Molecular responses to chilling in a warming climate and their impacts on plant reproductive development and yield.

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Highlight: This review discusses how plants respond to winter chilling, focusing on the effects of temperature variation during and after winter chilling on plant development and crop performance.
Abstract

Responses to prolonged winter chilling are universal in temperate plants which use seasonal temperature cues in the seed, vegetative and reproductive phases to align development with the earth’s orbit. Climate change is driving a decline in reliable winter chill and affecting the sub-tropical extent of cultivation for temperate over-wintering crops. Here we explore molecular aspects of plant responses to winter chill including seasonal bud break and flowering, and how variation in the intensity of winter chilling or de-vernualisation can lead to effects on post-chilling plant development, including that of structures necessary for crop yields.

Key words: chilling, temperature, vernalisation, vernalization, dormancy, bud dormancy, temperature, climate change, de-vernualisation, crop yields.
Introduction

One of the most important consequences of climate change for global agriculture this century will be a reduction in reliable chilling for over-wintering annual and perennial crops (Luedeling, 2012). Adequate chilling is essential for multiple aspects of plant reproductive development, but especially for the production of high performing flowers, fruits and seeds. Moreover, requirements for winter chilling determine the sub-tropical extent of cultivation of many temperate crops. In warm temperate zones such as Southern Brazil, southern United States, the Mediterranean basin and parts of Australia reductions in reliable winter chill have been recorded that are predicted to worsen under all reasonable climate scenarios (Wrege et al., 2010; Luedeling et al., 2011; Darbyshire et al., 2013; Parker and Abatzoglou, 2018; Campoy et al., 2018; Rodriguez et al., 2019). Even cool temperate regions such as Northern Europe, and tropical upland regions, are also at risk of declining chilling, with some orchards at risk losing economic viability without adaptive measures (Darbyshire et al., 2013; Campoy et al., 2018; Fadon et al., 2021).

In this review we will cover aspects of winter chilling that are conserved between different species, showing how variation in winter chill acts at the molecular and genetic level to affect plant development and crop yields. In model species such as Arabidopsis thaliana the mechanism of the initial chilling response is understood in detail and has been reviewed frequently elsewhere (e.g. Bloomer and Dean, 2017). Instead we will focus on the study of long term chilling responses in the field, their role in life history and the likely mechanisms of disruption for yield. Finally, we will consider the implications and outcomes of warming winters on plant development in the context of changing climates.

In plants that do not require vernalisation, plant growth rate increases in response to rising temperature, through a combination of passive effects of temperature on reaction rates, and effects on phytohormone signalling pathways, reviewed elsewhere in this special issue. At its simplest, the promotion of development through chilling is a subversion of this standard relationship between temperature and plant growth, such that warmer temperatures no longer shorten the time to flowering or growth resumption in spring, but instead induce a delay to the next developmental transition. In a previous review we proposed that a generalised plant response to cool temperatures was induced by temperatures below 17°C (Penfield, 2008). 17°C represents the upper limit of vernalisation in many species (Tommey and Andrews, 1991; Porter and Gawith, 1999; Wollenburg and Amasino, 2012; Duncan et al., 2015). At this temperature the Arabidopsis circadian clock remodels and low temperature responses such as cold acclimation begin to be induced. Furthermore, in some perennial species including common pear (Pyrus communis) and birch (Betula sp.), cooler autumn temperatures make a major contribution to seasonal growth cessation and bud set (Heide and Prestrud, 2005; Cooke et al., 2012). Indeed for modelling the earth’s climate the most parsimonious model of seasonal growth is simply that plant growth ceases as the mean temperature falls below 10°C, and restarts as temperature increases above this same threshold in spring (Clarke et al., 2011).
Thus although vernalisation has traditionally been considered a transient response to winter, in practise plants growing in cool temperate regions are likely to experience vernalising temperatures from early autumn through to late spring. Ultimately, this means that rather than being a winter-phenomenon, vernalisation is a complex ongoing process that can last several months, whereby chilling begins in autumn and plants may proceed through several phases of vegetative and reproductive development as they grow throughout autumn and winter, all under vernalising conditions of first increasing and then decreasing intensity. As such, it is quite difficult to reproduce natural plant responses to chilling in simple laboratory systems that shift plants abruptly between summer and winter conditions. Yet much of our understanding of the molecular basis of chilling responses comes from the study of flowering time in the laboratory, especially in model species such as *Arabidopsis thaliana*. Therefore, it is important that knowledge gained from the laboratory is transferred to plants growing in real field conditions.

