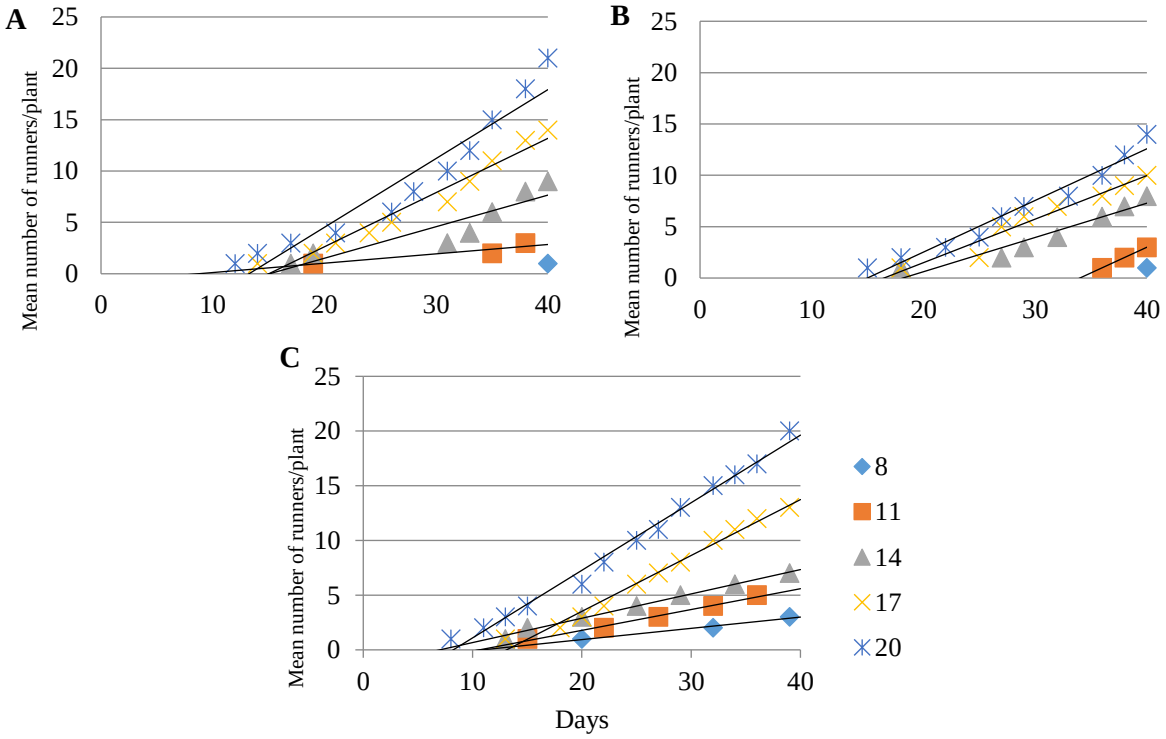


Figure 6.11 Mean initial runner production per plant across chill and spring treatment combinations; the letter in the top left corner represents the chill treatment. Linear lines of best fit have been included to aid comparison of runner production between spring temperatures

As for leaf production and runnering, the rate of flower production increased with spring temperature; with the greatest rate of flower production observed at the warmest spring temperature (20°C) and the lowest at the coolest temperature (8°C) for all chill treatments (Figure 6.12). There was some variation in time to flower emergence between treatments, with earliest flower emergence recorded in chill treatment C for the warmer spring temperatures (17°C and 20°C).

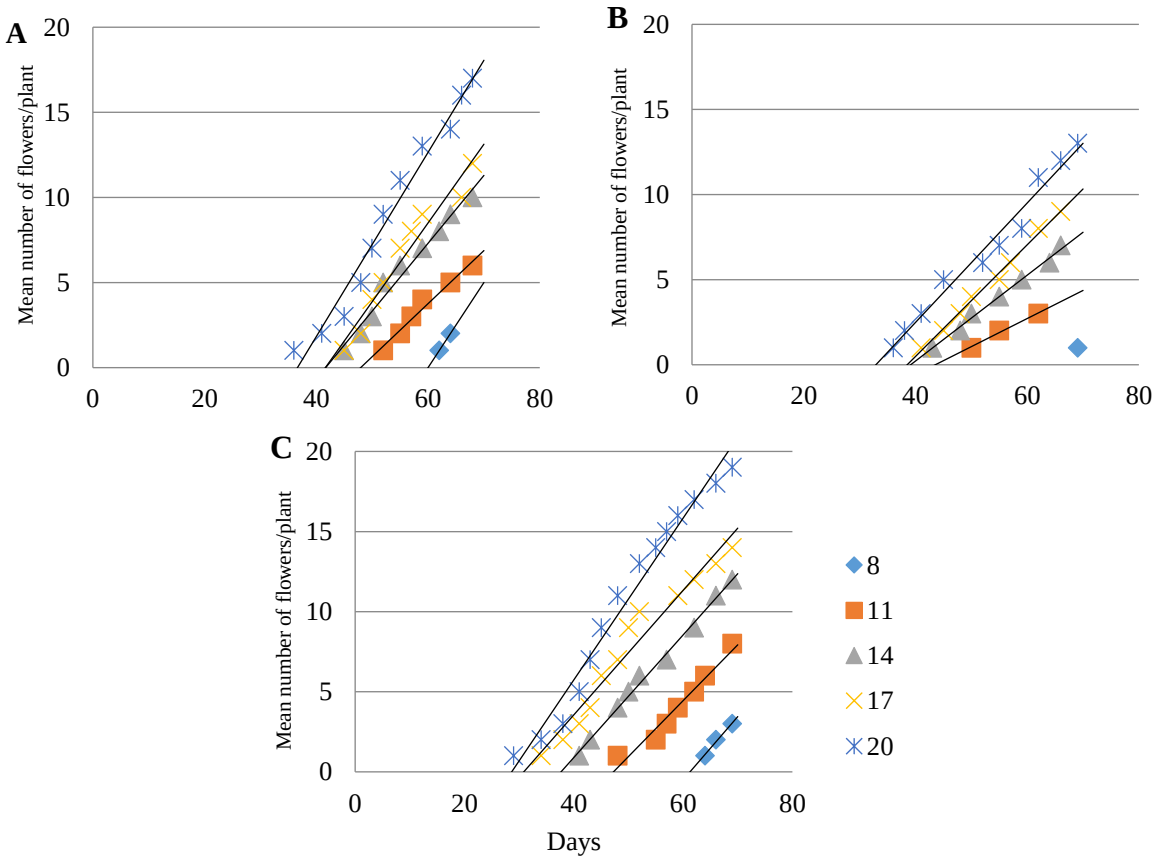


Figure 6.12 Mean flower production per plant across chill and spring treatment combinations; the letter in the top left corner represents the chill treatment. Linear lines of best fit have been included to aid comparison of runner production between spring temperatures

All plants produced leaves and runners during the experiment but some plants did not produce flowers (Figure 6.13). This variation in the proportion of plants flowering was not consistent across chill treatments but was lowest at the lowest spring temperature.

To address specifically the question of whether the growth and development response of *F. vesca* confirmed the ideas presented by Cannell, time to first emergence of runner and flowers was measured from when plants were transferred to forcing conditions. Data were collected for all plants across treatments, and average time to first emergence calculated and presented. However,

due to insufficient replication (as a result of plant death) in chill treatment B, only chill treatments A and C were analysed statistically.

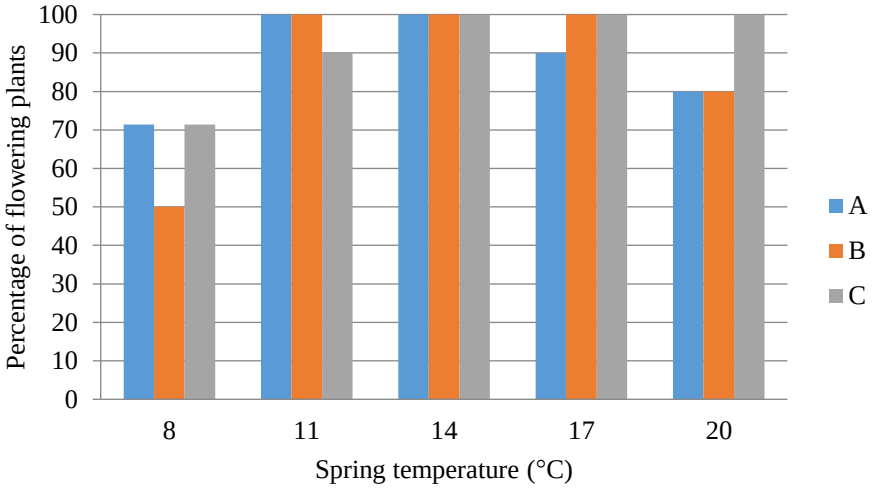


Figure 6.13. The percentage of plants that flowered during the experiment

Initial observations were made of the raw and reciprocal data before a generalized linear model (GLM) was used to analyse time to runner emergence in response to spring temperature and chill treatment. Chilling ($F_1 = 62.74$; $p = 0.00$) and spring temperature ($F_4 = 43.80$; $p = 0.00$) were both found to have a significant effect, and the interaction between these two factors was also significant ($F_4 = 4.96$; $p = 0.00$); chill treatment C showed significantly earlier runnering across all spring temperatures (Figure 6.14).

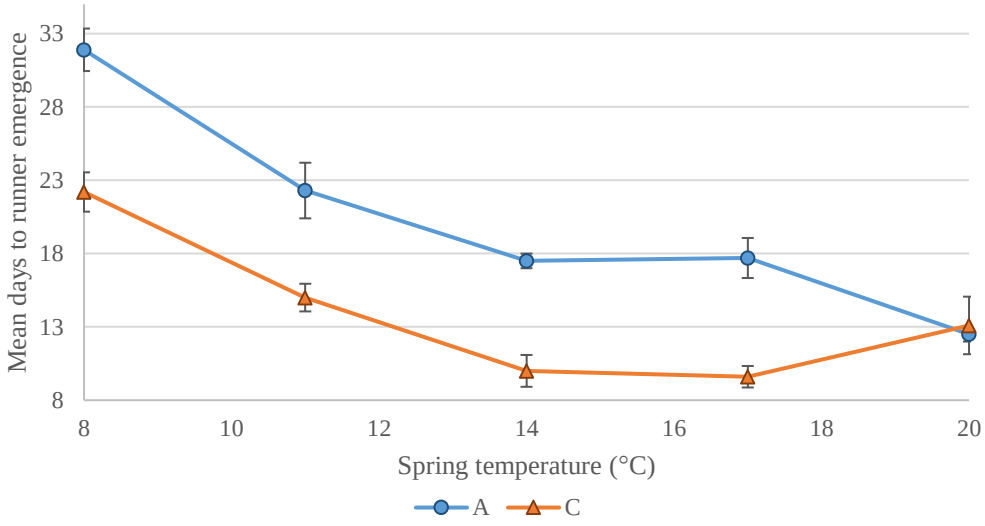


Figure 6.14 Mean days to runner emergence in relation to forcing temperature for chill treatments (A = 870 CU and C = 1550 CU). Standard errors of the mean are shown

Initial observations were also made of raw and reciprocal data for flower emergence, before a statistical analysis was performed using a GLM; this produced similar results to runner emergence. Chilling (F_1 = 45.70; p = 0.00) and spring temperature (F_4 = 170.67; p = 0.00) were both found to have a significant effect, and chill treatment C showed significantly earlier flowering across all spring temperatures (Figure 6.15).

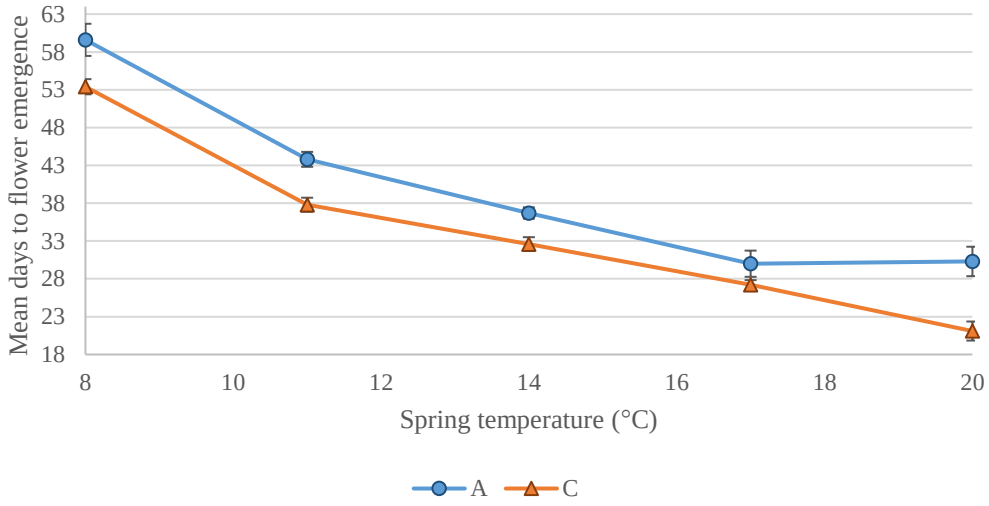


Figure 6.15 Mean days to flower emergence in relation to forcing temperature for chill treatments (A = 870 CU and C = 1550 CU). Standard errors of the mean are shown

One of the most striking results was the emergence of runners before flowers across chill and forcing treatments; typically flowers are reported to emerge before runners in *F. vesca* (Sønsteby and Heide, 2006; Walpole, 2015). One potential hypothesis to account for this reversal was the use of a LD photoperiod across forcing treatments, which may have promoted runnering more than flower emergence.

6.3.2: 2015-2016 experiment

To test whether the reversed order of emergence between runners and flowers observed in the 2014-2015 experiment was due to LD photoperiod, SD treatments were included at 13 and 20°C. The two photoperiod treatments were compared and showed little effect on time to runner production, with almost no difference in timing of emergence at 20°C and no consistent effect at 13°C (Figure 6.16). There was no significant difference in time to runner production between photoperiod treatments (F_1 = 0.04; p = 0.838), and photoperiod had no significant interaction with chilling (F_4 = 0.45; p = 0.77) or forcing (F_1 = 0.00; p = 0.94). For flower emergence there was no clear difference at 13°C, but LD consistently advanced time to emergence across chill treatments

at 20°C (Figure 6.17). This meant that at 20°C the gap between flowering and runners was narrowed, although runnering still came first. Analysis of the data with both forcing temperatures combined showed there was no significant difference in days to flower emergence as a result of photoperiod (F_1 = 3.32; p = 0.07) or significant interaction of photoperiod and chilling (F_4 = 0.78; p = 0.54), although the interaction between forcing temperature and photoperiod was significant (F_1 = 5.23; p = 0.02).

