Environmental regulation of life history phenology in *Arabidopsis thaliana*

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Abstract

The seasonal timing of plant development is regulated by environmental cues. Flowering time is influenced by the temperature and photoperiod experienced during vegetative growth, while germination timing is affected by temperatures during seed maturation and after dispersal. The timing of each developmental transition also determines seasonal conditions experienced during subsequent life stages, however the significance and stability of these interactions are not well understood. This work aimed to further an understanding of the environmental regulation of plant phenology by creating a multi-stage life history model based on *Arabidopsis thaliana*.

Laboratory and field studies were used to inform predictive models of seed development and seed dormancy. The time required to complete seed development was mainly affected by temperature, and was therefore sensitive to seasonal flowering time. Mean daily temperatures at the end of seed maturation had the greatest influence on rates of primary dormancy loss, and post-dispersal temperatures determined rates of secondary dormancy induction. Germination probabilities were predicted by modelling frequencies of primary and secondary dormancy within the seed population. This revealed an abrupt switch from low to high germination when mean daily temperatures exceeded 14°C. Thermoinhibition was also predicted at high temperatures due to rapid secondary dormancy induction.

Combining models with a previously described model of flowering time provided a framework for investigating the effects of perturbations on entire life history phenology. Seed set timing in spring and winter annuals was consistently predicted to coincide with mean daily temperatures of 14°C in locations across Northern Europe, resulting in the production of both dormant and non-dormant offspring. Phenotypic plasticity at each growth phase also served to buffer against modest perturbations in germination date, flowering date, and climate in order to maintain these specific dispersal conditions. This result was interpreted as evidence for a robust bet-hedging germination strategy.

Table of Contents

List of Figures

List of Tables

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Author’s Declaration

I declare that the work submitted in this thesis is my own unaided work, unless otherwise acknowledged in the text. No part of this thesis has been previously submitted for any other degree at this, or any other university.

Chapter 1: Introduction

1.1 Phenology

Phenology is the study of the timing of seasonal events and the factors that influence them (Leith, 1974). Examples of such events include bud burst and flowering times in plants, bird migrations, and insect emergence. Usually these phenomena are sensitive to seasonal environmental factors, for example in extratropical regions the phenology of the dominant forest tree species is sensitive to winter chilling, photoperiod, and temperature (Körner and Basler, 2010). Because temperature sensitivity is often important, changes in phenological events are frequently used as indicators of climate change. For example, an influential study of 385 British plant species reported that spring flowering has advanced an average of 15 days compared to 50 years ago (Fitter and Fitter, 2002), and advancing spring phenology closely matches the pattern of warming in Europe over the last few decades (Menzel et al., 2006b).

However, not all species respond to changing environments in the same way and there is concern that mismatches in phenological shifts will result in disruption within ecosystem communities (Durant et al., 2005). Some disruption has already been observed in vulnerable ecosystems such as in the arctic (Post and Forchhammer, 2008). Sowing and harvest dates of many spring and winter crop varieties are also advancing, although not as quickly as in wild plants (Menzel et al., 2006a). This suggests farming practices may not be adapting quickly enough to keep pace with the current rate of warming. Understanding the processes that regulate

phenological responses is therefore an important goal, and is necessary to anticipate the consequences of current and future climates.

Substantial progress has already been made towards understanding the genetics behind important phenological traits in model systems such as *Arabidopsis thaliana* (Wilczek et al., 2010). This annual weed is an ideal subject due to its ability to grow easily and rapidly in laboratory conditions. It also has a wide geographic distribution encompassing central Asia, Europe and North America, and different ecotypes possesses substantial variation in life history phenology (Nordborg and Bergelson, 1999). It is predominantly a winter annual which germinates in autumn, over winters as a vegetative rosette, and then flowers and sets seed the following spring (Baskin and Baskin, 1983). Spring annuals can also germinate, flower, and set seed during spring or early summer. In some locations *Arabidopsis* has also been known to flower and set seed in autumn (Thompson, 1994), and is capable of completing multiple generations in a single year; known as 'rapid cycling'. *Arabidopsis* is therefore a useful tool to investigate plant phenology and the factors that determine life history.

1.2 Germination

Germination marks the end of seed dispersal; when completed seedlings are fully committed to growth in their immediate environment. The timing of this event is crucial since it not only determines the seasonal conditions during seedling establishment, but also which seasons will be experienced during the adult and reproductive phases. Inappropriately timed germination can reduce reproductive fitness, and can even be fatal (Biere, 1991). Germination timing is therefore a trait

which is subject to intense natural selection (Donohue et al., 2005c), and the regulation of germination by seed dormancy is a subject of continuing research.

Germination itself is a process which involves a series of metabolic and structural changes in the seed. The full process includes water uptake, reserve mobilization, mRNA and protein synthesis, cell division, embryo growth, and finally culminates in the protrusion of the radicle. Plant hormones play a central role in promoting or suppressing the onset of germination. Gibberellins (GA) not only promote germination, but are absolutely required for its completion. This was demonstrated by GA-deficient mutants which were unable to germinate without addition of exogenous GA (Karssen et al., 1989; Hilhorst and Karssen, 1992; Derkx and Karssen, 1993b). GA is now understood to perform a dual role by increasing the growth potential of the embryo, and also weakening the outer tissues covering the radicle (Groot and Karssen, 1987; Groot et al., 1988). Collectively these processes are coordinated by a complex pattern of GA-regulated gene expression, which also includes crosstalk between GA and other plant hormones (Ogawa et al., 2003).

Abscisic acid (ABA) is a hormone which acts antagonistically to GA by inhibiting germination, and promoting seed dormancy. This was demonstrated in lettuce, where treatment with ABA led to inhibition of light and GA induced germination (Khan, 1968). Maintenance of dormancy requires ABA synthesis, and the use of ABA inhibitors such as norflurazon is an effective way to alleviate dormancy and promote germination (Debeaujon and Koornneef, 2000). Transcriptomic analysis has also revealed that a high proportion of genes expressed by dormant seeds contain ABA responsive elements, indicating that ABA signalling has an important role to play in dormancy (Cadman et al., 2006).