In Arabidopsis, winter chilling acts predominantly to down-regulate a key floral repressor, *FLOWERING LOCUS C* (*FLC*; Michaels et al., 1999; Sheldon et al. 2000). The molecular details of the vernalisation response, particularly the complex transcriptional and chromatin dynamics at the Arabidopsis *FLC* locus, have been reviewed in detail elsewhere (Bloomer and Dean, 2017) and will not be covered here in detail. Suffice to state that in Arabidopsis the duration of chilling needed for floral induction can vary due to allelic variation at the *FRIGIDA* (*FRI*) locus that controls the starting expression level of *FLC* in seedlings or through allelic variation at *FLC* itself which affects either the starting *FLC* transcript level or rate of silencing (Hepworth et al., 2020). This two gene model, initially proposed by Napp Zinn (1957), explains the majority of variation of the effect of chilling on flowering time in Arabidopsis accessions (Caicedo et al., 2004). During vernalisation itself two key processes occur at the *FLC* locus which result in cell-autonomous silencing of *FLC*. In warmer climates an early, slow phase of transcription shutdown is followed by a faster phase as extended exposure to cooling induces expression of a transcriptional co-factor *VERNLISATION INDEPENDENT 3* (*VIN3*). In colder climates these two responses can be induced simultaneously (Hepworth et al., 2018). Indeed *VIN3* is cold and circadian regulated in a manner resembling that previously described for many cold-induced genes in Arabidopsis (Covington et al., 2008), likely explaining the multiple temperature inputs to the regulation of *VIN3* transcript levels (Antoniou-Kourounioti et al., 2018). Transcriptional shutdown of *FLC* is accelerated at first by the absence of warm temperatures (Hepworth et al., 2018) but as autumn progresses freezing temperatures additionally induce anti-sense *FLC* expression which further increase the rate of *FLC* transcriptional shutdown. (Zhao et al., 2021). Antisense transcription is a widely conserved phenomenon at *FLC*-like genes across species (Jiao et al., 2019).
Temperature sensing and control of the floral transition in winter annuals

In older literature a simple model of Arabidopsis flowering time control was popularised from laboratory experiments whereby vegetative plants vernalise over winter and transition to flowering in the spring in response to lengthening days and warming temperatures. In the laboratory, where plants are often transferred from chilling directly into warm long days, the latter conditions enable the transition to flowering because target genes of FLC, such as SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) and FLOWERING LOCUS T (FT), are activated by warmth and red light in the evening (Michaels et al. 2005; Valverde et al., 2004). This model accurately explains events during and after vernalisation treatments given in the laboratory, which are often far shorter than those experienced by plants in the field and ended by an abrupt transition into warm conditions.

However, in the field Duncan et al (2015) showed that Arabidopsis growing in Sweden had already experienced at least 12 weeks of vernalisation and saturated its vernalisation requirement during autumn, before winter had even begun. These plants make flowers over winter ready to open in spring. In Brassica napus grown in the United Kingdom, flower buds are formed in mid-autumn and overwinter before bolting the following spring (O’Neill et al., 2019). Like B. napus the short-lived mountain perennial A. alpina will undergo the floral transition while in vernalisation if left in chilling conditions for longer durations (Wang et al., 2009; Lazaro et al., 2018). These observations tally with findings from theoretical studies which predict that the vernalisation requirement for A. thaliana and B. napus is fulfilled in autumn (Habekotte, 1997; Wilczek et al., 2009).