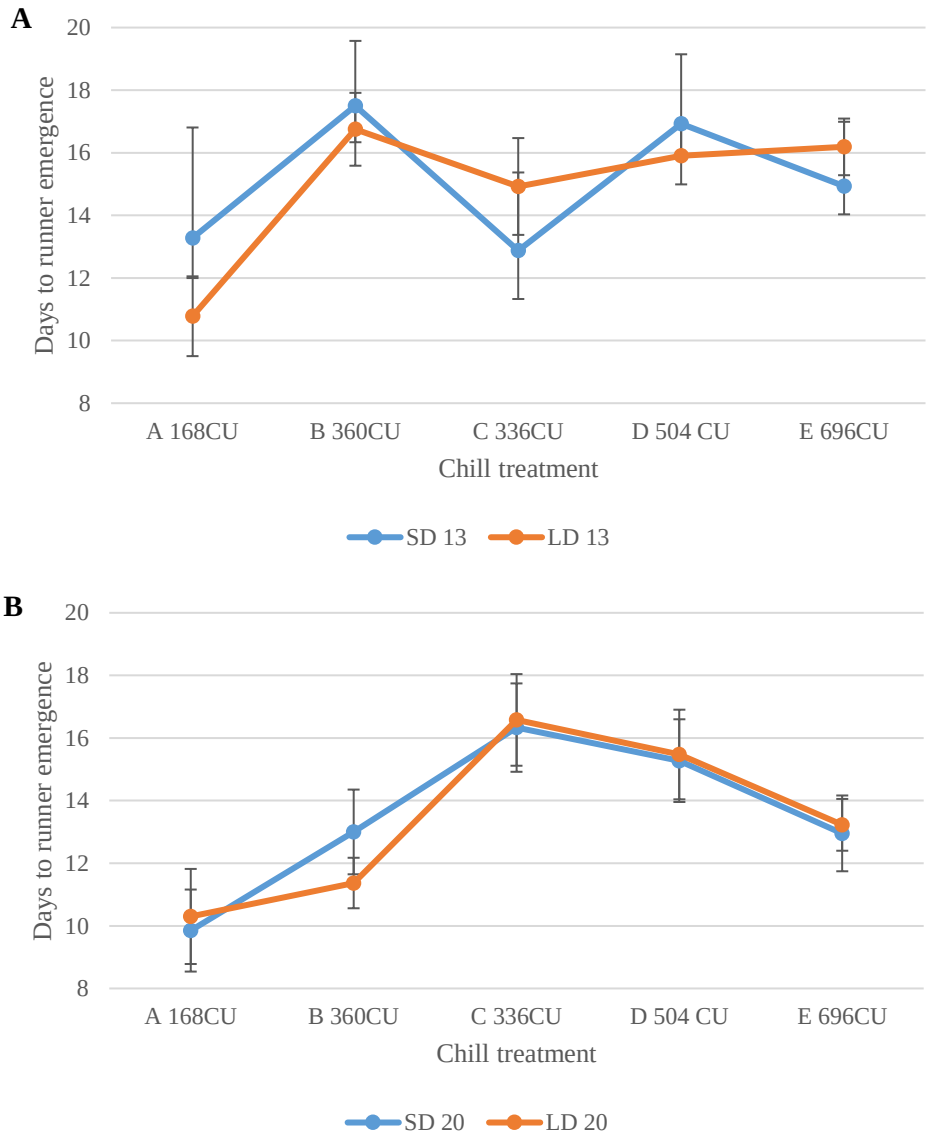


Figure 6.16 Mean days to runner emergence as a function of progressive chilling (chill accumulation increased from treatment A to E) for forcing at 13°C (A) and 20°C (B), under SD and LD. Standard errors of the mean are shown

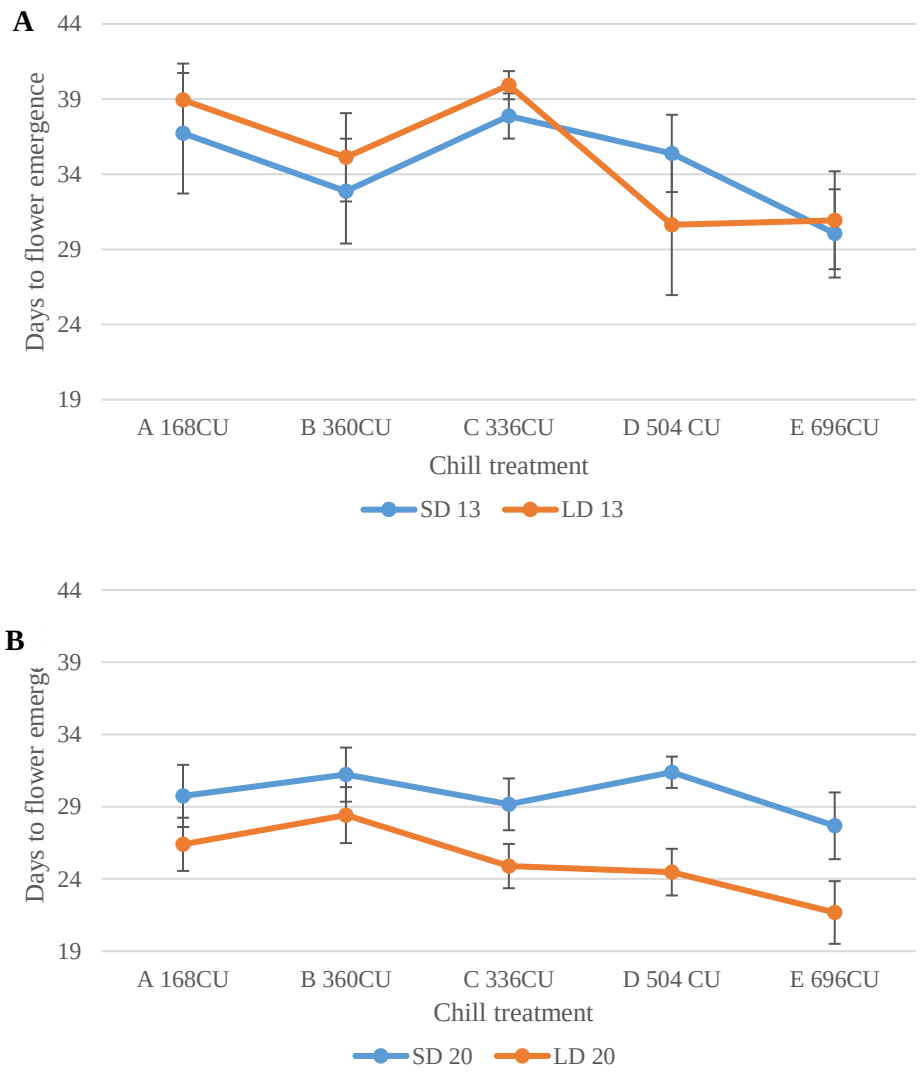


Figure 6.17 Mean days to flower emergence as a function of progressive chilling (chill accumulation increased from treatment A to E) for forcing at 13°C (A) and 20°C (B), under SD and LD. Standard errors of the mean are shown

Days to the beginning of runner production and flower emergence were recorded after plants were transferred to forcing conditions and these two parameters analysed separately. Time to runnering and flower emergence were recorded for each plant individually and averaged to provide the mean number of days to emergence for each chill treatment under each of the forcing treatments. The data are presented in relation to both forcing temperature and chilling, in order to highlight clearly how each of these factors influenced the response. Only data for plants grown in LD conditions are presented here.

Means days to flower emergence showed some similarity to the response described by Cannell (1989): days to flower emergence advanced with chill accumulation and increasing spring

temperature (Figure 6.18B), with chilling (F_4 = 14.39; p = 0.00) and forcing (F_3 = 41.09; p = 0.00) significantly influencing days to flower emergence. The interaction between these two factors was also significant (F_{12} = 3.40; p = 0.00), so that chilling had an effect on flowering which was significantly influenced by forcing and vice versa. There was a clear distinction between spring temperatures in the lower chill accumulation treatments (A, B and C), whereas further chilling (treatment D and E) was associated with less difference in days to flower emergence in the forcing treatments (Figure 6.18A).

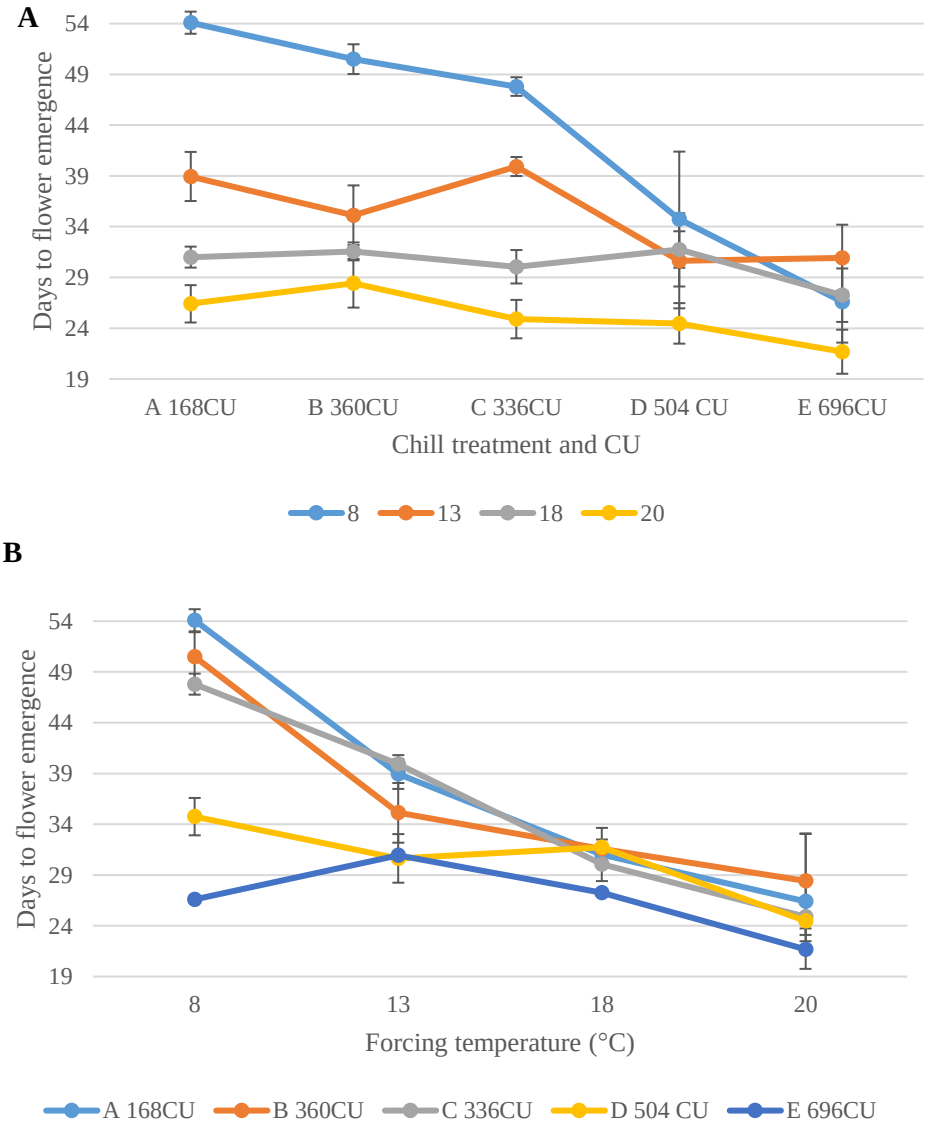


Figure 6.18 Mean days to flower emergence, under LD, as a function of: (A) progressive chilling (chill accumulation increased from treatment A to E); (B) forcing temperature. Standard errors of the mean are shown

The influence of chill accumulation was more prominent at 8°C in comparison with the warmer temperature treatments, with mean days to flower emergence decreasing by 28 days at the chill extremes (A and E). By comparison there was only a mean decrease of four days in flower emergence time between chill extremes for plants grown at 20°C; this reduced difference in days to flower emergence with chilling was also observed at 18°C (Figure 6.18B). Plants grown at 13°C showed a less clear effect of chilling on flower emergence time: there was an overall difference of eight days between chill treatment A and E, and this temperature treatment was more similar to the higher forcing temperatures than to the 8°C forcing temperature (Figure 6.18B). In general these results are consistent with the hypothesis by Vegis (1964), that additional chilling has a more pronounced effect at low temperatures, with the effect of chilling most pronounced at 8°C.

It was anticipated that time to the start of runner production would show a similar response to flower emergence, with the expectation that days to first runner production would have decreased across the chill treatments from A to E; and that the warmer the forcing temperature, the earlier runner production would have begun, with any particular chill treatment. However, the effect of chilling and subsequent forcing on days to the beginning of runner production was more complex than expected (Figure 6.19A and B).

Days to first runner production for plants from chill treatments B, D and E showed roughly the expected response: for example, days to first runner of plants in chill treatment B took twice as long for plants grown at 8°C in comparison to those at 20°C (Figure 6.19B). This difference was also observed for plants exposed to the two greatest chill accumulation treatments (D and E); however, for both these treatments plants grown at 18°C produced runners first of all the spring temperature treatments. Forcing temperature (F_3 = 11.75; p = 0.00) and chilling (F_4 = 7.90; p = 0.00), as well as the interaction between these factors (F_{12} = 3.14; p = 0.00), significantly influenced days to runner production. The interaction between chill and forcing appeared had the most apparent effect at low forcing (8°C), with the greatest variation between chill treatments observed at this temperature.

Unlike treatments B, D and E, chill treatments A and C did not show a clear effect of forcing across temperature treatments, with little difference between any of the temperature conditions in days to first runner production (Figure 6.19A). There appeared to less variation in response between chill treatments for all forcing temperatures above 13°C. The effect of progressive chilling on days to runner production may have been a result of the unexpected response of chill treatments A and C, in comparison to B, D and E (Figure 6.19A).