The opposing roles of ABA and GA in promoting dormancy and germination respectively led to the formulation of the hormone balance theory (Karssen and Laçka, 1986). This theory suggests that germination depends on the balance between growth promoters (e.g. GA) and growth inhibitors (e.g. ABA). Environmental factors such as light and temperature are thought promote either dormancy or germination by altering the ratios of these hormones.

1.3 Seed Dormancy

Seed dormancy is the mechanism which enables seeds to regulate the timing of germination. Most seeds have some level of dormancy at maturity, which allows them to delay their germination until after a suitable dispersal period. Delayed germination also promotes the formation of a soil seed bank. Previous studies have estimated that as many as 70,000-90,000 seeds per m^2 can accumulate in the top 15-25cm of cultivated soil, and 95% of these were thought to originate from annual species (Kropac, 1966; Roberts, 1981; Baskin and Baskin, 1985). Buried seeds can also remain viable for many years, and one study found seeds of some species were still able to germinate after 100 years of burial (Kivilaan and Bandurski, 1981). The existence of a soil seed bank facilitates the long term persistence of a population, and is also important for recolonisation following natural disasters such as fire. Seed banks also make annual weeds notoriously difficult to eliminate from agricultural systems, and an improved understanding of seed dormancy is an important goal for the purpose of improving weed management strategies.

Despite the ecological significance of seed dormancy, until relatively recently it was said to be one of the lest well understood aspects of seed biology (Finch-Savage and Leubner-Metzger, 2006). One of the difficulties faced by dormancy researchers in

the past has been a lack of a universally agreed definition. At present, there is no way of directly measuring dormancy, which must instead be measured indirectly via germination tests. As a result, dormancy is occasionally used as a term to mean 'not germinating' and has also been confused with persistence in soil (Thompson et al., 2003). However, a lack of germination does not necessarily equate to dormancy, and could also be caused by a lack of suitable environmental conditions (e.g. light, moisture). The state of being non-dormant, but not germinating due to a limiting environment has been described as 'pseudo-dormancy' or 'enforced dormancy', although using the term dormancy to describe this state may be misleading. An alternative term 'quiescence' has therefore been suggested (Baskin and Baskin, 2004). Researchers have also disagreed on whether light requiring seeds should be considered dormant (e.g. Bewley and Black, 1994), or whether light is simply required by quiescent seeds to initiate germination (e.g. Vleeshouwers et al., 1995; Baskin and Baskin, 2004). However, one of the most commonly used definitions is one which attempts to cover these issues, and simply states that dormancy is "the failure of an intact viable seed to complete germination under favourable conditions" (Bewley, 1997).

Another interesting point is that germination in a single seed can only be viewed as an all-or-nothing trait. However, the depth of dormancy is a continuous measure and can take on any value between fully-dormant and non-dormant. Changes in dormancy depth can be observed at a population level as a change in the germination frequency in particular conditions. Studies of exhumed seeds have also shown that seasonal changes in dormancy depth are associated with changes in the range of conditions that permit germination (Baskin and Baskin, 1985). In general, seeds with the lowest dormancy germinate in the widest range of conditions, whereas increasing

dormancy results in a narrowing of this range, until germination is no longer possible in any conditions. For this reason, dormancy is also sometimes defined as a seed property that determines the range of conditions that permit germination (Vleeshouwers et al., 1995). According to this definition, environmental factors such as light would only be considered to act on dormancy if they altered the range of permissive germination conditions (Finch-Savage and Leubner-Metzger, 2006).

There are now known to be five major classes of seed dormancy, and confusion in the past may have also arisen because the type of dormancy being studied was not always stated (Baskin and Baskin, 2004). The work in this thesis focuses on physiological dormancy (PD) which is the type of dormancy attributable to *Arabidopsis* seeds, and is also the most common throughout the plant kingdom (Finch-Savage and Leubner-Metzger, 2006). PD can also be further subdivided into three levels; deep, intermediate, and non-deep (Baskin and Baskin, 2004). Non-deep PD can usually be alleviated by a period of dry storage (after-ripening) or a period of moist incubation (stratification) at either a high or a low temperature, depending on the species' requirements. Non-deep PD may also be alleviated by scarification or removal of the seed coat, or by treating with GA. In contrast however, seeds with deep and intermediate PD generally require longer periods of stratification or after-ripening before they will germinate, and removal of the seed coat or addition of GA is usually ineffective (Baskin et al., 2005). A further distinction is also usually made between primary and secondary dormancy.

1.3.1 Primary dormancy

Primary dormancy is the type possessed by most seeds at maturity, and is established during seed maturation with the involvement of ABA. *Arabidopsis* mutants which are ABA deficient or insensitive have reduced primary dormancy and a tendency for precocious germination while still maturing on the mother plant (Karssen et al., 1983; Koornneef et al., 1984). Pre-harvest sprouting (vivipary) is also responsible for substantial yield losses in crop species such as wheat (Clarke et al., 2005), which are often less dormant than their wild relatives as a result of domestication and selective breeding. In contrast to reduced dormancy phenotypes, overexpression of ABA biosynthesis genes leads to an accumulation of ABA, increased primary dormancy and delayed germination (Frey et al., 1999; Nambara and Marion-Poll, 2003).

There is substantial variation in levels of primary dormancy within *A. thaliana* ecotypes (Evans and Ratcliffe, 1972; Ratcliffe, 1976), making this species a useful test subject for studying the genetic basis of this variation. The commonly studied laboratory strains Columbia (Col) and Landsberg *erecta* (L*er*) have relatively low dormancy, whereas the Cape Verdi Islands ecotype (Cvi) is much more dormant when grown in the same conditions. The genetics of primary dormancy establishment are still mostly unknown, however one important gene has so far been cloned and indentified. This gene, called *DELAY OF GERMINATION 1* (*DOG1*), is expressed specifically in seeds during maturation, and coincides with the development of primary dormancy (Bentsink et al., 2006). Expression of *DOG1* was also shown to require the ABA-mediated sugar signalling pathway (Teng et al., 2008), and variation in *DOG1* expression levels are thought to account for some of the variation in primary dormancy between ecotypes (Chiang et al., 2011).