In contrast to laboratory experiments where exposure of vernalised plants to warm long days activates FT expression in leaves, because many field-grown winter annuals complete early floral development in autumn or winter FT expression is low because of the short daylength (O’Neill et al., 2019; Hyun et al., 2019). In particular Hyun et al (2019) showed that if Arabis alpina plants were maintained in vernalisation for longer durations the floral transition occurred in an FT-independent manner, with the orthologue of FLC, PERPETUAL FLOWERING 1 (PEP1) instead acting on the SQUAMOSA PROMOTER-BINDING-LIKE PROTEIN 15 (SPL15) gene to promote the transition to flowering. Instead, in the field FT is more likely to be important in later events in inflorescence, flower and seed development which occur in the lengthening days of spring (Chen et al., 2014). Indeed spring induction of FT expression is more closely associated with bud break or bolting than with the floral transition in winter annuals of multiple species including sugar beet, Brassicas and poplar (Rinne et al., 2011; Pin et al., 2010; O’Neill et al., 2019; Hyun et al., 2019), and may only be important for the control of the floral transition in summer annuals.
In cereals and other monocots winter annual behaviour is also generated by sensing of seasonal temperature changes through a process of vernalization. The cereal vernalisation response appears conceptually similar to those in other species in that the permissible temperature range for vernalisation fulfilment and the transition from vegetative to reproductive growth is between -5°C and 18°C, with an optimum around 5°C (Porter and Gawith 1999; White et al. 2011; Dixon et al. 2019). Thus for cereals growing in cool temperate climates, temperatures can be presumed to be broadly vernalising throughout autumn, winter and spring as we have previously discussed for dicotyledonous plants. In addition to the vernalisation temperature, the growth temperature after vernalization is also important for flowering time in wheat where warm temperatures can accelerate or delay flowering, depending on the genotype (Dixon et al., 2019).

Genetic studies have revealed three principle loci that are important for winter cereal responses to chilling, although there is still some uncertainty as to which genes are the primary responders to seasonal temperature changes. VERNALISATION1 (VRN1) is an orthologue of Arabidopsis APETALA1/FRUITFUL and increases in expression in response to low winter temperatures (Yan et al., 2003; Trevaskis et al., 2003). Strong alleles that increase expression of VRN1 act dominantly to generate a spring wheat life history (Fu et al., 2005). VRN2 encodes a CCT-domain protein which functions as a floral repressor necessary to prevent flowering before winter in winter cereals, and which represses VRN1 expression. VRN2 expression is down-regulated by chilling, possibly due to repression by increasing VRN1 activity, because VRN2 is not required for VRN1 responses to chilling (Hemming et al., 2008). In the absence of VRN1, the up-regulation of the cereal orthologue of FT, FT1 (Yan et al., 2006), does not occur in response to long days, but loss of VRN2 sees a rapid increase in FT1 expression and transition to flowering (Hemming et al. 2008).

The VRN1 locus was originally proposed to be the primary responder to chilling temperatures. Like FLC, the VRN1 gene contains a large first intron which has been shown to be the site of epigenetic reprogramming of both loci (Questa et al., 2016; Oliver et al., 2013). However, another candidate for the primary responder to chilling was described as ODDSOC2 (Greenup et al., 2010). ODDSOC2 is part of a large clade of monocot MADS box transcription factors that show closer similarity to Arabidopsis FLC and SOC1 (Greenup et al., 2010; Ruelens et al., 2013; Sharma et al., 2017). Interestingly the ODDSOC2 gene responds to chilling temperatures by reducing its expression in a manner similar to FLC, both in barley and Brachypodium (Greenup et al., 2010; Sharma et al., 2017). Furthermore, it has been shown that silencing of ODDSOC2 precedes the activation of VRN1 in the winter chilling response in Brachypodium (Sharma et al., 2017). Ruelens et al (2013) identified a larger clade of FLC-like transcription factors in cereals that contains ODDSOC2 and many others: several of these have since been shown to respond to chilling, in addition to ODDSOC2 (Sharma et al., 2017; Jiao et al., 2019; Kennedy and Gueten, 2020).