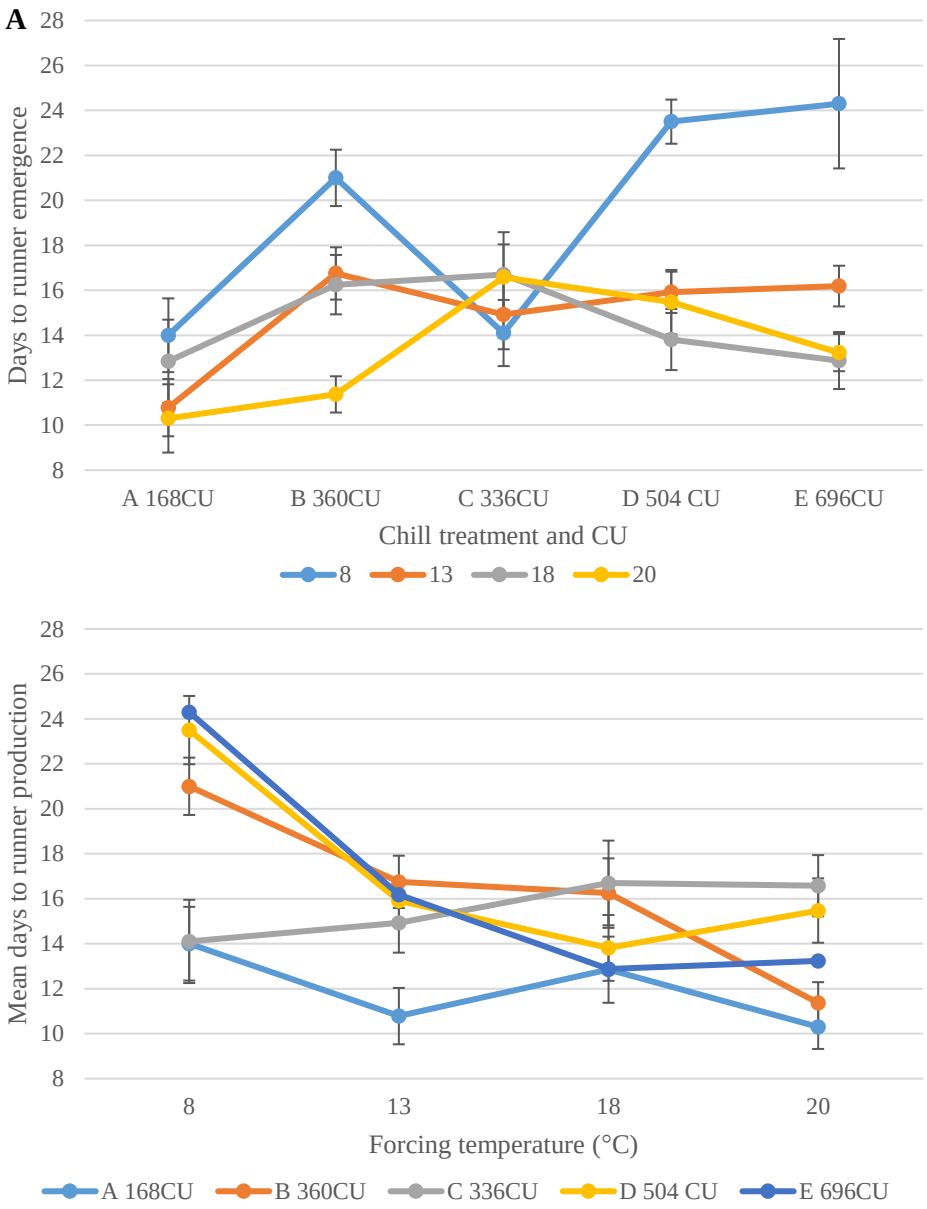


Figure 6.19 Mean days to runner production, under LD, as a function of: (A) progressive chilling (chill accumulation increased from treatment A to E); (B) forcing temperature. Standard errors of the mean are shown

Similarly to results from the 2014-2015 experiment, all treatments in the 2015-2016 experiment also produced runners before flowers; under LD forcing, runner production was observed approximately 10-25 days after transfer to forcing, whereas time to flower emergence took approximately 20-55 days (Figure 6.20). Regardless of this difference in time to first appearance, plants showed similarity in their general response to forcing temperature, with a more varied response in time to runnering and flowering at the low forcing temperature across chill treatments.

Comparison of these two phenological traits, in relation to forcing temperature, highlighted possible differences in their sensitivity to chilling.

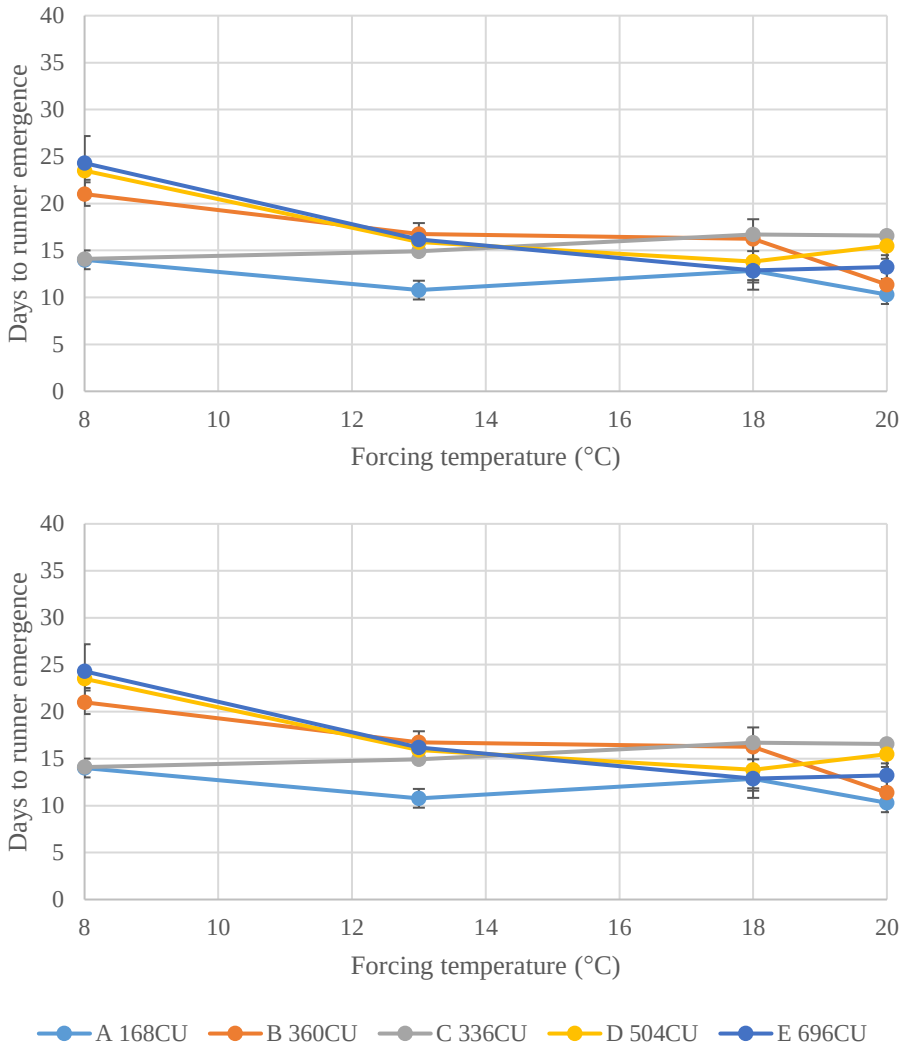


Figure 6.20 Comparison of mean days to runner production and flower emergence as a function of forcing temperature (in LD conditions) for each chill treatment. Standard errors of the mean are shown

As described time to runner production showed a more varied response at low forcing, however, the influence of increased chill accumulation and forcing temperature showed a more linear response than observed for flowering (Figure 6.20). This implied that runner production might be less sensitive to chill accumulation and increased forcing temperature than time to flower emergence, as increased forcing temperature had a more pronounced effect on reducing time to flowering than observed for runnering (Figure 6.20). This floral response also implied a potential saturation of chill accumulation; chill treatments D and E, which accumulated more than 504 CU, showed a more linear response to forcing temperature than chill treatments A-C, which accumulated less than 360 CU. This difference in flowering response between these groups of chill treatments suggested that chill accumulation for flower emergence may have been reached

and saturated between 360 and 504 CU, whereas for runner production plants appeared less sensitive to chilling across forcing temperatures (Figure 6.20).

6.4 Discussion

The two experiments described in this Chapter, carried out during the winter and spring months of 2014-15 and 2015-16, aimed to describe quantitatively the response of spring growth to winter chilling in *F. vesca*. In the first year the focus was on determining whether the relationship between chilling and thermal time (to budburst or flowering) described by Cannell for forest trees, could be found in the herbaceous perennial *F. vesca*, where the equivalent developmental events could be considered to be runner production and flower emergence. The expected response was that the more chilling is fulfilled, the wider the range of spring temperatures at which plants would grow; and that as chilling progressed, the thermal time required to reach spring phenophases (here runner production and flower emergence), would progressively decrease. These expectations were partially met: runners and flowers appeared earlier at higher forcing temperatures, and chilling generally decreased time to these developmental events. However, there were some limitations in this experiment (discussed in more detail below). In particular, chilling was accumulated naturally outside (in the 2014-2015 experiment) at a relatively high level compared to some estimates of the chilling requirement of *Fragaria* (see Atkinson *et al*., 2005). It was therefore possible that all the plants could have been over-chilled, potentially obscuring developmental responses; further, the impact of warm temperatures during the chilling period was not addressed systematically, a dimension of importance in relation to understanding the effects of predicted climate change. Finally, one of the most striking findings was that in all treatments runners began to be produced before flowers emerged, in contrast to the generally observed order of emergence, in which flowers are followed by runners in the natural environment (see below). One possible reason for this effect was that forcing was carried out under LD, conditions which are generally associated with promotion of runnering in *Fragaria* (see, for example, Battey *et al*., 1998).

The second experiment, in 2015-16, was designed to address the questions that arose from the first experiment, as well as to confirm and extend understanding of the chilling response of *F. vesca*. Chilling treatments were given in controlled environments, at a level less than in the first experiment, and included warm temperature breaks to allow potentially negating effects on chilling (or direct, promotive effects on growth via forcing) to be analysed. In the discussion that follows both experiments are reviewed together in order to allow the full range of chilling treatments to be included. Considering flower emergence and runner production in turn, the

results are compared to those predicted based on the work of Cannell and others; anomalies are highlighted and an attempt is made to account for them in terms of both heat (growing degrees) and chill operating together. Finally, the consistent tendency of controlled environments during forcing to advance runner production relative to flower emergence is highlighted, and an explanation offered in terms of the regulation by temperature of distinct plant developmental processes (meristem growth driving leaf and runner production, compared to expansion of pre-existing inflorescences). This contrasting view of runner production and flower emergence may also help to explain the anomalous effects of some of the chilling treatments.

6.4.1 Predicted responses

It was hypothesized that the growth responses of *F. vesca*, recorded as flower emergence and runner production, in relation to chilling and forcing would be that found for woody perennials (trees) by Cannell (1989). These are shown as generalized 'days to emergence' (of flowers or runners) (Figure 6.21):

- Graph A: as forcing temperature increased the time to emergence was predicted to decrease linearly, as shown by Campbell and Sugano (1979), providing that the forcing temperature remained below supra-optimal. Chilling would advance time to emergence across forcing temperatures with progressive chill accumulation.
- Graph B: as chill was accumulated the rate of emergence was expected to increase exponentially, as suggested by Cannell (1989). The influence of accumulated chilling on emergence was expected to be more apparent at low forcing temperature, as chilling was expected to alleviate growth restraint at low temperatures (Vegis, 1964).

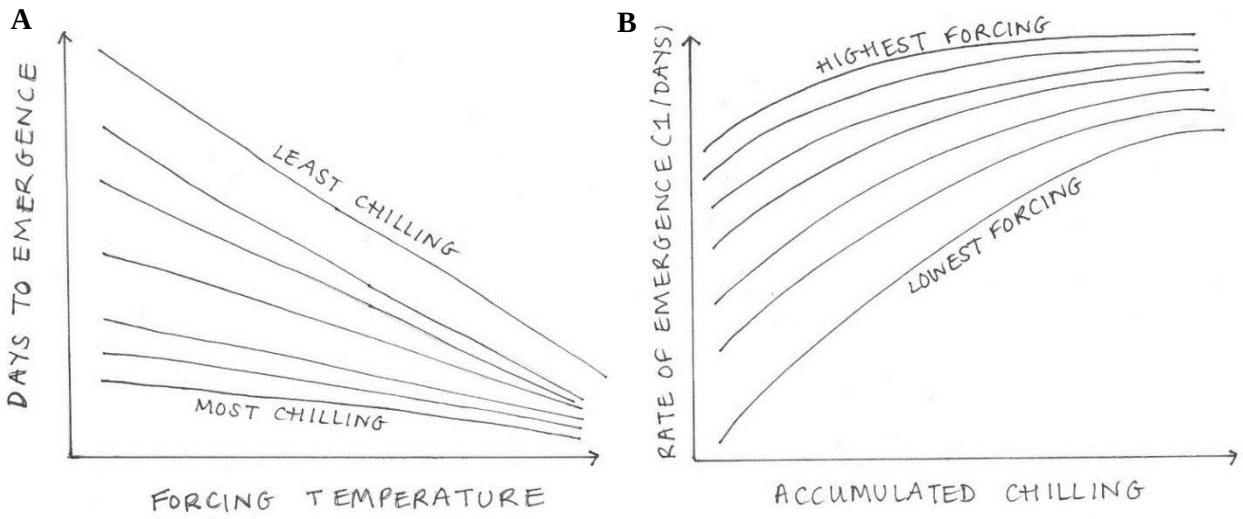
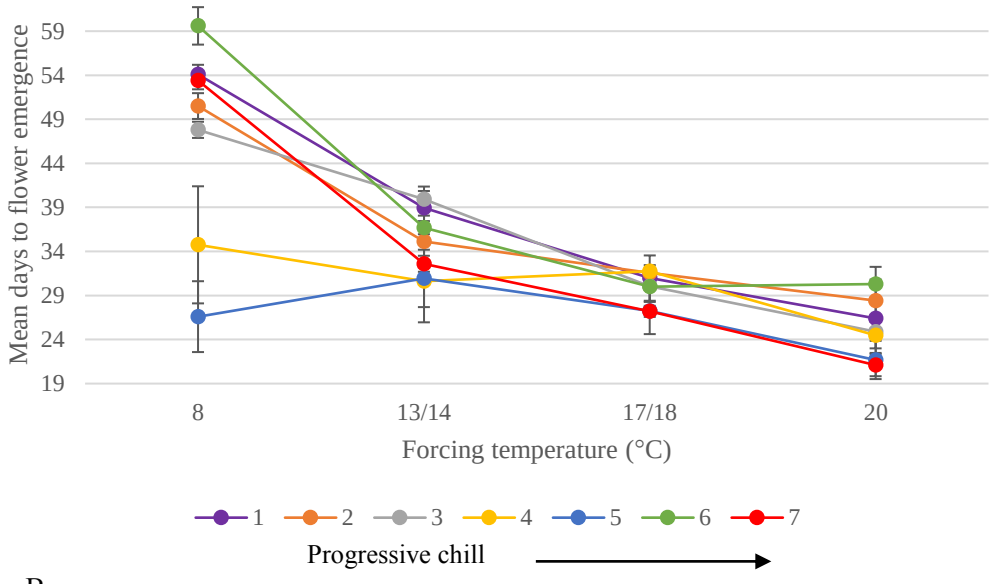


Figure 6.21 Hypothesised growth response to A) forcing temperature and B) chilling

6.4.2 Flower emergence

The treatments from the 2014-15 and 2015-16 experiments, renamed 1-7 in order of increasing chill accumulation, are presented together in Figure 6.22. In general, plants exposed to greater chill accumulation showed earlier flower emergence, with chilling shown to significantly influence time to flower emergence in both experiments ($P < 0.05$); even though in 2014-15, chilling was given naturally under field conditions, whilst in 2015-16, plants were exposed to constant controlled temperatures (Figure 6.22). As well as the way in which chilling was accumulated, the experiments differed in timing of forcing: the duration of the chill period in 2015-16 was the same across all the chill treatments, regardless of differences in total chill accumulation, with plants transferred to forcing conditions on 11/12/2015. In contrast, in 2014-15 the natural chill accumulation resulted in varied chill duration and time of forcing between treatments, with treatment 6 (2014-15 A) transferred to forcing conditions on 09/01/ 2015 and treatment 7 (2014-15 C) transferred on 16/02/2015. This difference in timing of forcing in relation to chilling did not appear to influence the timing of flower emergence, and forcing was shown to significantly influence time to flower emergence in both experiments ($P < 0.05$).