The environment during seed maturation is also known to affect levels of primary dormancy (Fenner, 1991). Temperature has the largest effect, with low temperatures generally resulting in seeds with increased dormancy (Schmuths et al., 2006; Kendall et al., 2011; Penfield and Springthorpe, 2012). For example, strong correlations between dormancy and the mean average daily temperature in the last 30 days of seed ripening have been demonstrated in *Rosa* (VonAbrams and Hand, 1956) and *Chenopodium bonus-henricus* (Dorne, 1981). However in some cases, a low temperature treatment can reduce dormancy if given at the appropriate time (Wiesner and Grabe, 1972). Furthermore, seed dormancy can even be affected by temperatures experienced by the parent plant during the vegetative growth phase (Thomas and Raper, 1975; Sawhney et al., 1985). The mechanism which allows such long term signals to be transmitted from parent to offspring is unknown, however low temperatures during seed maturation are associated with elevated *DOG1* expression (Chiang et al., 2011).

Many other factors, including photoperiod (Munir et al., 2001), rainfall (Baskin and Baskin, 1975) and even the position of seeds on the parent plant (Gutterman, 1980) can affect levels of primary dormancy, although to a lesser extent compared to temperature. These so called 'maternal effects' also determine the length of stratification or after-ripening required to break dormancy (Donohue et al., 2008; Footitt et al., 2011; Kendall et al., 2011), and this phenotypic plasticity allows plants to produce offspring with dormancy characteristics specific to a particular dispersal environment. Primary dormancy is therefore a complex trait determined by both genetic and environmental factors, meaning that seasonal reproductive timing is central to germination phenology.

1.3.2 Secondary dormancy

After dispersal, seeds may enter secondary dormancy if conditions remain unfavourable for germination. Relatively little is known about secondary dormancy, as the majority of research has so far focused on primary dormancy. However, it is thought that seeds can transition through different levels of secondary dormancy in response to seasonal changes, creating an annual dormancy cycle (Baskin and Baskin, 2004). Many studies of buried weed seeds have demonstrated these annual dormancy cycles by showing that exhumed seeds tend to germinate best at certain times of year (Baskin and Baskin, 2001). Evidence has also been published showing that dormancy cycling in buried *Arabidopsis* seeds is associated with seasonal patterns of gene expression, which are also correlated with temperature (Footitt et al., 2011; Footitt et al., 2013).

Distinguishing between states of primary and secondary dormancy is difficult, and no clear criteria have yet been established. However, there is some evidence that the two types of dormancy operate through distinct mechanisms. For example, secondary dormancy in wild oats (*Avena fatua*) can be alleviated with nitrate, however seeds with primary dormancy require both after-ripening and nitrate (Symons et al., 1986). Similarly, primary dormant seeds of the annual weed *Sisymbrium officinale* are insensitive to GA, however during dormancy cycling GA sensitivity remains high and seeds become insensitive to light and nitrate (Derkx and Karssen, 1993a). Despite some differences, there is also evidence that a common transcriptional mechanism involving ABA signalling is common to different dormancy states (Cadman et al., 2006).

1.3.3 Light

Germination in many species requires light. In *A. thaliana*, light stimulates the production of GA (Derkx and Karssen, 1993b) via activation of GA biosynthetic genes, which are themselves regulated by phytochromes (Yamaguchi et al., 1998). Phytochromes are light sensitive photoreceptors which are synthesised in an inactive, red light absorbing form (Pr). Upon exposure to red light, Pr is converted to an active far-red light absorbing form (Pfr), and this activation is photoreversible. The promotive effect of red light and inhibitory effect of far-red light on germination was first discovered in lettuce seeds, however the significance of the phytochromes was not realised until their role in the control of flowering was also discovered (Cone and Kendrick, 1986).

It is now known that the *Arabidopsis* genome contains 5 different phytochrome genes (*PHYA* to *PHYE*), each with specific sensitivities and functions (Sharrock and Quail, 1989). Distinct germination responses to different fluences of red light have also been found (Cone et al., 1985b; Kendrick and Cone, 1985). The low fluence response (LFR) is mediated by PHYB and permits germination after exposure to approximately 1-1000µmol m^{-2} red light (Cone et al., 1985a; Botto et al., 1995; Shinomura et al., 1996). The very low fluence response (VLFR) is mediated by PHYA, and permits germination after exposure to 10^{-4} – 10^{-2}µmol m^{-2} 660nm light resulting in just 10^{-4} – 10^{-2}% of the total phytochrome as Pfr (Cone et al., 1985a; Botto et al., 1996). The VLFR is thought to allow germination under dense canopy shade. However, since most seed batches contain low levels of endogenous Pfr, the VLFR can also lead to germination in complete darkness, unless Pfr is first inactivated by a saturating pulse of far-red light (Cone et al., 1985a).

The downstream responses to light are mediated by a family of transcription factors called PHYTOCHROME INTERACTING FACTORS (PIFs), which bind directly to activated phytochrome proteins. The first of these to be discovered was PIF3, which was found to interact with both PHYA and PHYB in *A. thaliana* and rice (Ni et al., 1998). Genome sequencing also led to the identification of additional members of the family based on sequence homology to *PIF3*. Consequently, some of the family members were also named PIF3-like factors (PILs). For example, PIF1 is also known as PIL5. This particular protein was found to have a key role in regulating germination in response to light, and loss-of-function mutant seeds are capable of germination after a normally inhibitory pulse of far-red light, and also in darkness (Oh et al., 2004; Penfield et al., 2005) showing that PIL5 is required for dark and far-red inhibition of germination. PIL5 also inhibits germination by promoting the production of two growth repressing DELLA proteins; RGA and GAI (Oh et al., 2007), which function to repress GA signalling. In addition to this, PIL5 also activates genes for GA catabolism and ABA biosynthesis, thereby promoting dormancy. Upon light irradiation, the PIL5 protein binds to activated phytochromes leading to its degradation (Oh et al., 2006; Shen et al., 2007). Light induced degradation of PIL5 therefore promotes germination by simultaneously reducing ABA synthesis, and promoting GA accumulation and signalling.