Importantly, transcript levels of VRN1, VRN2 and VRN3/FT1 remain highly sensitive to the growing temperature right through early floral development in wheat (Dixon et al., 2019), with temperatures in the vernalising range promoting much higher levels of VRN1 and VRN3/FT1 in developing spikes.
Thus vernalisation temperature continues to affect gene expression levels long after the traditional vernalisation requirement for flowering has been fulfilled.

**Chilling responses in woody perennials**

In perennial species short days or declining temperatures in late summer and autumn can induce the state of endodormancy (Lang et al., 1987). In endodormant buds growth is repressed by warm temperatures. Endodormancy is ‘broken’ by a winter chilling experience that strongly resembles vernalisation, after which buds transition to ecodormancy, a state defined by the ability of warmth to again promote growth. Recently it has become clear that the molecular basis of endodormancy release is shared in the most part with vernalisation (Figure 1), and thus it seems likely that stable rather than transient silencing of floral repressors is important in the endodormancy/ecdormancy transition.

In *A. thaliana* the floral repressor FLC acts in tandem with SHORT VEGETATIVE PHASE (SVP) to inhibit flowering in summer and autumn. SVP is a promiscuous MADS-box transcription factor that will form heterodimers and multimers with both floral repressors and promoters to control the floral transition and downstream aspects of floral development (Smaczniak et al., 2012). For example, in Arabidopsis FLC and SVP together promote continuing vegetative development, but SVP can also bind APETALA1 to promote flower development from inflorescence meristems, and inhibit gene expression specific to inflorescence meristems in developing flower buds (Gregis et al., 2013).

In perennial species, SVP-like genes have important roles during endo-dormancy induction and release. In species of the Rosaceae, the DORMANCY ASSOCIATED MADS BOX (DAM) genes are closely related to SVP and are expressed during all dormant stages. DAM genes were first described in peach, where deletion of a large array of DAM genes was shown to underlie the evergreen (*evg*) mutation which prevents vegetative buds of peach from entering winter bud dormancy (Bielenberg et al., 2008). The *EVG* locus contains six *DAM* genes each of which has a unique seasonal expression profile, but all of which fall in expression before or during loss of endodormancy. Heterologous expression of apricot *DAM6* in poplar showed that individual *DAM* genes are sufficient to induce bud dormancy (Sasaki et al., 2011). SVP-like genes can also induce dormancy in apple (Wu et al., 2017) and form complexes with DAM proteins (Falavigna et al., 2021). Just as in Arabidopsis, the composition of these multimeric MADS-box transcription factor complexes impacts binding specificity to target genes, due to nucleotide specificity conferred by different complexes and their interaction with CArG DNA motifs. In apple buds DAM proteins do not simply undertake the role of FLC: there are genes more closely related to *FLC* in apple that peak in expression during summer. *MdFLC1* and *MdFLC3* are most highly expressed in the juvenile phase, perhaps hinting at a role in repression of the reproductive program, rather than responses to chilling in flower buds (Kagaya et al. 2020).
The key role of SVP in bud dormancy has most clearly been demonstrated in hybrid aspen (Singh et al., 2018, 2019; Tylewicz et al., 2018). Loss of the poplar orthologue of SVP (known as SVP-Like or SVL) leads to failure of vegetative meristems to enter winter dormancy, as with the evg mutant in peach. In hybrid aspen the mechanism of winter bud dormancy induction appears strikingly similar to that described for lateral meristems of Arabidopsis (Gonzalez-Grandio et al., 2017; Maurya et al., 2020), via a BRC1-mediated up-regulation of the ABA biosynthesis gene NCED3, and GA2 oxidases which reduce levels of active gibberellins (Singh et al., 2018). In A. thaliana SVP also has a conserved role in the control of gibberellin metabolism (Andres et al., 2014). However, chilling only has a very weak effect on SVL mRNA levels, suggesting that SVL is not the primary transcriptional responder to prolonged chilling during endodormancy loss. In hybrid aspen the dormant state is maintained by the feedback up-regulation of SVL transcription by ABA and closure of the plasmodesmata in buds, symplastically isolating dormant buds from the rest of the plant (Tylewicz et al., 2018).