A



B

	2015-2016 A	2015-2016 B	2015-2016 C	2015-2016 D	2015-2016 E	2014-2015 A	2014-2015 C
Renamed treatment	1	2	3	4	5	6	7
Chill units	168	360	336	504	696	870	1550
Chilling	Controlled environment – weekly transfers between cold (6°C), mid (10°C) and warm (15°C) chill temperature conditions, depending on treatment; total chill duration was the same for all treatments, so forcing started in unison across treatments					Field conditions – natural temperature conditions; plants transferred to forcing conditions at intervals	

Figure 6.22 Flower emergence in the 2014-2015 and 2015-2016 experiments. A) Mean days to flower emergence as a function of forcing temperature for each of the seven chilling treatments; B) Chilling treatments, accumulated chill units and the method of chilling was given

It is noteworthy, however, that this difference in experimental design meant that there was also a difference in the photoperiod associated with chill treatments: the controlled environment facilities used for chilling in 2015-16 provided a constant SD (8h) across all chill treatments, whilst plants in 2014-15 were exposed to natural photoperiod during chilling, which would therefore have varied across the treatments. Nevertheless, all chill treatments across both experiments would have been exposed to SD. Low temperature (6°C) was shown to nullify the dormancy-inducing effect of SD by Sønsteby and Heide (2006); therefore, considering that all plants were subjected to SD, it was assumed that differences between treatments were primarily due to differences in chill pattern and forcing temperature, rather than photoperiod. This assumption appears to be borne out by the broad similarity in the response of flower emergence to forcing temperatures between the two experiments.

The difference in flower emergence time between chill treatments was more apparent at low (8°C) than at high forcing temperatures (Figure 6.22A). A total difference in flower emergence of 33 days was observed at 8°C (between chill treatments 5 and 6); whilst at the highest forcing temperature (20°C), a difference of nine days was recorded (between treatments 6 and 7). These results suggest that in general progressive chilling acts to release inhibition of flower emergence in *F. vesca*, consistent with the expected effect of progressive chilling on widening the temperature range over which growth can occur (Vegis, 1964). An example of this response has been described for budburst in elm, with the depth of dormancy reflected by the range of temperatures promoting growth: the deeper the dormancy, the narrower the range (Ghelardini *et al*., 2010). In a similar type of response, breaking of summer dormancy by high temperatures acted to decrease growth inhibition at high temperatures in a perennial grass (*Poa bulbosa*) (Ofir, 1986).

There were, however, some anomalies in the general pattern of response. For example, chill treatments 4 and 5 showed little effect of forcing temperature on time to flower emergence in comparison to other treatments; as a result timing of emergence for these two treatments was significantly earlier at low forcing temperature (8°C), whereas at mid-warm forcing temperatures (13-20°C) flower emergence was more in line with other treatments (Figure 6.22). This lack of forcing temperature response was not observed for treatments with less or more chill accumulation (for example, treatments 3 and 6), consistent with the idea that total chill accumulation was not the primary reason for this lack of forcing temperature response. Further, the pattern of chill accumulation prior to forcing was similar to that in treatments 2 and 3 (Table

6.4), suggesting that this effect was not a consequence of exposure to warm temperatures before forcing. One potential explanation is that these were the highest chill treatments in the 2015-2016 experiment and that the response to chilling had saturated.

Trt	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Total CU
A	H	W	H	W	C	H	W	W	168
B	H	H	W	W	C	H	W	C	360
C	H	H	W	W	C	W	C	W	336
D	H	W	C	H	C	W	C	W	504
E	W	W	C	C	W	W	C	C	696

Table 6.4 Sequence of temperature exposure for chill treatments in SD in controlled environments in the 2015-16 experiment. H = 15°C; W = 10°C; C = 6°C

The differences in the method of chill accumulation in the 2015-2016 experiment, compared to the 2014-2015 experiment, could have led to advanced flower emergence at low forcing temperatures in treatments 4 and 5 relative to treatments 6 and 7, even though the latter accumulated more chill units overall. This may be explained by the fact that in the 2014-15 experiment plants received supra-optimal chilling. According to Vegis (1964), dormancy determines the range of temperatures at which plants are able to grow: progressive chilling gradually removes dormancy, decreasing the minimum temperature allowing growth, but above a threshold chilling increases this minimum temperature again (Figure 6.23). This would lead to a lack of expected responsiveness at low forcing temperatures in treatments 6 and 7.

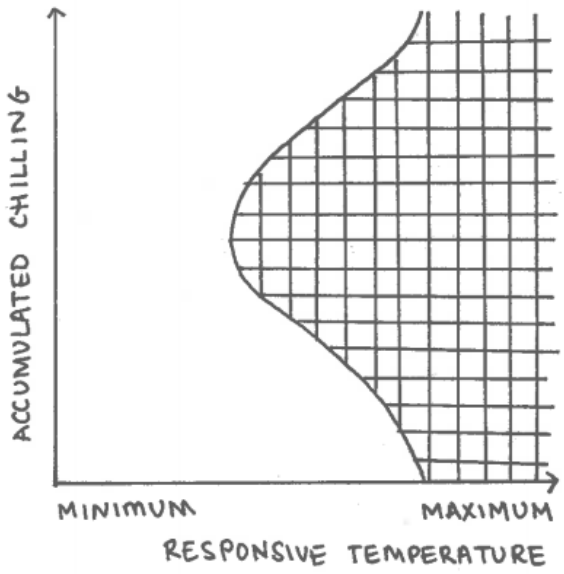


Figure 6.23 Diagrammatic representation of the potential promotive temperature range (area with hatching) for flower emergence with changes in accumulated chilling. A widening of the responsive range through a decrease of the minimum temperature, is followed by narrowing, with additional chilling resulted in an increase in the minimum promotive temperature

6.4.3 Runner production

As for flower emergence, runner production generally began earlier with increasing forcing temperature and forcing temperature significantly influenced time to first runner in both experiments ($P < 0.05$). There was greater difference in timing between chill treatments at low than at higher forcing temperatures: there was a maximum 18 day difference (between chill treatments 1 and 6) at 8°C, whilst at 20°C the maximum difference was only seven days (between treatment 1 and 3) (Figure 6.24). Chill treatments 1, 3 and 7, however, showed less clear trends across forcing temperatures, and treatment 6 had the longest time to runnering at low-mid forcing temperatures (8-17°C). As treatment 6 was a high chill treatment it was expected to show earlier runner production, regardless forcing was shown to significantly influence time to first runner in both experiments ($P < 0.05$).

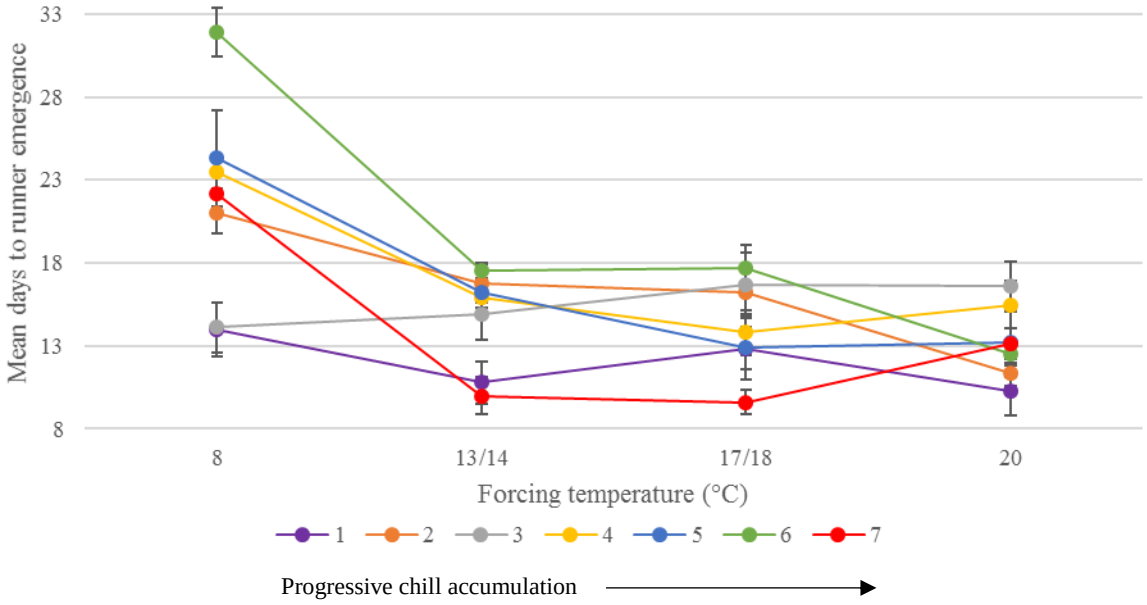


Figure 6.24 Mean days to runner production in relation to forcing temperature across different chill treatments in the 2014-2015 and 2015-2016 experiments

One explanation for the unexpectedly early runner emergence in chill treatment 1 across forcing temperatures could have been the pattern of chilling. Chill treatment 1 received a three week period of warm temperature (10-15°C) before transfer to forcing, which was not experienced to the same degree by any of the other chill treatments; as a result treatment 1 had the highest heat units accumulated (see below for further analysis). This warm temperature could have been promotive for growth, regardless of low chill accumulation, and forcing for this chill treatment may therefore have begun prior to the start of the forcing treatment. This pattern was not, however, observed for flowering in this treatment, which suggests that the regulation of runner production and flower emergence differs; the SD and warm temperatures experienced by chill treatment 1 towards the end of the chilling period were apparently not sufficient to advance flower emergence but they were for runner production. While the promotive conditions for runnering formation have been established to be LD and high temperatures (Battey *et al.*, 1998), SD at warm temperatures have been shown in this thesis to be equally promotive for runner production (see Chapter 4).

6.4.4 Runner production and flower emergence: evidence for differential regulation

Overall the difference in time to emergence between forcing extremes (8 and 20°C) was less for runner production than for flower emergence, with a difference of four and nine days at chill treatment extremes (1 and 7), in comparison with 23 and 32 days for flower emergence (compare Figures 6.22 and 6.24). Chilling therefore had a greater effect on time to flower emergence in relation to forcing temperature than it did on time to runner production.

Temperature was found to have a major impact on flowering time in *F.* x *ananassa* (Le Mière *et al.,* 1998) and Verheul *et al.* (2007) identified an optimal photoperiod of 12h and a day/night temperature of 18/12°C for flowering time. Low day and night temperature (6°C) and LD reduced flower emergence, and cultivars showed variation in optimal conditions and sensitivity to temperature and photoperiod (Verheul *et al.,* 2007). The conditions promotive for runner initiation and subsequent emergence have been characterised as LD (greater than 14-16h) and high temperatures (above 17-20°C optimal) (Battey *et al.,* 1998). There is thought to be a competitive interaction between runner and flower emergence at a physiological level, and the relationship between the development of flowers and runners under LD has been described as antagonistic (Gaston *et al.,* 2013). Perrotte *et al*. (2016a) reviewed the interaction between runners and flowers, and stated that environmental factors (temperature and photoperiod) are known to alter the balance between vegetative and floral development in strawberry (see also Bradford *et al*., 2010).

It is therefore generally unsurprising that differences in forcing responses were observed between runner production and flower emergence in response to chilling. This is, however, the first detailed description of the way in which these responses differ. It is important to note that spring runner production requires the initiation of new leaves in the spring (the first axillary bud to begin growth in the spring becomes the first runner) (Guttridge, 1959; Dana, 1980; see Chapter 3 Introduction). It is therefore, principally a reflection of SAM growth, as well as requiring cell expansion of the newly produced runner. Flowers, on the other hand, are initiated during the autumn, and their spring emergence occurs solely a result of cell expansion in these pre-existing structures (Hollender *et al.,* 2012). It is therefore reasonable to infer that runner production and flower emergence are mainly regulated by the environmental constraints on meristem activity and cell expansion, respectively.

The differences between runner production and flower emergence also highlight an important difference between *F. vesca* and the tree species studied by Cannell and others. Trees undergo budburst in the spring, the leaves in the bud being initiated and undergoing early development before/during dormancy, ready to emerge in the spring (Spann *et al.,* 2007; Lauri and Cochard, 2008). In *F. vesca*, on the other hand, the leaves which emerge in the spring are mainly initiated

at that time, and runners along with them. There is no bud. The herbaceous perennial habit of *F. vesca* therefore means that the spring growth of its runners and flowers would be expected to differ in detail from the more unified development of tree buds.

6.4.5 Quantifying chill and heat accumulation to explain anomalous responses to chilling

For the research carried out here long-term phenological data were not available, so methods based around the Dynamic Model and partial least squares regression analysis (see Chapter 6, Section 6.1) could not be used to distinguish between and quantify chill and heat components of the runner production and flower emergence responses. The influence of negating temperatures and accumulated heat units has, however, been addressed in accumulation models which have a more physiological approach to plant response, such as the Utah model (Richardson *et al*., 1974). This model was initially established to include a negating influence of supra-optimal chilling temperatures (Richardson *et al*., 1974). The Utah model was therefore tested here, alongside the simple cumulative chill method employed in both the 2014-2015 and 2015-2016 experiments (<7.2°C method). The Tehranifar (1997) model was also tested as it was developed from the Utah model specifically for *F.* x *ananassa* ('Elsanta'). Unlike the Utah model, Tehranifar's model did not include a negating effect of temperature above the maximum and minimum threshold. Chill accumulation calculated using these three alternative models gave similar results: in all cases chill treatment 1 accumulated the least chill units, and chill treatment 7 the greatest (Table 6.5). There were however, some differences between models, with the greatest overall difference between treatments shown by the Tehranifar model. The same order was nevertheless maintained regardless of chill model, and regardless of the inclusion of negating temperatures in the Utah model. The Utah and Tehranifar models for chill accumulation therefore offered no further explanation for the anomalous responses observed.