1.3.4 Temperature

Temperature is one of the most important factors governing both seed dormancy and germination (Roberts, 1988). However, it is sometimes difficult to determine whether an observed difference is due to an effect of temperature on germination or an effect on dormancy. For example, ambient temperatures are correlated with

germination rates, however reducing dormancy by pre-treating seeds at low temperatures can also lead to increased germination rates (Schmuths et al., 2006). Changes in dormancy are also known to affect sensitivity to environmental factors, including the temperature ranges under which germination will occur (Baskin and Baskin, 1985).

As mentioned previously, the temperature during seed maturation can have a substantial effect on the levels of primary dormancy in mature seeds, and in most species low temperatures during seed ripening are associated with increased primary dormancy. However, low temperature imbibition, or cold stratification, is also commonly used to break dormancy. This dormancy alleviation is thought to be caused by an increase in the levels of bioactive GAs found in seeds imbibed at 4°C in comparison to those imbibed at 22°C (Yamauchi et al., 2004). The increased GA levels at low temperatures also coincide with increased expression of genes promoting GA biosynthesis, and reduced expression of genes promoting GA degradation (Yamauchi et al., 2004). This suggests an important role for GA metabolism during low temperature induced primary dormancy loss during imbibition.

The mechanism which allows low temperatures to have opposite effects on dormancy in imbibed seeds compared to maturing seeds is not well understood. However, recent evidence has suggested a role for C-REPEAT BINDING FACTORS (CBFs), which are transcription factors known to be involved in the cold acclimation pathway (Medina et al., 2011). It was shown that CBFs are required for low temperature regulation of DOG1 during seed maturation (Kendall et al., 2011). In imbibed seeds however, CBF expression levels were insensitive to temperature, suggesting that CBF inactivation may be important to the promotion of germination

by low temperatures during imbibition (Kendall et al., 2011). Interestingly, a role for a gene known primarily for its involvement in the regulation of flowering; *FLOWERING LOCUS C* (*FLC*), has also recently been discovered. High levels of *FLC* expression were found to promote germination at low temperatures, however this promotion was also maternally controlled and influenced by seed maturation temperatures (Chiang et al., 2009).

In contrast to imbibition at low temperatures, high temperatures can induce dormancy and suppress germination; a process called thermo-dormancy or thermoinhibition (Negm et al., 1972). This process is thought to rely on maintaining high levels of ABA via an increase in gene expression promoting ABA biosynthesis and suppressing GA synthesis and signalling (Toh et al., 2008). In contrast however, some studies suggest ABA levels are similar in seeds imbibed at different temperatures (Ali-Rachedi et al., 2004), and instead increased sensitivity to ABA may be responsible for dormancy induction at high temperatures (Leymarie et al., 2008).

Although progress has been made in understanding how environmental factors can influence the concentrations and sensitivities of key hormones that regulate seed dormancy (Figure 1.1), the way in which these factors combine to regulate germination timing in the field is not well understood. Summer annuals are generally presumed to lose dormancy during winter chilling, which allows germination in spring; while winter annuals are thought to lose dormancy during dry after-ripening in summer, allowing germination in autumn (Baskin and Baskin, 1985). However, some studies have suggested that prolonged chilling over winter may be more likely to induce dormancy (Donohue et al., 2007; Penfield and Springthorpe, 2012), and in damp temperate climates, after-ripening may not be possible for winter annuals

(Footitt et al., 2011). Maternal effects also add an additional layer of complexity to the subject of dormancy regulation, and the potential impact of altered climates and flowering phenology on seed populations is almost totally unknown.