Currently it is unclear whether FLC itself can play a role in bud dormancy, alongside DAM and SVP although FLC can clearly induce dormancy in seeds. However, circumstantial evidence for the role of FLC-like genes in dormancy in flower buds comes from the study of A. alpina, where it has been shown that there are indeed two separate responses to vernalisation that promote flowering in spring, both of which are mediated by the silencing of the FLC orthologue PEP1. In addition to the direct regulation of well-known promoters of the floral transition, PEP1 also acts to repress gibberellin levels (Tilmes et al., 2019). In A. alpina, increases in gibberellins after vernalisation act to promote the floral transition, but also affect the growth rate of bolting stems, an effect consistent with a role in elongation growth after bud break (Tilmes et al., 2019). Interestingly early gene expression analyses of Arabidopsis flc mutants showed that even in leaves FLC has large effects on the expression of genes closely linked to ABA responses such as ABA-INSSENSITIVE 3 and ABA-INSSENSITIVE 5 (Edwards et al., 2006), and FLC and PEP1 can both affect dormancy of seeds (Chiang et al., 2009; Hughes et al., 2019). In apple FLC/SVPa complexes can bind the important ABA biosynthesis gene NCED4, which could be important in dormancy induction (Falavigna et al., 2021). Because ABA and GA are central regulators of plant dormancy, these findings are consistent with a role for FLC-like genes in chilling responses during winter dormancy loss.

De-vernisation and floral repressor responses to non-chilling temperatures

Climate change is changing the frequency, duration and geographical extent of warm spells during chilling. Depending on the time of year and gene expression state of chilling-responsive floral repressors, warm spells have the potential to delay chilling responses, and even reverse the effects of chill in some circumstances. For instance, in autumn one warm day is sufficient to lower VIN3 transcript levels (Hepworth et al., 2018), a consequence of the necessary short half-life of cold and circadian regulated genes.

In Arabidopsis chilling initially marks an area of the first intron of FLC with H3K27me3, and subsequent movement of vernalised Arabidopsis plants to 22°C induces spreading of H3K27me3
across the FLC locus (Yang et al., 2017). This is associated with stabilisation of the vernalised state. This stabilisation phenomenon was first documented in experiments vernalising imbibed seeds prior to germination which is possible in species which lack a vernalisation-unresponsive juvenile phase such as rye, wheat, wild lettuce and Arabidopsis (Purvis and Gregory, 1945; Napp Zinn 1957; Marks and Prince, 1977). Indeed, vernalisation can even begin during seed development on the mother plant (Reid and Murfet, 1978). Yearly cycles of vernalisation and de-vernralisation in buried seeds enables annual plants of the same species to be capable of both winter annual and summer annual phenology, depending on the time of year of eventual germination from the soil seed bank (Prince and Marks, 1982). Vernalisation of seeds is reversed in the field by summer temperatures, and can often be reversed in the laboratory by short high temperature treatments given before germination, showing that the vernalised state in seeds is not stable in respect to future temperature variation (Purvis and Gregory, 1945). Instead these studies showed that if germination and establishment was allowed to proceed in warm conditions, subsequent high temperature treatments were much less effective at reversing the vernalised state. Aspects of growth such as cell division may therefore be important not for vernalisation itself but its stable memory in warm post-vernalisation conditions.