Table 6.5 Chill units accumulated for the chill treatments in the 2014-2015 and 2015-2016 experiments using three methods of chill quantification: the cumulative chill model (<7.2°C), the Utah model and the Tehranifar model

			Accumulated chill units		
Experiment	**Treatment**	**Renamed**	**<7.2°C**	**Tehranifar**	**Utah**
Cannell2	A	1	168	519.12	504
Cannell2	B	2	360	597.6	612
Cannell2	C	3	336	681.84	684
Cannell2	D	4	504	738.96	768
Cannell2	E	5	696	1010.4	1044

Cannell1	A	6	870	1228.56	1123
Cannell1	C	7	1550	2030.7	1738.5

One key factor highlighted by Tehranifar (1997), however, was the difference in chilling requirement between *F.* x *ananassa* plants which had been chilled in the field in comparison with a cold store. He concluded that field chilling was more effective than that received by plants in cold store (controlled environment), particularly for vegetative growth resumption. The most obvious difference between these two environments is the occurrence of naturally oscillating temperatures under field conditions. Erez and Couvillon (1987) showed that warm breaks enhance chilling in species such as peach, with chill efficiency of low temperatures increased by cycling with moderate temperatures (15°C); the effect of promoting chill effect was greatest in the latter stages of the rest period. In other species, such as blackcurrant, studies have shown a reversal of accumulated chilling by exposure to warm temperatures (Jones *et al.*, 2013). In the two experiments described here, plants received warm breaks during chilling, either naturally (2014-15) or as a deliberate part of the treatments (2015-16). Therefore warm breaks had the potential either to enhance or negate chilling in these experiments.

A development of the Utah model (Anderson and Richardson, 1986) offered the opportunity to quantify the effects of warm temperatures alongside those of chilling by the accumulation of heat units (HU), otherwise referred to as growing degree days (GDD) (Perry and Wehner, 1996; Snyder *et al.*, 1999). Heat accumulation is calculated by summing the difference between the mean daily temperature and the base/threshold temperature necessary for a given growth or developmental process (typically budburst or flowering):

GDD = mean daily temperature – base temperature

If daily mean temperature is less than the base temperature, GDD is set to 0, so that GDD is always positive. The base temperature was here set at 8°C, because for both experiments 7.2°C was set at the maximum threshold for chill accumulation. The choice of 8°C was also deemed appropriate given that when plants were grown at low forcing temperatures, all plants showed

runner production and flower emergence at 8°C (in 2015-16), while at 6°C (in 2014-15) they did not. As well as heat unit requirement, the time at which heat accumulation should begin was not known, so two potential methods were tested (Table 6.6), beginning heat accumulation from:

1) the time chill units began to be accumulated, which differed from the start of the chilling treatment (timing differed between chill treatments in the 2015-16 experiment, 23/10/2015 for treatments 4 and 5 and 06/11/2015 for treatments 1-3; 03/11/2014 for all 2014-15 experiment chill treatments);

2) the start of the experiment, which was the same time within each experiment (08/10/2015 – 2015-16 experiment; 01/11/2014 – 2014-15 experiment).

Table 6.6 Heat unit accumulation, as growing degree days (GDD), prior to forcing for treatments from start of chill accumulation (method 1) and the start of the chilling treatment (method 2)

		Heat units (GDD)	
Renamed	**Chill treatment**	**Method 1**	**Method 2**
1	C2 A	79	217
2	C2 B	63	196
3	C2 C	30	163
4	C2 D	79	149
5	C2 E	28	58
6	C1 A	33	45.1
7	C1 C	37.3	49.4

Both methods revealed differences between chill treatments, with greater heat units overall across the chill treatments using method 2 (Table 6.6). The differences were most apparent in the lowest chill treatments (1-4), indicating that plants from the lowest chill treatments had the most heating (forcing). Most strikingly, the generally early runner production (across all forcing temperatures) in chill treatment 1 was correlated with a high heat accumulation, particularly when calculated according to method 2: although this treatment had the least chilling, it also had the most heat. This may have offset the lack of chilling, promoting generally earlier runnering. However, this explanation does not account for the anomalous response of chill treatment 3, which showed similar early runnering to treatment 1 but had relatively low heat unit accumulation. The delayed runnering of treatment 6 at low forcing temperatures was not obviously related to heat accumulation. Therefore, heat accumulation quantified using these simple methods did not

account for all the forcing responses observed. The inclusion of heat units did, however, highlight the complexity involved with chilling and consideration of the combination of chill and heat accumulation provided some additional explanatory power. The mix of chill-heat conditions seems likely to have complicated the expected treatment differences, resulting, in some cases, in greater similarity in emergence responses than expected.

6.4.6 The order in which flowers and runners were produced and emerged

In the natural environment, flowers typically emerge from *F. vesca* and *F.* x *ananassa* plants in the spring, and runner production follows (Battey *et al.*, 1998; Carew and Battey, 2005; April 2017 dissections, Chapter 3). This behaviour is described as precocious (Darrow, 1966; Hytönen *et al.*, 2004), in contrast to the hysteranthous order of leaves and flowers in tree species such as apple (see below). The precocious flowering (in relation to runnering) is illustrated for *F. vesca* in Figure 6.25, in an experiment carried out by MSc student Jan Walpole. She recorded phenological timing in a range of ecotypes at two experimental sites (Exeter and Torquay) (Walpole, 2015). In all ecotypes, regardless of experimental site, flowers emerged before runners. There was some variation between ecotypes in timing of emergence of runners and flowers; but the order was the same nonetheless. The Park Wood ecotype (Reading; UK9) included in this experiment was used in both the 2014-2015 and 2015-2016 experiments described here.

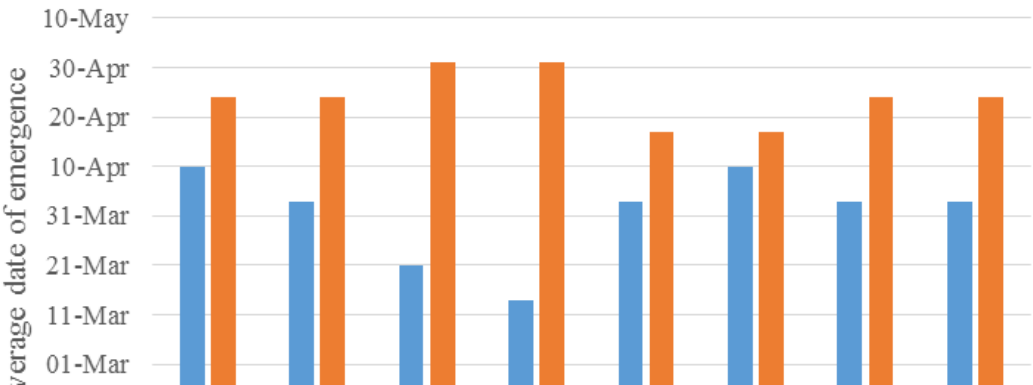


Figure 6.25 Mean emergence date for runner production (DTR) and flower emergence (DTF) for four *F. vesca* ecotypes (E – Exeter; T – Torquay; S – Scotland; R – Reading) grown at two experimental sites E – Exeter; T – Torquay) (data adapted from Walpole, 2015)

It is therefore very striking that in both the experiments described in this chapter, runner production consistently began before flowers emerged (see Figures 6. 14, 15, 16, 17 and 18). This observation was noted in the 2014-2015 experiment. To test whether it was a response to LD during forcing, the 2015-2016 experiment included a SD treatment at 13 and 20°C. Regardless of this, or the different methods of chill accumulation, on average runners were produced before flowers in all forcing/chilling treatment combinations in the 2015-2016 experiment.

The lack of effect of photoperiod on the order of emergence suggests that it does not solely determine the relative timing of runner production and flower emergence. However, photoperiod was shown to have a significant interaction with forcing temperature on timing of flower emergence ($P < 0.05$), but this was not shown for runnering. The interaction of photoperiod and forcing temperature on flowering has been previously reported, with inflorescence development promoted by long photoperiods (>14h) and warm temperatures (18-20°C) (Darrow, 1966).

Runner production is also promoted by LD and warm-high temperatures (Hytönen *et al.*, 2004; Hytönen and Elomaa, 2011). Hytönen and Elomaa (2011) showed an interaction between temperature and photoperiod: at high temperatures (21-27°C) runners were produced regardless of photoperiod (8-24h); at intermediate temperatures (15-18°C) LD promoted runner production, while under SD it greatly declined; and at low temperatures (9-11°C), runner production was reduced regardless of photoperiod. To test whether runner production showed a similar response here, it was analysed for the first few weeks of forcing in the 2015-2016 experiment (Figure 6.26). Initial analysis showed that there was no significant effect of chilling on runner production ($P > 0.05$), but it was significantly affected by, and directly proportional to, forcing temperature ($P < 0.05$). The response to photoperiod also followed the pattern described by Hytönen and Elomaa

(2011): runnering under SD was significantly reduced at both forcing temperatures (13°C and 20°C) in comparison to LD ($P < 0.05$) (Figure 6.26). These results indicate an interactive effect of temperature and photoperiod on runner production. The lack of a significant effect of chilling suggests that the response of runner production to forcing was sufficiently great to obscure any previous influence of chilling. It is also striking that the effect of temperature and photoperiod on time to first runner production was different, and more complex than that for runner production itself.

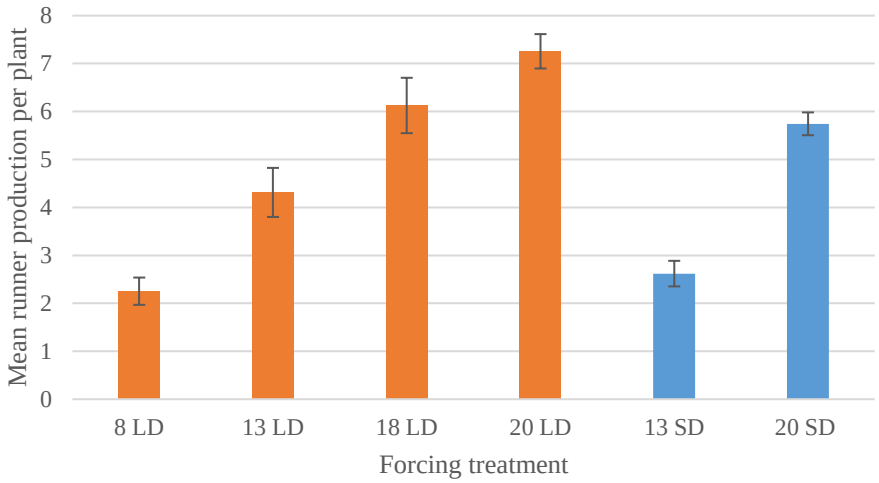


Figure 6.26. Mean runner production for the first four weeks after transfer to forcing conditions. Analysis revealed no effect of chilling within each forcing treatment, so chill treatments were combined to calculate means for each forcing treatment. Standard error of the means are shown

The emergence of flowers before runners has also been observed in experiments which included the use of controlled environment facilities, such as those of Ling (2011), who exposed *F. vesca* plants to a range of chilling treatments of varying durations at 4, 8 and 12°C in SD; plants were then forced in LD at 20°C. The numbers of inflorescences (flowers) and runners were recorded rather than timing of emergence, and time to emergence was inferred from the mean number of runners/inflorescences. Flowers emerged before runners across all treatments, and for one chill treatment (12°C/8 weeks) flower emergence began while plants were still under chill conditions; all other chill treatments showed flower emergence following transfer to forcing conditions by week 10 at the latest.

On the other hand, runner production was only observed once plants were transferred to forcing conditions, and was not observed for all treatments until week 11. Sønsteby and Heide (2006) also focused on numbers of runners and flowers, but in *F.* x *ananassa*, and similarly to Ling (2011) timing of emergence can be inferred during forcing (in a controlled environment with

constant temperatures). Plants were grown under contrasting conditions (SD or LD) following a period of controlled floral induction and a period of chilling, the duration of which varied between treatments (0-6 weeks). The response of inflorescence and runner production was recorded for two cultivars under LD and SD growth conditions, and for both cultivars flowers emerged slightly before runners regardless of photoperiod (this response was inferred from production data presented by Sønsteby and Heide, 2006). In a field-based study of *F.* x *ananassa*, Antanaviciute (2016) recorded flower emergence on average 12 days before runner production.

The results of these four studies are summarized in Table 6.7, alongside the data from the experiments described in this Chapter. In all cases, apart from the experiments described here, flower emergence preceded runner production. However, it is interesting that the difference in timing was typically much less pronounced under controlled environments. For instance, apart from the work described here, the difference between the beginning of inflorescence production and that of runners was not greater than seven days for both *F.* x *ananassa* and *F. vesca* when grown under controlled environments (Sønsteby and Heide, 2006; Ling, 2011). For plants grown in the field, on the other hand, there was a more varied response, with differences in timing from 7-21 days, depending on the study and species (Walpole, 2015; Antanaviciute, 2016). This suggests that controlled environments may promote runner production more than field conditions so that the difference in timing is reduced, possibly as a response to constant (warm) day/night temperature. This hypothesis accounts for the reversed order of emergence/production recorded in both experiments in this Chapter, where controlled environments employed constant temperatures throughout forcing.

Table 6.7 Summary of timing of flower emergence (often inferred from the number of inflorescences) and

Runners	17/05	Week 5/6	24/04	17/04	Week 10	13.2
Difference	12 days	0-7 days	21 days	7 days	7 days	19 days reversed

Species	Study	Conditions	Experiment	Flowers
F. x *ananassa*	Antanaviciute (2016)	Field	2013 data for genotype RH, irrigation WW	05/05
F. x *ananassa*	Sønsteby and Heide (2006)	Controlled	Chill duration and photoperiod – minimum of 2 weeks chilling	Week 5/6
F. vesca	Walpole (2015)	Field	Park Wood ecotype grown in Exeter	03/04
			Park Wood ecotype grown in Torquay	10/04
F. vesca	Ling (2011)	Controlled	Mean across chill treatments	Week 9
F. vesca	Bedry (this thesis)	Controlled	Chill treatment 5, forced at 20°C	21.7

The effect of constant or varied day and night temperature on growth and development has been widely studied, with a general response observed by Roberts (1943) for a number of species that night temperature, more so than day, was responsible for determining plant response to temperature. Went (1944) also considered the effect of varied temperature and proposed the term thermoperiodicity following work on tomato (*Lycopersicon esculentum*), to describe the greater rate of growth and development for plants grown in diurnally fluctuating temperatures in comparison to constant temperatures. Work with tomato has also shown that the leaves of this species are damaged by constant light but that varied day/night temperature (DIF, difference between night temperature and day temperature, of 10°C) reduced the severity of leaf injury (Matsuda *et al.*, 2014). The manner and extent of variation between day and night temperatures had an influence on the plant response, as a lack of DIF was observed to result in slower leaf appearance and thinner leaves in tomato plants under constant light (Matsuda *et al.*, 2014); this also negatively impacted photosynthesis (Shibaeva and Sherudilo, 2015). Interestingly, a

negative DIF (warmer night than day temperature) caused a decrease in growth and development (dry weight of overall plant, leaf, root and fruit, as well as stem length) for a range of Solanaceous species; while a positive DIF, especially when combined with high day temperature tended to promote dry weight accumulation in almost all plant organs (Inthichack *et al.*, 2013).