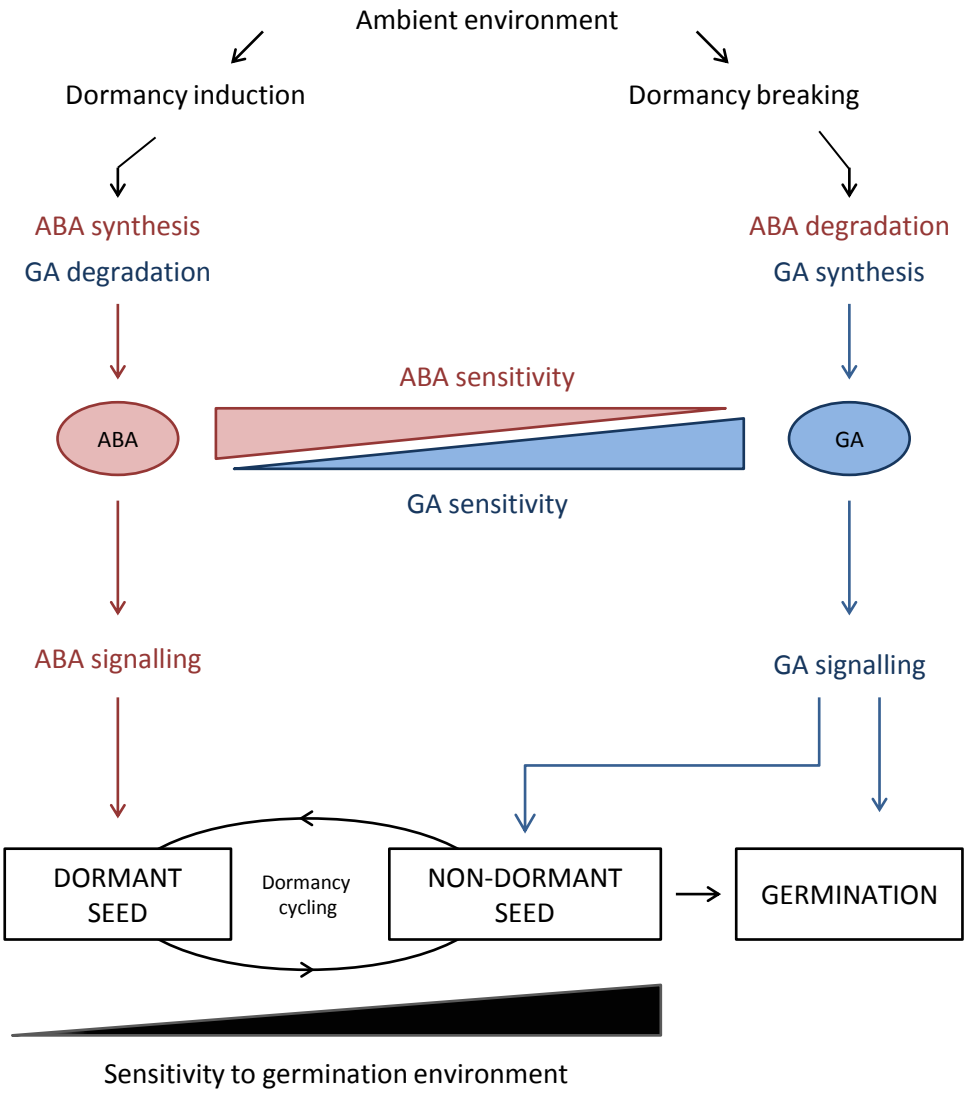

Figure 1.1 Environmental regulation of dormancy and germination by ABA and GA

Environmental factors such as temperature affect the balance of growth regulating phytohormones ABA and GA. Dormancy induction is characterised by an increases in ABA biosynthesis and a simultaneous increase in GA catabolism, while dormancy loss is associated with increased GA biosynthesis and ABA catabolism. Sensitivity to these hormones also changes with dormancy status, and feedback with environmental signals can lead to dormancy cycling. The depth of dormancy also alters sensitivity to environmental signals, and germination occurs when seed germination requirements overlap with ambient conditions. Adapted from Finch-Savage and Leubner-Metzger, 2006.

1.4 Seed Development

Successful seed production is crucial to plant fitness. Following fertilization, seed development in most higher plants can be broadly divided into two phases; morphogenesis and maturation. During morphogenesis, the zygote undergoes a defined pattern of cell division and differentiation. Once the basic plant architecture is established, cell division stops and the maturation phase begins (Raz et al., 2001). This phase involves cell growth and expansion, accumulation of storage proteins and lipids, acquisition of desiccation tolerance, and establishment of primary dormancy. The whole process is completed in approximately 20 days under standard laboratory conditions (Baud et al., 2002).

Seed development is controlled by a network of at least four master regulators; *FUSCA3* (*FUS3*), *ABSCISIC ACID INSENSITIVE3* (*ABI3*), *LEAFY COTYLEDON1* (*LEC1*), and *LEC2* (Raz et al., 2001). Mutations at these loci result in a broad spectrum of seed maturation defects including reduced primary dormancy, fewer seed storage proteins and reduced desiccation tolerance (Wobus and Weber, 1999; Raz et al., 2001; Gutierrez et al., 2007; Holdsworth et al., 2008). LEC1 controls embryo morphogenesis, and ectopic expression is sufficient to induce embryonic development in adult vegetative tissue (Lotan et al., 1998). LEC2 activates the transcription of genes expressed during the maturation phase of seed development, including major dormancy regulator *DOG1* (Braybrook et al., 2006). LEC2, FUS3 and ABI3 also have partially redundant roles, such that constitutive expression of *ABI3* or *FUS3* can rescue most of the *lec2* mutant phenotypes (To et al., 2006). Interactions have also been found amongst these four genes, which together form an interconnected network for the regulation of seed maturation (Kroj et al., 2003; Kagaya et al., 2005; To et al., 2006) (Figure 1.2).

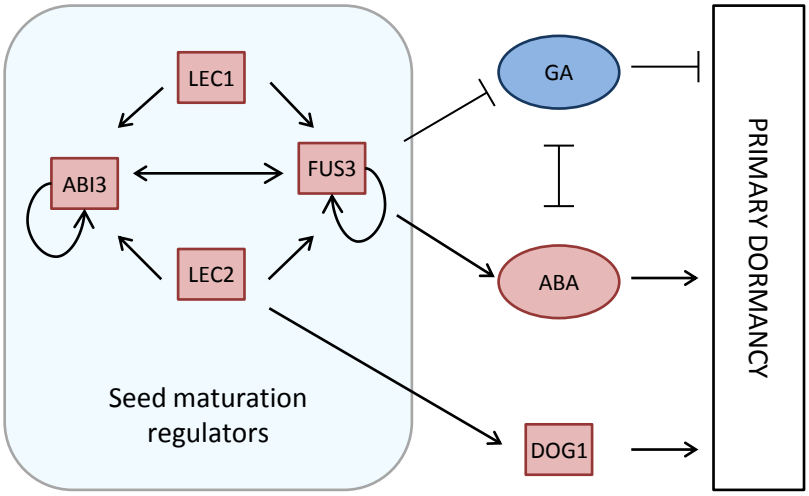

Figure 1.2 Regulation of seed maturation and primary dormancy establishment

Four major regulators of seed maturation form an interconnected network which coordinates seed development. Primary dormancy is induced by the simultaneous promotion of ABA and inhibition of GA. Major dormancy gene *DOG1* also promotes primary dormancy in response to low seed maturation temperatures. Adapted from Holdsworth et al., 2008.

1.5 Regulation of Flowering

The transition from vegetative growth to flowering is another pivotal event in plant development, and has been the subject of a large volume of research over the past century. From an evolutionary perspective flowering is critical to successful reproduction, and in order to ensure success the environment must be suitable for the production of seeds. The seasonal timing of flowering is therefore essential and is subject to regulation by a complex network of genetic pathways, which are sensitive to a variety of environmental signals. Two major seasonal cues are photoperiod and temperature, however as many as five genetic pathways regulating flowering in *Arabidopsis* have been described (Srikanth and Schmid, 2011) (Figure 1.3).