In Arabidopsis similar observations have been made (Napp Zinn, 1957) and high temperatures immediately after vernalisation prevent H3K27me3 deposition at FLC, but not when given after a period of stable growth at 22°C (Bouché et al., 2015). Furthermore, in buried Arabidopsis seeds FLC expression does indeed proceed through an annual gene expression cycle of activation and de-activation (Footitt et al., 2011), showing that in non-growing seeds FLC silencing is freely reversible at normal spring and summer soil temperatures in the United Kingdom. Although most studies suggest that growth is required for the stabilisation of the vernalised state rather than vernalisation itself, growth may additionally accelerate vernalisation in Arabidopsis via dilution of an activator of FLC expression during cell division (Zhao et al., 2020). Reversal of the effect of vernalisation has been termed de-vernralisation (Purvis and Gregory, 1945).

In Arabidopsis de-vernralisation is part of a wider spectrum of FLC responses to non-vernalising temperatures (summarised in Table 1). As temperature increases from 12°C right up to 27°C Arabidopsis FLC levels continue to decrease (Blazquez et al., 2003; Balasubramanian et al., 2006). These FLC gene expression changes have consequences for the plant because they affect flowering time (Blazquez et al., 2003; Balasubramanian et al., 2006), cold-responsive gene expression (Penfield, 2008) and seed dormancy (Chen et al., 2014). The ambient temperature-regulation of FLC occurs through a similar epigenetic process to vernalisation because late flowering induced by higher FLC activity at cool ambient temperatures can be reversed by vernalisation (Chen and Penfield, 2018). High temperatures can also cause FLC activation via H3K27me3 demethylation and in this way limit the floral promotive effect of extreme warmth (Gan et al.,2014). H3K27me3 demethylation is also observed by giving heat immediately after vernalisation (Bouché et al., 2015). Thus the temperature range in which FLC can mediate developmental responses to temperature is quite broad and is not restricted to stable sensing of chilling temperatures, and temperatures around 16°C or above 29°C have the potential to raise FLC gene expression levels, even if FLC is silenced by H3K27me3.
Table 1. Summary of known responses of *A. thaliana* FLC to different temperatures. Table describes the known effects of the indicated temperatures in left most column when FLC sense expression is in the indicated states in the second column.

| Temperature | Starting FLC gene expression state | Effect of indicated temperature | Reference |
|-------------|-----------------------------------|---------------------------------|-----------|
| 0°C         | high                              | Strongly vernalising            | Napp Zinn, 1957; Michaels et al., 1999; Wollenburg and Amasino, 2012 |
| 0°C-10°C    | high                              | Weakly vernalising              | Wollenburg and Amasino, 2012; Duncan et al., 2015; |
| 10°C-16°C   | High or low                       | No effect.                      | Napp Zinn, 1957; Michaels et al., 1999; Sheldon et al., 2000 by implication. |
| >24°C       | vernalised                        | De-vernalisation in seeds       | Napp Zinn, 1957 |
| 17-27°C     | low                               | Expression increases with decreasing temperature | Blazquez et al., 2003; Balasubramanian et al. 2006; Chen et al., 2014 |
| >29°C       | low                               | De-vernalisation in seedlings. Increases expression due to H3k27me3 demethylation | Gan et al., 2014; Bouché et al. 2015 |

Thus although FLC silencing is commonly described as ‘stable’, the most stable state is most commonly seen in the laboratory in conditions of moderate warmth. The duration of prior chilling and chilling temperature required to reach this state is strongly variable in different FLC haplotypes and in different species. Nishio et al (2020) directly tested the time of year at which H3K27me3 spreading was observed in the field in *A. halleri*. H3K27me3 spreading was mainly observed in late winter and early spring when plants began to bolt, at temperatures in a daily range of 5-15°C. But by the time the plants flowered FLC re-activation had already begun. Measurements of FLC gene expression responses to temperature show that in *Arabidopsis halleri* FLC has the strongest correlation with temperature over the preceding 6 weeks (Aikawa et al., 2010). Whether annual species show similar expression dynamics during flowering or greater stability of silencing remains to be tested.