The effect of night temperature has been considered for a range of species with varying results. Frantz *et al*. (2004) studied the influence of a range of night temperatures on growth and carbon use efficiency in lettuce, tomato and soybean, and showed a varied response between species; soybean was the most sensitive to night temperature, with increased temperature decreasing leaf and root biomass, but significantly increasing shoot biomass. The effect of night temperature can also vary depending on the phase of growth and development. For example in maize, increased night temperature during the vegetative phase significantly increased the rate of maturity and subsequently senescence, but did not have a detrimental effect on vegetative yield; whereas high minimum (night) temperature during the grain-filling period decreased efficient grain production, negatively impacting yield (Hatfield and Prueger, 2015).

In strawberry specifically, varied day and night temperatures have been reported as optimal for general plant growth, as well as for flower and inflorescence emergence. Verheul *et al*. (2007) observed optimal and earliest flower emergence under varied day/night temperature at 18°C/12°C. They also showed an interaction in response to day and night temperature: flower emergence was more responsive to increased night temperature, especially when combined with low day temperature (Verheul *et al.*, 2007). Wang and Camp (2000) also studied the effect of diurnal temperature on *Fragaria* and showed that physiological processes differed in the optimal day/night temperature for growth and development which for leaf and petiole growth was 25/12°C, while for roots and fruits it was 18/12°C (Wang and Camp, 2000). These responses indicate that the optimal conditions driving growth and development of runner production and flower emergence in *F. vesca* are likely to differ. Further work is needed to establish the exact nature of these differences.

To investigate whether in the experiments included here the change in the timing of flowering, or of runnering, might be the dominant factor in the reversal in emergence order, data for the Park Wood ecotype (UK9) were obtained for two field studies, one in southern England (Exeter/Torquay – Walpole, 2015) and the other in Reading (Alzahrani R., 2015, University of Reading, personal communication). The latter data were from the same field site where the experiments in this Chapter were carried out. For the plants grown at Reading, runners typically emerged seven days after flowers, whereas further south, there was a difference of 14 days (Table 6.8). Although the time to first flower and first runner varied in these two studies, there was more

consistency in date to first runner than to date for first flower (Table 6.8). This suggests that timing of runner emergence may be less influenced by environmental conditions than flower emergence. On the other hand, time to first runner changed more than time to flower emergence in controlled environments. It may be that, in general, time to runnering responds more strongly to constant temperatures than time to flower emergence, although this hypothesis needs to be directly tested. It is also of interest that there was less variation in time to emergence (of runners and flowers) in plants grown in controlled environments compared to those in the field (Figure 6.27).

Table 6.8 Mean time to first emergence of flowers and runners for *F. vesca* studies, using the Park Wood ecotype, with plants grown in the field or in controlled environments

Study		Date of first flower	Date of first runner
Field (Exeter)	Walpole (2015)	03/04/2015	24/04/2015
Field (Torquay)		10/04/2015	17/04/2015
Field (Reading)	Alzahrani (2015) (pers. comm.)	22/04/2015	29/04/2015

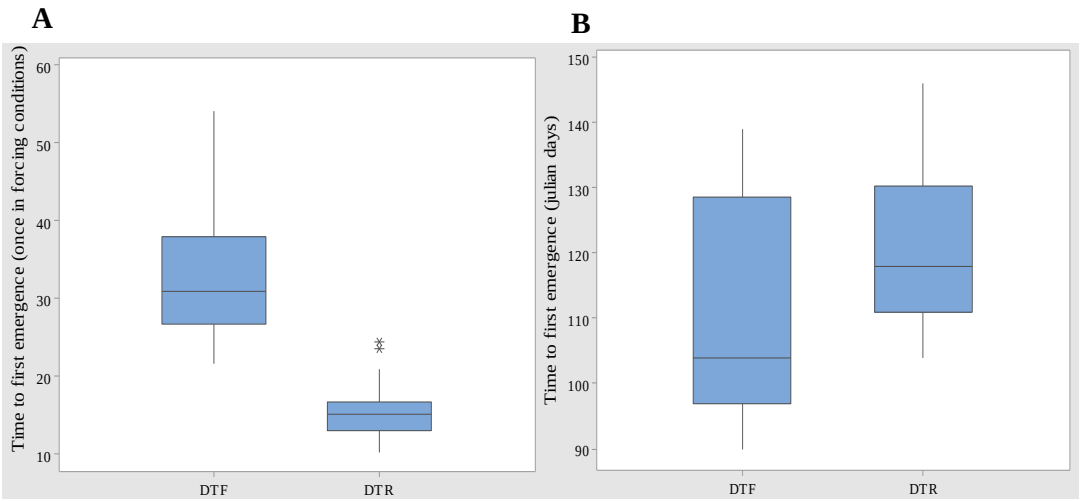


Figure 6.27 Boxplot representation of data for time to first emergence of flowers and runners in (A) Bedry (2015-2016) and (B) Alzahrani (2015) (pers. comm.)

The promotion of runner production relative to flower emergence under constant day/night temperatures may reflect the fact that runner production arises a direct consequence of new spring vegetative growth, with the first runner initiated in the axillary of the first new leaf to be initiated in the spring (Guttridge, 1959; Dana, 1980). The conditions driving runner production would therefore be those that promote vegetative growth at the apical meristem, as well as promoting cell expansion once the runners have been formed. Runners emerge as a consequence of emerging leaves, which is driven by growth of the primordium until it can no longer be contained with the

stipules of the youngest emerged leaf (Arney, 1953a). Leaf growth prior to emergence is primarily due to cell division, whereas growth after emergence is predominantly due to cell enlargement and vacuolation (Arney, 1953a; Jahn and Dana, 1970a). The interval between time of leaf emergence decreases with increased temperature (Arney, 1953b), which suggests that temperature plays a crucial role in influencing leaf production rate at the SAM and therefore influencing the rate of runner production and emergence.

Flower emergence, on the other hand, occurs from structures initiated the previous autumn (Heide *et al.*, 2013) and can therefore be considered to be driven principally by cell expansion rather than apical meristem activity. It is suggested that the conditions, possibly temperature thresholds and/or heat requirements, for these physiological processes differs. Flower emergence may be favoured by oscillating temperatures, whereas vegetative growth (time to runner production) may occur more rapidly under constant temperature conditions.

The order in which morphological structures arise in the spring has been shown to differ between species, with organisation being precocious in, for example, *Prunus avium* (flowers first, then leaves); or hysteranthous, in *Malus* x *domestica*, where leaves unfold before flowers. Guo *et al.* (2014) showed that differences in heat requirements between vegetative and floral buds were associated with hysteranthous behaviour in apricot and mountain peach, more than differences in chill requirements. This phenomenon has not been subject to systematic study under controlled environments, however; the data presented here suggest this topic is worthy of more detailed investigation in the future.

6.4.7 Conclusions

- Increased forcing temperature advanced time to first runner and first flower, but the effect of temperature differed. There was less difference in time to first runner between forcing extremes in comparison to flower emergence.

- Although there were some similarities, chilling had a more complex effect on time to first runner and first flower which was different in detail from that described by Cannell (1989) for budburst and flowering in deciduous trees.

- It is suggested that the lack of expected response, particularly for time to first runner might have been due to crucial differences in morphology between *F. vesca* and deciduous trees. Budburst is recorded as spring growth in trees, but *F. vesca* does not

have buds, instead growth resumes from vegetative meristems. This suggests that *F. vesca* might not be such a good model species for vegetative spring response in comparison with perennial trees. However, there are many valuable features of *F. vesca* as a model perennial (see Battey *et al.*, 1998), such as similarity in flower initiation, development and emergence behaviour.

- The order of flower and runner emergence was reversed in both experiments, compared to that in other studies. Photoperiod during forcing was not shown to significantly influence the order of emergence. It is suggested that time to first runner might have been more responsive to constant temperatures (in controlled environments), whereas flower emergence may be more dependent on oscillating temperatures.

Chapter 7: General Discussion

7.1 Setting the scene

The research in this thesis was designed to investigate the annual cycle of development of the herbaceous perennial *F. vesca*, with the objective of characterising the way in which the environment regulates vegetative growth, flowering and semi-dormancy in the context of the natural sequence of environmental changes in the temperate northern hemisphere. The overarching concept was to build upon knowledge gained using: 1) other species, for example Cannell's quantification of chill requirement of forest tree species and the development of chill/forcing models to define winter-spring development in temperate forest trees; 2) *Fragaria* (*F.* x *ananassa* and *F. vesca*) in controlled environments (for example, knowledge of flower induction and runnering); 3) common garden experiments to define ecotypic variation in response to a single environment. These different approaches were combined to provide an integrated conception of the regulation of the developmental phases in the annual cycle of *F. vesca*.

7.2. Getting closer to F. vesca in its natural developmental state

A baseline of understanding was achieved by a continuous study of development using plant dissections at intervals during the natural growing season (Chapter 3). This yielded data which were basically consistent with the following established views of *Fragaria*: spring growth is associated with flowering and runner production; branch crowns exist always at the base of the plant but begin to develop there and at higher nodes in late summer and autumn, associated with flower induction; semi-dormancy is associated with a lack of runner production and the presence of flower initials in the terminal and basal positions on the main crown. However, an important and unexpected finding was that after June the terminal SAM made very little growth, as measured by leaf initiation and the production of new runner initials. This was deduced not to be associated with limitations of plant culture (e.g. nutrition, pot size, growing in or outside the glasshouse), or cohort; rather, it was proposed that growth of the SAM itself might be regulated by the presence of actively growing runners in axillary positions by the end of June. This correlative effect has not been reported previously, possibly because in many experimental studies runners are removed. The significance of this practice was supported by data from Chapter 5, in which more sustained SAM development occurred when runners were removed in August/September (in both forcing and natural conditions). Further data from Chapter 4 (AXB4) confirmed the dominating effect of runners on SAM growth.

It has long been established that in many plants the outgrowth of buds is suppressed by active growth at the shoot apex (see Domagalska and Leyser, 2011). This was investigated in early studies using apical decapitation, which prompted the outgrowth of lower buds; auxin was established as an important regulator of bud outgrowth (Thimann and Skoog, 1933). Apical dominance has been extensively studied in *Arabidopsis* and it has been shown that auxin is

produced mostly in young expanding leaves at the shoot apex and is transported basipetally down the stem via the polar auxin transport stream, inhibiting shoot branching (Domagalska and Leyser, 2011). The influence of auxin on bud outgrowth is aided by strigolactones, which have been identified in a range of species including: *Arabidopsis*, rice, pea and petunia, and linked with repression of bud activity (Waldie *et al*., 2014). In direct contrast to this activity, cytokinins promote bud outgrowth and are synthesised mostly in the roots, resulting in acropetal transport (Domagalska and Leyser, 2011). Cytokinins act to allow bud activation even in the presence of high auxin, thus enabling buds to escape apical dominance (Müller *et al*., 2015). The research described in this thesis suggests that in *F. vesca* apical dominance may be weak, as active lateral growth was observed by runnering and branch crown development throughout the growing season. The nature of this lateral growth implied a reversed apical dominance, because axillary growth came rapidly to dominate overall plant growth and resulted in little activity at the SAM, shown by a slow rate of leaf initiation. It is unclear how apical dominance is regulated to alleviate restraint on lateral bud outgrowth in *F. vesca.* Lateral outgrowth has also been studied in *Trifolium repens* and the correlative inhibition associated with axillary shoots indicates that auxin may not be the primary factor influencing bud outgrowth in species with prostrate habit (Thomas and Hay, 2015). In *T. repens*, the net root-derived stimulus (NRS) has primary control of axillary bud outgrowth; as a result of this, as the horizontal main stem grows away from its basal root system the most basal six-eight axillary buds grow out vigorously into branches, but the branching vigour of successively later-formed distal axillary buds declines, although the formation of a nodal root further along the main crown can trigger unemerged bud outgrowth (Thomas and Hay, 2004, 2007, 2015). Distance from the root system does not directly influence the availability of NRS, with uniform distribution to all buds (apical and lateral); however, the sensitivity of a bud to its stimulatory signals is greater for those located near the basal roots (Thomas and Hay, 2008). In *F. vesca*

F. vesca has a complex growth habit, which can be interpreted as neither strictly orthotropic (like *Pisum*/*Arabidopsis*) nor prostrate (like *T. repens*). The main crown of *F. vesca* is orthotropic, although the internodes on the main crown are relative small and the orthotropic period of the SAM is short in comparison to other rosaceous tree species e.g. apple and cherry (Costes *et al.*, 2014). Another factor complicating the growth habit of *F. vesca* is the development of epigenous runners, which are plagiotropic (and create a prostrate growth habit similar to *T. repens*); if rooted these runner daughter plants become independent orthotropic plants (Nultsch, 1971). As a result of this lateral establishment of runners, *F. virginiana* has previously been described as prostrate (McPhee *et al.*, 1997).

As in *T. repens*, apical dominance in *Fragaria* appears weak; this is expressed as a low rate of leaf initiation at the SAM and its rapid dominance by axillary growth (Chapter 3 and 4). There is, nevertheless, some apical dominance in *F. virginiana*: the removal of shoot apices reduced apical dominance and increased branching intensity (McPhee *et al*., 1997). Overall it seems that in *F. vesca*, as in *T. repens*, apical dominance has some control on bud outgrowth, but does not act as the primary regulatory mechanism. Gibberellins have been identified as regulators of runner development and axillary bud fate in *F.* x *ananassa* (Hytönen *et al.,* 2009; Hytönen and Elomaa, 2011); but further research is required to investigate how in *F. vesca* axillary bud outgrowth is promoted throughout the main crown, with the exception of the very basal axillary buds (often observed as arrested buds). Axillary bud outgrowth may occur in response to: (i) low apical dominance (not inhibiting axillary bud development); (ii) promoters (such as cytokinin and gibberellin) actively stimulating bud outgrowth; or (iii) another regulatory process.

The naturalness of not removing the runners was the reason the experiments in Chapter 4 and 6, and the annual cycle study in Chapter 3, were performed in this way. In Chapter 5, runners were removed for consistency with the collaborating partners. This raises the question of the best way to conduct experimental studies of *F. vesca*. The practice of runner removal assumes that the correlative effects of this practice are not important for developmental/physiological studies and the conclusions drawn from them. It is legitimate to ask, however whether this assumption is valid, particularly in relation to questions about plant performance in the natural environment, present and future. Ecological studies have emphasised the importance of runners in the population ecology of *F. vesca*. The degree of resource transfer among runner plants has a genetic basis, and while ecotypes differ in their potential for resource sharing, one study showed that disconnection of runner plants from the parent and from each other eliminated ecotypic differences in biomass accumulation (Alpert *et al.,* 2003). It was concluded that regardless of variation in potential for resource sharing, all runners had a sink effect on the parent. Furthermore, the demand of connected runners can be long lasting, with daughter plants still receiving resourcing from the parent six weeks after establishment (Roiloa and Retuerto, 2005). The ability to produce runners and the nature of resource sharing is of key benefit to plants such as *Fragaria* in maintaining ecotype performance, especially in areas of resource heterogeneity (Zhang and Zhang, 2013). Although connected daughter plants may act as sinks to the mother plant, this connectivity increases the likelihood of survival in comparison to disconnected daughter plants, and is not considered to decrease mother plant survival (Roiloa and Retuerto, 2005; Atkinson and Else, 2012). The issue of time over which research is conducted on clonal plant responses was highlighted by Roiloa and Retuerto (2006), who suggested that many ecological studies report short-term responses, which cannot be directly extrapolated to the long-term.