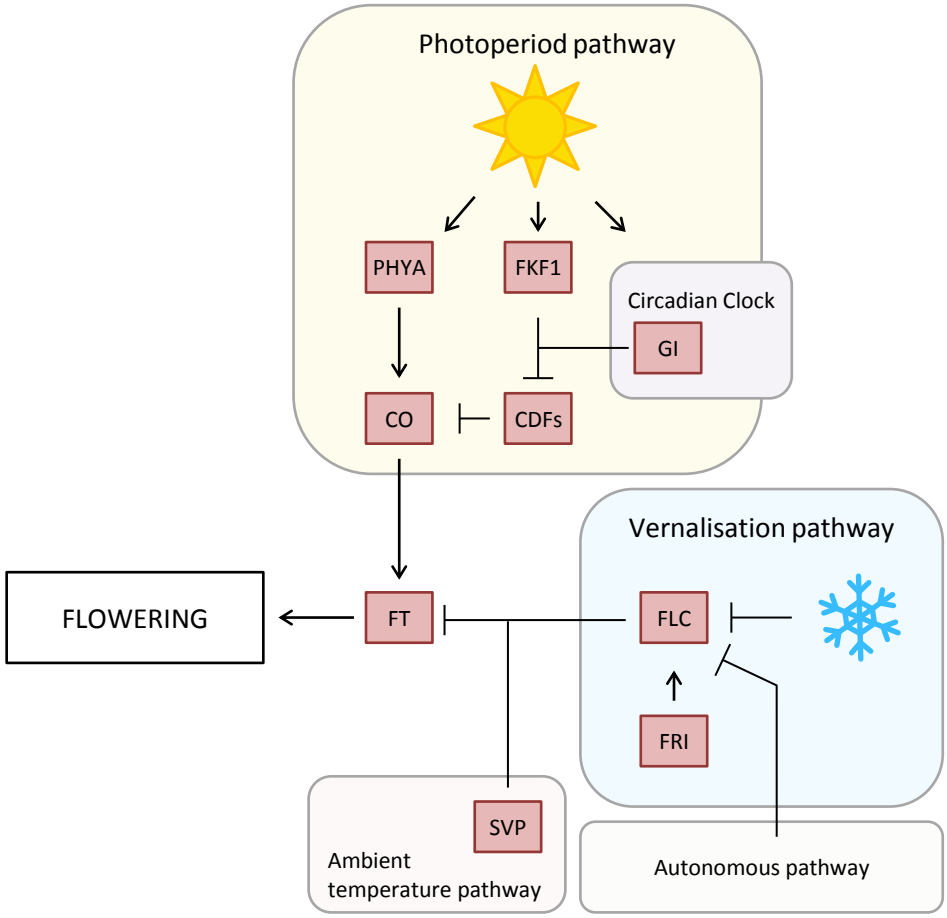

Figure 1.3 Genetic pathways involved in the regulation of flowering in *A. thaliana*

Several environmentally controlled pathways converge to regulate the major floral integrator FT. Light is perceived by photoreceptors in leaves, such as PHYA, which promote the stability of CO in long days. Circadian clock protein GI interacts with the photoreceptor FKF1 to promote the degradation of CO-repressing CDFs. The CO protein is therefore stabilised in long days, leading to transcriptional activation of FT. Repression of flowering is also mediated by FLC, which together with FRI confer a vernalisation requirement. This repression is removed by chilling via the vernalisation pathway. The autonomous pathway also promotes flowering via suppression of FLC. Ambient temperatures affect expression of an additional flowering repressor, SVP, which combines with FLC to inhibit FT.

1.5.1 Regulation of flowering by photoperiod

Early studies identified that flowering in many species was regulated by day length (Garner and Allard, 1920). Plant species were subsequently classified as long-day, short-day, or day-neutral depending on the day length that promoted flowering. *Arabidopsis thaliana* is a long-day plant, and the study of flowering time mutants led to the identification of several genes involved in the photoperiodic control of

flowering. In particular, the genes *GIGANTEA (GI), CONSTANS (CO)* and *FLOWERING LOCUS T (FT)* were found to have major roles in this pathway (Putterill et al., 1995; Fowler et al., 1999b; Kardailsky et al., 1999). *FT* encodes a transcription factor that induces flowering, and acts as a long distance signal between leaves and the shoot apical meristem (Jaeger and Wigge, 2007). Activation of *FT* expression in leaves requires CO, a zinc finger transcription factor which is under control by the circadian clock (Suarez-Lopez et al., 2001).

Transcription of *CO* mRNA is promoted by an interaction between GI and a light sensitive ubiquitin ligase called FLAVIN KELCH F BOX 1 (FKF1) (Sawa et al., 2007). In long days, this interaction results in the degradation of CO repressors known as CYCLING DOF FACTORS (CDFs) (Imaizumi et al., 2005). In addition, CO protein is stabilised at the end of a long-day photoperiod, but is otherwise quickly degraded by the proteasome (Valverde et al., 2004; Laubinger et al., 2006; Liu et al., 2008). As a result *CO* mRNA is more abundant in long days, leading to CO protein accumulation, and initiation of flowering via *FT* activation.

1.5.2 Regulation of flowering by temperature

Many plant species require a prolonged cold treatment (vernalisation) before flowering can occur. In winter annual ecotypes of *Arabidopsis, FRIGIDA (FRI)* promotes the expression of the flowering repressor *FLC* (Michaels and Amasino, 1999; Geraldo et al., 2009). FLC binds directly to FT and other flowering promoters to suppress their action (Helliwell et al., 2006), however the *FLC* gene is silenced in response to chilling (Gendall et al., 2001). The effect of prolonged chilling is

therefore to remove the *FLC* repression of *FT* which would otherwise inhibit flowering.

Much of the flowering time variation observed between natural ecotypes of *Arabidopsis* has been attributed to variation at the *FLC* and *FRI* loci (Koornneef et al., 1994; Gazzani et al., 2003; Stinchcombe et al., 2004; Shindo et al., 2005; Stinchcombe et al., 2005a). Mutations resulting in non-functional *FRI* alleles have arisen at least twice during the evolution of *Arabidopsis* (Johanson et al., 2000). This is thought to confer an early flowering phenotype in the absence of vernalisation, therefore allowing some ecotypes to behave as summer annuals.