A second feature of vernalisation in the field that is rarely considered is the threshold level of FLC repression required for the floral transition to occur. In *B. napus* we found that several FLC genes remain expressed at high levels even after the floral transition, and none were fully silenced (O’Neill et al., 2019). We could show that in the field in developing reproductive tissues at least two FLC
genes remained expressed and temperature-sensitive. These different FLC isoforms exhibit variable gene
expression dynamics in response to chilling in the laboratory (Calderwood et al., 2021). To add
further complexity work in A. alpina has also shown that buds produced during chilling have less
propensity for FLC silencing (Lazaro et al., 2018). Perennial A. alpina relies on this mechanism to
maintain vegetative lateral meristem for the next growing season. So even single copies of FLC can
exhibit different gene expression relationships to temperature in different plant tissues.

Both de-vernralisation and proliferation of copies with different seasonal dynamics can potentially
explain the apparent paradox that although FLC is apparently silenced during vernalisation, in both
in A. thaliana and A. alpina seasonal sensing of temperature by FLC in the mother plant can also
control progeny seed dormancy (Chiang et al., 2009; Auge et al., 2017; Hughes et al., 2019). In A.
thaliana vernalisation of the mother plant reduces seed dormancy, and ambient temperature
variation during early reproductive development results in a temperature memory at FLC that can be
observed in flower, fruit and seed tissues (Auge et al., 2017; Chen et al., 2014; Chen and Penfield,
2018).

Consequences of reduced chilling or de-vernralisation for flower development and yield

Climate change is affecting the accumulation of chilling in temperate zones, and reducing the
subtropical extent of safe reliable chill on every continent. Thus it is timely to consider possible
molecular mechanisms by which variation in chilling temperature affects reproductive development
and yield. Surprisingly few studies consider the molecular effects of unusually warm temperatures at
different stages of reproductive development. The reason this is interesting is that a key aspect of
reproductive development in angiosperms involves transcription factors which mediate the
environmental regulation of the floral transition have secondary roles in the development of the
reproductive tissues themselves. For instance, SVP is also expressed in floral meristems and is
required alongside AP1 for the early stages of flower development (Gregis et al., 2008) and for ovule
development (Simioni et al., 2012). FLC has redundant roles in floral development (Deng et al.,
2011). This is consistent with multiple studies which show that in a wide range of species insufficient
chilling can have multiple developmental consequences for flower, fruit and seed structures
resulting in yield losses. Incomplete chilling often determines the sub-tropical extent of cultivation of
perennial crops (Atkinson et al., 2013).

Most attention has been paid to the role of incomplete chilling in the prevention or delay to bud
break (Luedeling, 2011). This can result in low yields in several species, including grapevine, kiwi, and
perennial fruit-bearing trees and shrubs such as peach, apricot or blackcurrant. However, in addition
to the delay in bud set, incomplete chilling also causes a separate delay to anthesis in pear (Pyrus
communis; Atkinson and Taylor, 1994) showing that growth-delaying effects of incomplete chilling
last beyond the loss of bud dormancy itself. Further effects of chilling deficiencies were helpfully
collated by Atkinson et al (2013). In some species warm winters lead to flower abscission, with bud
drop defining the sub-tropical limit of the common pear (Yamamoto et al., 2010) and affecting
multiple crops such as apple, apricot and cherry. Defects in flower development have also been observed, leading to yield or quality penalties. For instance in peach incomplete chilling leads to long thin fruits whose skin can part at the tip, whereas strong chilling leads to round fruits (Salvador et al., 1998; Sherman et al., 2003; Wert et al., 2007; Li et al., 2016). Effects on fruit shape are also found in apricot, whereas in sweet cherry (Prunus avium) both flower and fruit size are similarly affected by chilling. In apple (Malus x domestica) chilling affects the rate of cell division in the ovary prior to anthesis, and in this way affects fruit size (Grebeeye and Berg, 2000). However, overexpression of DAM or SVP alone in apple increase bud dormancy but do not alter fruit development (Wu et al., 2017). Interestingly, known targets of SVP in Arabidopsis include meristematic regulators such as PHABULOSA, CLAVATA1 and ATHB8 (Gregis et al., 2013), suggesting that continued activity of SVP complexes with floral repressors may be important for the effects of reduced chilling or devernalisation on yield traits via control of meristem functions or early organ growth. Taken together this suggests that the activity of chilling-responsive factors in developing flowers can limit the growth of flowers and fruit tissues, and thus affect yields.