Thus plants with runners intact can be argued to be the relevant entities for discussion which aims to relate environmental regulation of development to present and future climate, and the developmental regulation of whole plants, including runners and even rooted runners, would be a fruitful area for future work. The runners maintained on the mother plants in the experiments reported here were unrooted, and as a result would have remained as sinks for the mother plant; whereas rooted runners can act as sources (water, photosynthate, nutrients), both to other daughter plants and the mother plant (Atkinson and Else, 2012). A key element of future experimentation would be to investigate the effects on overall plant development of runner rooting. *F. vesca* has been shown to differ in its response to runner rooting depending on environment: a heterogeneous environment favours rooting runners in the highest quality patches, but in homogeneous environments rooting of runners is more random (Roiloa and Retuerto, 2006). It would be challenging to replicate the natural environment in which *F. vesca* plants are situated, with many of the ecotypes in this thesis typically sampled from woodland edges, or under tree/over-storey cover. The effect of these variations on developmental decisions, especially those related to the mechanisms at work in *T. repens*, needs to be understood in order to predict responses to a changing environment. For example, in *T. repens* the formation of a nodal root distally in the main crown, or at a newly emerged node in a region of weak axillary bud activity, leads to a renewed burst of branch outgrowth, triggering unemerged bud outgrowth; this provides indirect evidence to suggest that the NRS is xylem-transported cytokinin (Thomas and Hay, 2007, 2015). The ability of rooting to promote bud outgrowth and in doing so alter the morphological structure of the plant in *T. repens*, raises the question of whether rooting of runner daughter plants in *F. vesca* might not only alleviate the pressure of such daughter plants as a sink of resources, but also regulate the growth of other axillary buds and/or the SAM.

One striking difference between axillary bud outgrowth in *Fragaria* and *T. repens* is that there is a number of abiotic regulatory factors reported to influence *Fragaria* runner habit and development, such as light and nutrient availability (Alpert and Simms, 2002). This has also been shown in other species which have a combination of plagiotropic and orthotropic shoots, such as *Glechoma hirsuta*, in which plagiotropic and orthotropic shoots vary in their plasticity to light availability; as a result shading can alter morphology and growth habit (Huber and Hutchings, 1997). By contrast, in *T. repens* the main influence on promoting bud outgrowth is considered to be NRS (Thomas and Hay, 2004, 2007, 2015).

7.3 Developmental choice in F. vesca: local determination autonomously from the terminal SAM

Another area of intensive study in *Fragaria* has been the interaction between temperature and photoperiod and its promotion or inhibition of runner, branch crown and flower initiation and development. The annual cycle of development is characterised as one in which runner production is promoted by warm, LD in spring and summer, while branch crowns and flower initiation are promoted by the shortening days and cooling temperature of late summer and autumn (see reviews by Guttridge, 1985; Battey *et al.*, 1998; Heide *et al.*, 2013). The experiments described in Chapter 4 were designed to investigate in detail the way in which developmental choice is made between runners and branch crowns in axillary buds: the timing of determination and its relationship to the time of origin of leaf initials and their subtended axillary buds on the SAM. The striking results obtained in AXB1 and AXB2 were that regardless of photoperiod, runners developed from existing axillary buds and from those few newly-initiated by the very slow growing SAM, a slow growth deduced to be associated with the presence of runners. It was thus not possible to establish the determination window for runner, as opposed to branch crown development. Future efforts to analyse this process might be feasible in the light of the result from AXB4, which demonstrated that runner removal promoted SAM leaf initiation. However, if runners were removed, there would be an attendant uncertainty over whether results obtained under these artificial conditions would be relevant to the natural situation.

It was possible, however, to show in Chapter 4 that branch crown formation was induced, and apparently necessarily associated with flower induction, by 11°C/SD; but that a four week treatment might have only transiently induced flower formation. Further investigation is required into whether full vegetative growth would be resumed under these conditions, or, as perhaps existing literature would favour, further flower initiation would continue though at a slower rate than in continuous inductive conditions. The subject of floral reversion is not typically discussed in the context of *Fragaria* studies, although the process of reversion is necessary for the re-establishment of vegetative growth in the spring. In commercial production of *F.* x *ananassa*, however, high temperatures during flower initiation have been shown to promote resumption of vegetative growth, although the flower initials present before reversion did not themselves revert and emerged normally (Kumakura and Shishido, 1995).

Perhaps the most crucial insight, and further question, which arises from Chapter 4, is whether in the natural, ecological context runners come rapidly to dominate SAM growth such that the key processes are not related to determination of axillary structures as they arise from the terminal SAM; but rather, the critical process is the lack of development of some axillary meristems into runners, so that with shortening days and cool autumn temperatures, these pre-existing, quiescent structures take on a branch crown and then floral fate, associated with the conversion of the dominated terminal SAM to a flower. Determination then would be local to individual axillary

meristems, and the role of the terminal SAM would be to initiate a limited number of leaves whose axillary meristems are a potential source of runners. The terminal SAM would itself become quiescent until late summer when inductive signals, and/or a decline in *TFL1* expression enable it to convert to a flower. It has been suggested that initiation of flowers in axillary positions is only possible if buds have reached competence to flower (Arney, 1953a; Hytönen *et al.*, 2004; Koskela *et al.*, 2012; Mouhu *et al.*, 2013). This could be the means by which vegetative meristems remain following the transition to flower. For example, Koskela *et al*. (2012) showed that high *FvTFL1* (floral suppression) was detected in the apices of axillary shoots that emerged after the end of the floral inductive treatment. This suggests that axillary buds developing during flowering might not be sufficiently competent to transition to flower and would thus remain vegetative. The processes which maintain specific meristems in an arrested state, rather than developing into runners are, however, unclear. Those at the base of the plant often develop as branch crowns early in the season, but others show very little development at all until flowering time. The factors which regulate the arrested nature of these key meristems is a critical area for future study. In *T. repens*, the key regulators of bud outgrowth, resulting in arrested axillary buds, are the availability of NRS and apical dominance (Thomas and Hay, 2007, 2008, 2011, 2015).

7.4 Re-establishing vegetative development and the effects of winter and spring environment

As discussed above, the induction of flowering is necessarily only semi-permanent in polycarpic perennials, because of their need to maintain some meristems in a vegetative state through the floral phase (Townsend *et al.*, 2006; Albani and Coupland, 2010; Bratzel and Turck, 2015). In the case of *F. vesca*, this manifests itself morphologically in the way the inflorescence structure allows for continued floral development in the autumn, and the return to vegetative growth in the spring (Figure 7.1). It is possible to see: 1) that a form of reversion could occur in marginal/transiently inductive conditions, simply by advancing the transition back to vegetative growth, in the absence of chilling (Chapter 4); 2) that the effects of forcing on autumn growth described in Chapter 5, where runner growth and flower emergence coincided, can be explained in a similar way; 3) that there is general consistency with the idea that warm, LD can partially substitute for the effects of chilling (Lieten, 1997; Sønsteby and Heide, 2006). Therefore, the way in which winter chilling influences spring growth in *F. vesca*, and its comparability to the same process in perennials showing true dormancy, becomes a critical area for understanding. This was addressed systematically over two seasons in Chapter 6.

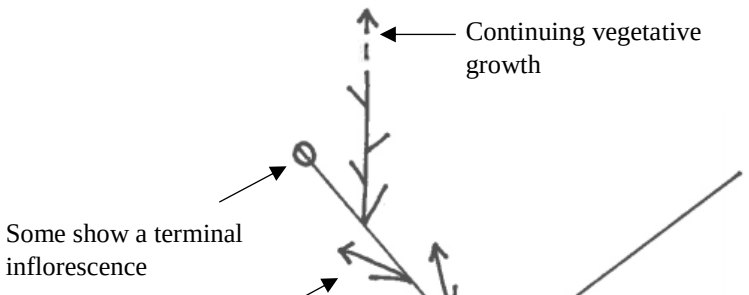


Figure 7.1 Continuing vegetative growth at the terminal SAM and in well-developed branch crowns (usually at the base of the main crown)

Vegetative growth in the spring (Chapter 3; April 2017) was comparable with that previously reported (Guttridge, 1955, 1985; Townsend *et al*., 2006). One striking difference, however, was the observation of runners in the axils of leaves in the branch crown subtending the terminal inflorescence(s), whereas Guttridge (1985), for example, only described branch crowns at these positions. It is not clear whether this is a difference between *F*. x *ananassa* and *F. vesca* (observed by Guttridge and in this thesis, respectively) or a variation due to environmental conditions.

The effects of chilling conformed in a general way to expectations based on research on forest trees by Cannell and others. Thus increased forcing temperature advanced time to first runner and first flower, and chilling generally decreased time to these events. Differences were, however, apparent which indicated that a key underlying distinction probably existed in the response to temperature of runner initiation/emergence, compared to flower emergence. It was suggested that this might reflect the differences in developmental mechanisms underlying these morphological processes: runner outgrowth in the spring being mainly associated with their initiation on the newly-activated SAM; while flower emergence was dominated by cell expansion associated with the emergence of pre-existing inflorescences. The detailed data on *F. vesca* were consistent with the possibility that flowering and runnering responded differently to constant and fluctuating temperature; this suggestion allowed interpretation of the varied timing of runner and flower emergence from the crown, according to winter and spring temperatures. It is interesting that in the experiment reported in Chapter 6 runners were not removed. By limiting SAM growth (see

above) this may have accentuated the early emergence of runners, relative to flowers, allowing more active runner growth in response to warmer spring forcing temperature.

The potentially different responses of flowering and runnering to temperature raises the question of the impact of predicted climate change on developmental timings in *F. vesca*. To allow a preliminary indication of the nature and extent of these impacts, UKCP09 data (http://ukclimateprojections.metoffice.gov.uk/) were collated to provide a prediction of climatic changes by 2080, focusing on South East England (Figure 7.2). From 1961 to 2006, mean daily temperature increased across all four seasons in this region, with the greatest increase over the winter and summer months (Figure 7.2).

Based on this analysis, by 2080 summer and winter temperature will have increased by 1.4-8.1°C and 1.4-5.7°C in summer and winter respectively. The predictions have not, however, been provided for spring and autumn, and also data are not sufficiently detailed to allow analysis of change in diurnal temperature range. Changes in temperature from 1961-2006 are provided for daily mean, maximum and minimum temperature. From 1961-2006, the mean change in spring daily maximum temperature was 1.7°C and 1.5°C for minimum temperature, in South East England. The similarity at both temperature extremes suggests there has been little change in the diurnal temperature range, assuming maximum temperature as the likely highest day temperature, and minimum temperature as the likely lowest night temperature. There has also been a similar increase in daily mean spring temperature over this time period (1.6°C; Figure 7.2), which implies that the temperature range has been maintained with a similar degree of increase in maximum, minimum and mean temperature. This indicates that the proposed difference in the response of flower and runner emergence to constant and fluctuating temperatures (described in Chapter 6) may not contribute to changes in response *in situ*, with little observed change in the extent of temperature fluctuation.

Predicted increase in mean daily temperature (°C) by 2080 for combined emissions scenarios

1.4 – 8.1

Summer

June - Aug

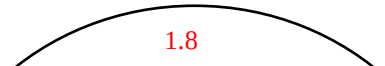


Figure 7.2 Annual cycle showing the changes in mean daily temperature from 1961-2006 (red) and the predicted range of mean daily temperature increase by 2080 (blue), combining emissions scenarios (low-high), for South East England. When predicting future changes in climate three emission scenarios are typically used, for low, medium and high emissions. A combination of these three scenarios was used to provide a range of likely temperature increase over the summer and winter months (by UKCP09). Data extracted from http://ukclimateprojections.metoffice.gov.uk/

The effect of the predicted increase in winter temperature on chill accumulation, based on the main chill model approach used in Chapter 6 can, however, be estimated. Approximate chill accumulation was calculated using the <7.2°C model and mean daily temperature for the winter period (specified by UKCP09 as December – February) for 2015-2016, extracted from the University of Reading Meteorological station (http://www.met.reading.ac.uk/) (A). This indicates that present winter temperature provides sufficient chilling for *Fragaria* (Table 7.1), given that Atkinson *et al.* (2005) suggested that the chill requirement of strawberries is 200-300 chill units, using a similar model of chill accumulation. UKCP09 reported that winter temperatures have increased by an average of 2°C from 1961-2006; if a similar rate of increase is maintained on top of current winter temperatures, chill accumulation would decrease but still be adequate for *Fragaria* requirement (Table 7.1B). However, predictions of potential temperature increase under a high emissions scenario suggests that mean daily temperatures could increase by 5.7°C over the winter; under such scenarios, chill accumulation would likely not be sufficient (Table 7.1C). This

suggests that although the low chill requirement of *Fragaria* makes it a relatively robust species, in comparison to other perennial horticultural crops (Atkinson *et al.*, 2005), under predicted climatic changes, even for this species there may be concerns over chill accumulation.

Table 7.1 Chill accumulation (chill units using the <7.2°C chill hours model) and mean spring temperature under: current temperatures (A), in relation to past warming (observed from 1961-2006) (B), and maximum predicted increase in temperature by 2080 (under high emissions scenario) (C), for South East England

	2015-2016 (A)	Increase from 1961-2006 (B)	By 2080 (C)
Chill accumulation December – February	1008	600	96
Spring temperature March - May	9.2°C	10.7°C	13.7°C

A similar method of prediction can be used to calculate the effect of potential increases in spring temperature under proposed climate change. The average spring (March – May) temperature for 2016 was 9.2°C; during these months temperatures increased by an average of 1.6°C from 1961-2006; if temperatures continue to rise at this rate, then mean spring temperature may increase to 10.7°C. UKCP09 do not provide predictions for climatic changes in spring or autumn, but a proportional increase can be calculated for spring temperature by 2080 using the maximum predicted temperature increase for the winter period under a high emissions scenario. Under such conditions, mean spring temperature might increase to 13.7°C.