In addition to the acceleration of flowering by chilling, flowering can also be accelerated by elevated temperatures. This effect is partly due to an acceleration of growth at warmer temperatures, however *Arabidopsis* also flowers with fewer leaves compared to plants grown at cooler temperatures (Samach and Wigge, 2005; Balasubramanian et al., 2006). Suppression of growth rates in response to low temperatures during the day has also been linked to a transcription factor called SPATULA (SPT; Sidaway-Lee et al., 2010). The so called autonomous pathway is also known to play a role in ambient temperature sensing (Blázquez et al., 2003), together with another major flowering repressor; SHORT VEGEATIVAE PHASE (SVP; Lee et al., 2007).

1.5.3 Modelling flowering time

Understanding the nature of flowering and predicting when it will occur is of great agronomic importance. Modelling this process provides a potentially useful tool to assess the effectiveness of various management strategies such as irrigation regimes,

planting dates and crop varieties without the need for costly field trials. It is also becoming more important to predict the effects of climate change on crops and devise strategies to avoid disruption of global food supplies. Consequently, there are many examples of models which attempt to predict flowering phenology in annual crops (e.g. Summerfield et al., 1991).

Typically these models measure plant development in growth units which accumulate depending on environmental conditions. Thermal time models for example use cumulative daily temperatures as an approximation for physiological age, and models such as these are commonly used to predict phenological events such as seedling emergence, leaf initiation and flowering. The assumption is that plants at the same developmental stage will have accumulated the same number of units, regardless of their individual histories. Plants should therefore always reach flowering stage after accumulating a specific thermal time total. These thresholds are easily estimated from empirical data, making these models a convenient method of predicting plant growth in variable environments.

Thermal time models can also be modified to include the effects of various other environmental factors. For example, hydrothermal time has been used to model the effects of temperature and water potential on germination rates (Bradford, 2002), and photothermal models accumulate thermal units according to photoperiod (Nuttonson, 1955). The effects of vernalisation have been incorporated into a photothermal model of flowering in *Arabidopsis* (Wilczek et al., 2009). This particular model was also able to predict the behaviour of several photoperiod and vernalisation pathway mutants, and showed that expression of life history was sensitive to germination timing.

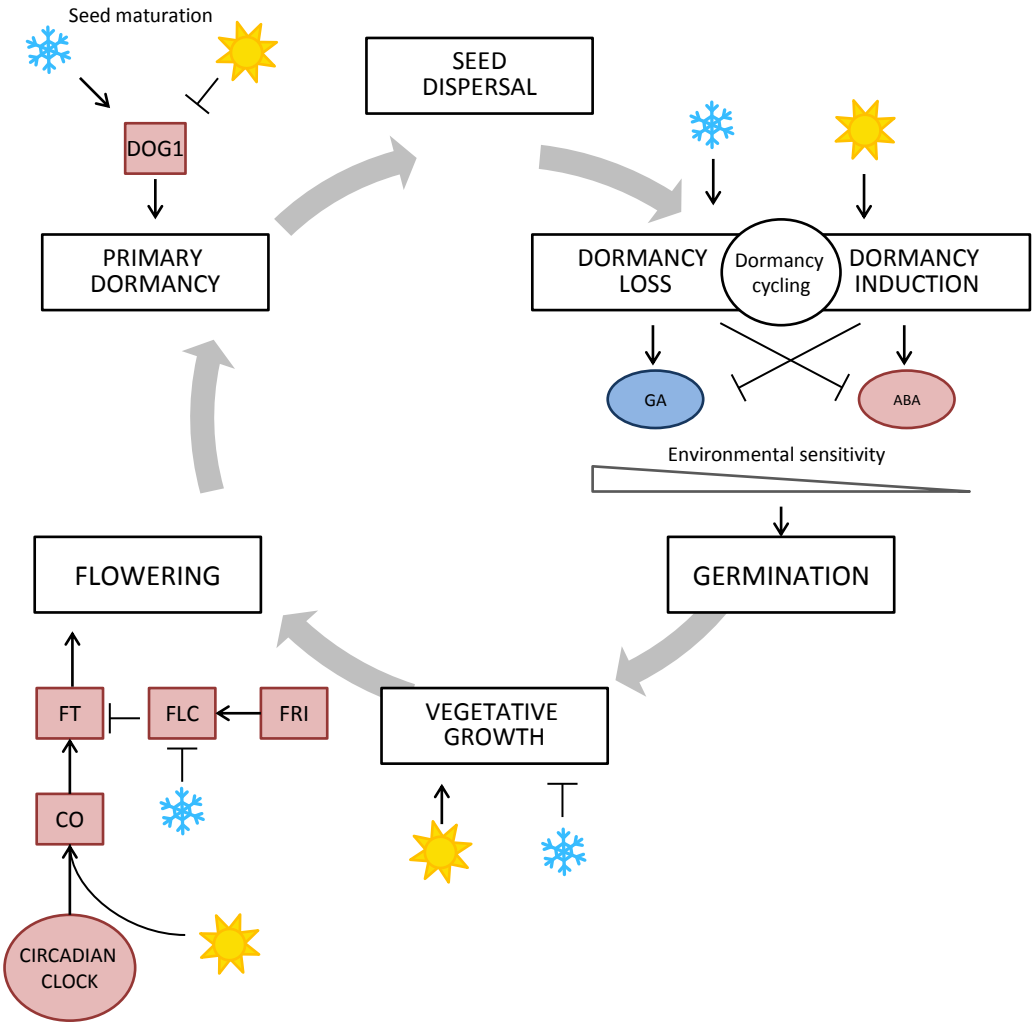

Figure 1.4 Environmental regulation of major life cycle transitions in *A. thaliana*

Ambient temperature during vegetative growth influences growth rates to determine how quickly seedlings reach adulthood. Chilling and long days accelerate flowering through the vernalisation and photoperiod pathways, which converge on the major flowering integrator gene *FT* (additional regulation by the autonomous and ambient temperature pathways are not shown). During seed development, temperature regulates levels of primary dormancy via *DOG1* expression levels. After dispersal, dormancy loss or induction is determined by environmental factors influencing the balance of GA and ABA levels, which may result in repeated cycling of dormancy states. Loss of dormancy is also associated with an increase in sensitivity to germination cues such as light, leading to germination when ambient conditions overlap with germination requirements.