Less is known about effects of reduced chilling on cereal inflorescence development, but as the core mechanisms of development are conserved, similar responses are plausible. Transcript levels of VRN1, VRN2 and VRN3/FT1 remain highly sensitive to the growing temperature right through early floral development in wheat (Dixon et al., 2019), with temperatures in the vernalising range promoting much higher levels of VRN1 and VRN3/FT1 in developing spikes. Thus vernalisation temperature continues to affect gene expression levels long after the traditional vernalisation requirement for flowering has been fulfilled in wheat. VRN1 and its close homologues FUL2 and FUL3 play roles in the control of spikelet number and number of floret per spike (Deng et al., 2015; Li et al., 2019), suggesting that temperature-regulation of VRN1 during reproductive development could be important for yield variation (Deng et al., 2015). FT1 and FT2 have similar roles in controlling spikelet number (Gauley and Boden, 2020). Furthermore, the same group of genes are important in determining the frequency of floret abortion in response to stresses such as lack of nutrients, heat or drought (Gol et al., 2017), just as has been reported in fruit bearing perennial species. Thus any variation in the response to chilling signals beyond the traditionally reported stable silencing/activation of gene expression can be hypothesised to have quantitative effects on crop performance and yield. This is important because in wheat de-vernallisation can be caused by exposure to high temperatures after chilling (Gregory and Purvis, 1945), or by simple variation of the growth temperature after vernalisation. The importance of this is clearly demonstrated by experiments reported by Dixon et al (2019) who show that allelic variation at VRN1 is important for flowering time responses to post-vernalisation temperatures as well as for vernalisation itself. In addition, when warm temperatures were applied after chilling expression of both the VRN2 and ODSOC2 genes was reactivated and the development of yield components such as spikelet number was affected (Dixon et al., 2019). Other arable crops may also be affected by variation in winter chill. For instance yield reductions in oilseed rape have been observed in both the United Kingdom and China in years with low level of early winter chill (Brown et al., 2019; He et al., 2017).
In conclusion, because the genes involved in vernalisation have secondary roles in the control of reproductive development, any failure to vernalise or subsequently de-vernalisation in the field can have consequences for flower fruit and seed development, as well as for seed behavioural traits such as seed dormancy. Because common genes control the response to chilling in both winter annual crops and perennial fruit crops, it is likely that warming winters will affect the yields of several crops through similar processes. Thus, breeding for adaptation to warmer winters may require manipulation of the same gene sets in very different crops.
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Figure Legends

Figure 1. Comparison of seasonal gene expression patterns during chilling in angiosperm buds during autumn winter and spring. Gene expression is indicated by the height of the filled shape for each gene. For each species a pictorial depiction of the reproductive developmental state is shown from vegetative meristems through to inflorescence meristem formation and flowering. Data are derived from O’Neill et al. (2019), Wu et al., (2017); Falavigna et al., (2019); Kagaya et al., (2020); Xie et al., (2019); Gauley and Boden, (2020); Vimont et al., (2019). DAM G1, G2, G3, group 1-3 DAM genes, as defined by Falavigna et al., (2019).
Figure 1