Integration of these data predictions and extrapolation of data collected in Chapter 6, suggest that the reduction in accumulated chilling and increase in spring temperature may advance timing of runnering and flowering; with timing of runnering predicted to advance by seven and 16 days under scenarios B and C, respectively (see Table 7.1 legend). Flowering may also advance by 24 and 22 days under scenarios B and C, respectively. This implies that increased spring and winter temperature may have a more profound effect on time of flowering, in comparison to runnering; which may result in a greater gap in timing between these two phenophases *in situ*. Sønsteby and Heide (2006) suggested that increased spring (forcing) temperature advanced time of runnering and flowering in *F.* x *ananassa*, but more so for runnering, provided plants were chilled sufficiently. Therefore if winter temperatures increase to such a degree to provide inadequate chill accumulation, the higher sensitivity of runnering to chilling suggests that the proposed difference in response between timing of flowering and runnering may increase even more.

7.5 Ecotypic variation in F. vesca

The experiments described in Chapter 5 showed that *F. vesca* collected from different localities and propagated vegetatively in Helsinki and then Reading had different responses to naturally changing late summer-autumn conditions in Reading, as measured by the timing and extent of flowering and runnering during forcing in a common environment (warm/LD). Repeating the experiments with selected ecotypes over two successive years showed that some remained relatively consistent in their response, while one (UK12) did not, a difference interpreted as most likely being a consequence of a carry-over effect, the nature of which could be the subject of fruitful further investigation.

There are two important caveats about these experiments: firstly, the word 'ecotype' was used, though it is not possible to claim anything from the data about the behaviour of natural populations, either *in situ* or at Reading. This is because the material was not collected in such a way as to reflect the local population. It was sampled so as to compare the response of a genotype (or perhaps limited range of genotypes), with genotypes from other sources, when grown in a common environment (at Reading). Secondly, the use of the forcing procedure to study flowering and runnering responses means that the natural variation in responses of these ecotypes is not known. A different experimental design, and recordings of *in situ* flowering and runnering, would be needed to make any statements about these. Nevertheless, the data presented do show natural variation in flowering and runnering responses to the environment; and that these can change over successive seasons. This is important information for future study of perennial flowering and vegetative development. In the context of the overarching conception of this thesis, the design of experiments to address natural, *in situ* flowering and runnering responses becomes central and is explored in the next and final section.

7.6 Future work on ecological development in F. vesca

Over the past 100 years the disciplines of plant developmental biology and plant ecology have followed divergent paths. The drive to understand plant development has led to study of plants in controlled environments at the physiological and molecular levels, with interpretation increasingly evolutionary in focus; ecology has also become increasingly evolutionary in focus, but more based on the natural context. In the light of the importance of predicting plant response to the natural environment as it changes in unprecedentedly rapid ways due to human activity (fragmentation, climate change, pressure on natural communities), plant developmental studies need, more and more, to address their subject in a natural context. This means that it is important not to over-simplify experiments, so they are actually relevant to plants in their *in situ*, ecological context. For perennials, which live a long time in environments which are rapidly changing

around them, the challenge of this requirement is even greater. Evidence of general awareness of the need for a change in research emphasis is apparent from the debate over cuttings *versus* whole trees (e.g. Laube *et al.*, 2014a, 2014b), and the issue of using juvenile trees; and large scale FACE studies (e.g. Ainsworth and Long, 2005; Leakey *et al.*, 2009) which are not, however, typically developmental in focus.

F. vesca has been promoted as a 'model perennial' (Battey *et al.*, 1998; Mouhu *et al.*, 2009; Rantanen *et al.*, 2014), and the work in this thesis has confirmed it has potential as a small plant, easily propagated and studied, but that care needs to be taken. If the plant is grown in a way too far from its natural habit (without runners, in a constant environment rather than continuously varying ones), useful information can be gained on its developmental responses, particularly in relation to questions about commercial cultivation (of *F.* x *ananassa*). The relevance of the information to the natural environment, and climate change, however, may not be easy to establish clearly. The annual cycles of *Malus, Prunus* and *Fragaria* were reviewed in Chapter 1.4 and suggestions made of the potential impacts of predicted climate change. Comparison of the growth cycles of these species highlighted key differences in the strictness of their environmental regulation with regards to key developmental processes (e.g. flower initiation and chilling).

The interpretation gained here suggests that *F. vesca* might not prove to be an ideal model perennial for phenophases such as budburst and true dormancy, because it shows only semi-dormancy, and lacks buds (and therefore budburst) which are found in trees, making it of limited value as a model for these aspects of perenniality. Nevertheless, its complex spring forcing response (Chapter 6) highlights key questions concerning the processes of chilling and forcing, especially in the light of climate change. *F. vesca* plants in Chapter 6 runnered and flowered, regardless of the chill-force treatment, which suggests that this species might be fairly robust in the face of predicted climatic changes. The relatively low chill requirement of *F. vesca* might also allow for comparison of chilling in low chill *Malus* and *Prunus* cultivars. This is important given that one concern raised for the approaches of modelling chill accumulation/growing degree hours is that they fail adequately to depict physiological processes under future climate conditions, where it is likely that the chilling and growth phases will be blurred (Darbyshire *et al*., 2014). The detailed response of *F. vesca* to chill-forcing treatments suggests that this is an area that requires further investigation.

F. vesca as discussed here reveals potential for developmental studies related closely to the naturally changing environment: an 'ecological development'. The research in this thesis emphasises the importance of correlative control, which is regulative of meristem function in a way less reductively mechanistic than molecular control, but perhaps equally significant. The

influence of the growth of the different parts of the plant for development elsewhere is crucial. Here the impact of runners on the terminal SAM has been emphasised (Chapters 3, 4, 6); but the influence of natural development of the root system, which differs in runner-derived and seed-derived plants, and which co-exist in the *in situ* context is likely to be even more profound. Correlative studies such as those discussed above for *T. repens* (Thomas and Hay, 2015) are likely to be a significant focus for ecological development. To exemplify the kind of future research envisaged for *F. vesca*, a series of experiments is proposed (Box 7.1). The initial focus of this future work would consist of a comparison between *F. vesca* growing in a simulated *in situ* context, with plants in experimental situations as described in this thesis (in particular Chapter 3) (Box 7.1, Appendix 7.1). As well as allowing a comparison between responses in an experimental and natural context this would also enable a repetition of Chapter 3 and AXB4 (in Chapter 4), to prove whether runner presence is the cause of the observed SAM slow-down in leaf initiation from June, or whether endogenous regulation is linked with this marked decline. The proposed future work would also establish whether the response reported in Chapter 3 varies between years and between ecotypes. Building upon this initial investigation, further experimentation would aim to integrate the findings from this comparison in order to explore and expand on the issues highlighted in the thesis, using simulated natural plots (described in Box 7.1, Appendix 7.1).

Box 7.1: Future research

Experiment 1

Aim: to contrast and establish baseline data of ecotypic responses in experimental and natural contexts

Null hypotheses

1) There is no ecotypic variation in response during the annual cycle
2) Plants will show the same responses in experimental and natural contexts

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Appendices

Appendix 7.1

Simulated natural context

For the natural plot to best reflect *in situ* conditions, nutrient analysis of soil *in situ* would be carried out. Light levels would be recorded and shade netting applied where necessary in the summer; typically these plants grow as under-storey species. Trees could be planted to create a more complete simulation. Each mother plant would be planted into the ground in such a way as to provide adequate space for runner rooting (Figure 7i). As runners develop, pins would be used where necessary to prevent crossing over of runners for ease of recording.

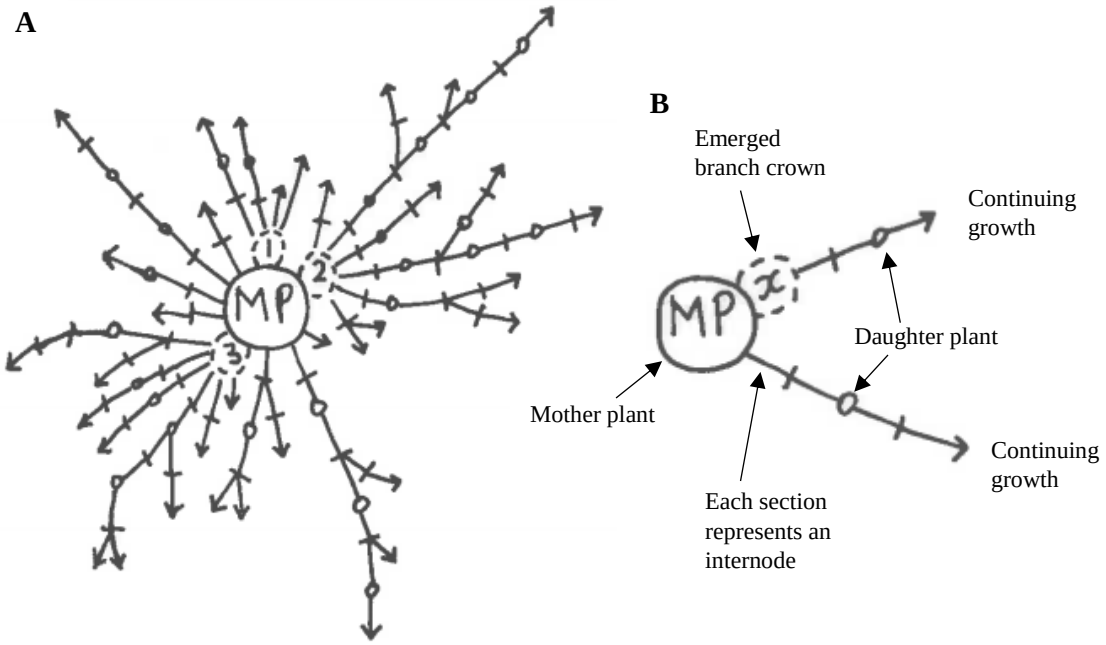

Figure 7i. (A) Schematic diagram of morphology for mother plant (MP) and runners under experimental conditions (adapted from data collected for Chapter 3, June 2016). Runners are observed from mother plants and developed, emerged branch crowns (B) Key to symbols used in A

Parameters to be recorded:

Number of: runners, daughter plants, rooted daughter plants

Architecture of runners – branching, internodes

SAM activity – inferred from the rate of leaf emergence.

Issues to consider:

- Repeatability of natural plot, between years – natural variation between replicates and years of runner and daughter plant numbers.
- Space required for sufficient replicates.
- Best means of recording.
- Habitats are naturally heterogeneous, which is shown to influence runnering behaviour.
- Lack of competition from other *F. vesca* plants and/or other species.
- Normally a mixed communities of runner- and seed-derived plants.
- Manipulation of runner placement within matrix, preventing crossing over/overlap of runners; the use of pins may promote rooting.

Experiment 1: comparison of simulated natural and experimental contexts and ecotypic variation

Regular observations would be made of growth and development, for all ecotypes in both treatments. The foci of recording would be as follows:

- SAM activity (inferred non-destructively through leaf emergence), in order to determine how runner maintenance and rooting influence SAM activity, and whether the marked decline in SAM activity from June onwards is primarily a response to runner growth or endogenously regulated. For example, leaf production in citrus typically occurs in flushes (Turrell, 1961; Syvertsen *et al*., 1981); if this applies in intact *F. vesca*, growth at the SAM might not be constant during the growing season, regardless of runner presence and/or environment.
- Runnering – duration of active production and the number of runners produced. This would address whether the period of runnering is the same for all ecotypes and whether treatments influence response to environmental regulation. Do plants, according to ecotype and/or natural *versus* experimental context, differ in their runnering behaviour: number of runners, internodes, branches and daughter plants produced? Establish when the runner connection between mother plant and daughter plants is naturally severed. How does treatment and ecotype influence the timing and/or the likelihood of this event? How does treatment and ecotype influence the timing of runner emergence in the spring and its timing in relation to flowering?
- Flower emergence – duration of flower emergence and the number of flowers and inflorescences produced.
- Branch crown production.
- Mortality – are plants in the simulated natural context more/less/equally likely to survive?

Experiment 2: response to environment of runner- and seed-derived individuals

The period of juvenility in seed-raised individuals would need to be established to ensure runner- and seed-derived plants were both able to respond to vegetative and floral cues.

Plants would be grown in the simulated natural context throughout the growing season, to compare growth response, similarly to Experiment 1. Additional plants would be grown to enable dissections throughout late summer and autumn to assess ecotypic and propagation-origin differences in time, rate and pattern of floral initiation. The following spring, natural timing of flower and runner emergence would be recorded, as well as the structure of inflorescences. Plant dissections once vegetative growth had resumed would establish whether ecotypes and runner-seed-derived material differed in their mean of re-establishing vegetative growth and to determine when the first emerged runners are initiated.

The following questions would be answered:

1) Whether all plants, regardless of ecotype or form of propagation, show flower and runner emergence at the same time in the spring and the order of emergence is consistent
2) Whether runner daughter plants produced during the growing season initiate flowers
3) Whether vegetative growth resumes from the same nodes and/or shows similar patterns of response to spring conditions in all ecotypes and regardless of propagation origin of material.

Experiment 3: the influence of runners and rooting on plant matrix growth and development

Plants would be grown in the simulated natural context and under current experimental conditions; the following treatments would be used:

1) All runner daughter plants rooted
2) No runner daughter plants rooted
3) All runners removed.

Non-destructive recording to be undertaken:

- Rate of leaf emergence of mother plant.
- Runnering response – number of runner, internodes, branches, daughter plants.

The following questions would be answered:

1) How does runner rooting influence plant morphology?
    a. At the SAM

i. Does runner removal promote SAM activity more than all other treatments?
ii. Do rooted runners promote SAM activity more than unrooted runners but less than removed runners?

b. Of itself
    i. Will runner rooting slow the growth of the daughter plant as it becomes a source for the plant as a whole, rather than a resource sink – need to consider with regards to hierarchical position of daughter plants?

c. Of the runner – other daughter plants and branching
    i. Will runner rooting enable more rapid development of runner – more daughter plants and greater branching?

d. Of other axillary buds
    i. Will runner rooting/removal promote outgrowth/development of higher axillary buds?
    ii. Is the quiescent state of basal arrested buds linked with floral initiation rather than due to inhibition by higher axillary buds?

Further experimental approaches

1. Does the rooting of individual daughter plants influence the response?

Compare the effect on terminal SAM activity and runner development of rooting all daughter plants, with only selected ones, for example, only the first, second or third daughter plant. This would address questions regarding the hierarchy of daughter plants along a runner. Does rooting only the first daughter plant have a greater influence on subsequent runner growth and SAM activity than independent rooting of subsequent, potentially less influential daughter plants?

2. Does node position in the main crown influence the impact of rooting runners?

Treatments would allow only specific daughter plants along the runner from a particular node (on the main crown) to root, to establish whether the potentially alleviating influence of rooted runners on SAM activity is affected by whether runners from the base, mid- or upper-section of the main crown are rooted. This might also indicate how the inhibiting or promoting effect of runners is signalled to the SAM.

3. How do girdled runners influence response?

Rooted or unrooted runners would be girdled (cf. Thomas & Hay, 2015) in order to determine whether this promotes SAM activity in a similar manner to runner removal. How girdling influences further runner growth (for rooted and unrooted daughter plants) would be investigated.