1.6 Summary and Aims

In summary, all stages of plant development, including major transitions such as flowering and germination, are sensitive to environmental cues (Figure 1.4). Flowering is largely dependent on temperature and photoperiod, while seed dormancy and the ability to germinate in favourable conditions is mainly regulated by temperature. Flowering time also exerts influence on seed dormancy by dictating the seed maturation and post dispersal environment, while germination timing dictates the conditions that will be experienced during vegetative growth, thereby influencing flowering time. The timing and environmental regulation of all developmental stages of plant growth are therefore intrinsically linked.

A few studies have attempted to address the effects of germination timing on flowering phenology (Donohue et al., 2005a; Galloway and Burgess, 2009; Wilczek et al., 2009), and researchers are also now beginning to appreciate the importance of reproductive timing on seed dormancy through maternal effects and the post-dispersal environment (Donohue et al., 2005b; Donohue, 2009). However, no study has yet attempted to understand the significance of these links in terms of whole life cycle phenology.

This study therefore aimed to reveal key features of *Arabidopsis* phenology by combining flowering, seed dispersal, and seed dormancy behaviours into a single, multi-stage life history model. A thermal time model of seed development was formulated to enable predictions of seed dispersal timing and maturation temperatures, and a model of seed dormancy was developed to predict germination responses to maturation and post-dispersal temperatures. The models were then combined to provide a framework for investigating environmental regulation of whole life cycle phenology. This model was then used to address questions including

why plants couple primary dormancy with temperature, and what is the significance of flowering at particular times. The effects of perturbing the system by altering germination or flowering time were also investigated with the aim of assessing the wider effects of particular loci governing these transitions. Effects of altered climates and varying locations were also investigated to understand the role and ecological significance of phenotypic plasticity.

Chapter 2: Materials and Methods

2.1 Plant Growth Media

2.1.1 Water agar medium

0.9% water agar was prepared using 9g agarose powder **(**A1296, Sigma) per litre of dH_20. Media was autoclaved and poured in a sterile flow hood.

2.1.2 Murashige and Skoog medium

Half-strength Murashige and Skoog (MS) (Duchefa Biochemie) medium was prepared in dH_2O, and the pH adjusted to 5.7-5.8 using KOH. Agarose powder was added (9g per 1L) and the medium was autoclaved before pouring.

2.2 Plant Material

Wild type accessions used were Columbia (Col-0) and Landsberg *erecta* (L*er*). *CCA1*-ox (Col background) was described previously by Wang and Tobin (1998). *ft-1*(L*er* background) described previously by Koornneef et al., (1991) and was obtained from the Nottingham Arabidopsis Stock Centre (NASC).

2.3 Seed Preparation

Each seed batch was sieved through a 250µm mesh (Fisher Scientific) to exclude poorly filled seeds. Ethanol bleach solution was made by dissolving 6 Klorsept

tablets in 100ml dH_20, and then diluting the required amount by 1:20 with 100% ethanol. Surface sterilisation of seeds was achieved by first washing with 100% ethanol, allowing to stand for 10 minutes in ethanol bleach solution, and then washing twice more with 100% ethanol. Any remaining ethanol was removed by allowing seeds to air dry in a sterile flow hood before use.

2.4 Stratification Treatments

2.4.1 For general plant growth

To promote uniform germination, sterilised seeds were placed on MS agar and stratified in darkness at 4°C for 2-3 days in a Sanyo MIR154, before transferring to the required germination conditions.

2.4.2 For dormancy experiments

To investigate seed dormancy, stratification treatments at different temperatures and of varying lengths were given prior to germination. Sterilised seeds (5 replicates of 25-50 seeds) were placed on 0.9% water agar plates which were promptly wrapped in foil to exclude light. Stratification treatments were carried out in growth cabinets operating at the required temperature. If a change in temperature was required, plates were transferred to a second cabinet operating at the required temperature, without removing foil. After the required total incubation time, foil was removed and plates were transferred to germination conditions. Occasionally, seeds germinated in darkness during stratification. These were counted before incubation in light, and dark germination was expressed as a percentage of the total number of seeds.

2.4.3 For circadian experiments

Sterilised seeds were pre treated in darkness at either 8°C, 12°C or 16°C, as described above. The aim was to determine whether any circadian rhythm could be detected in the dormancy of seeds incubating in darkness at constant temperature. Dormancy was therefore sampled after regularly spaced intervals (every 3 hours) by transferring a single plate from each treatment to germination conditions.

To ensure sampling could be carried out during normal working hours, the experiment was split into two parallel runs. Transcription of circadian clock genes is arrested in dry seeds, however the clock quickly restarts upon imbibition (Penfield and Hall, 2009). The parallel runs were therefore synchronised in anti-phase by a 12 hour difference in imbibition start time. An example of the sampling and imbibition times of both parallel runs is provided in Table 2.1.

Table 2.1 Example of imbibition and sampling times for circadian experiments

	Sampling time	Time since imbibition	
		Run 1	Run 2
Day 1	9.00 am	0 h	
	12.00 pm	3 h	
	3.00 pm	6 h	
	6.00 pm	9 h	
	9.00 pm		0 h
Day 2	9.00 am	24 h	12 h
	12.00 pm	27 h	15 h
	3.00 pm	30 h	18 h
	6.00 pm	33 h	21 h
Day 3	9.00 am	48 h	36 h
	⋮	⋮	⋮

2.5 Germination

All germination was carried out in a Sanyo MLR350 growth cabinet operating at 22°C in a long-day photoperiod regime (16h light, 8 hours dark), and light levels set at 70-80µmol m^2 s^{-1}. An exception was made when germinating seeds for growth rate experiments, which were transferred to the required growth conditions immediately after a 2-3 day cold stratification. Seeds were incubated for 10 days before transplanting into John Innes seed compost (Levington) in P40 trays.

For dormancy assays, numbers of germinated and ungerminated seeds were counted after 7 days. Germination was scored if the radicle could be seen protruding through the seed coat. Non-dormant seeds that germinated during stratification in darkness were easily identified by elongated hypocotyls, and were excluded from these counts. This was because the purpose of these assays was to observe changes in dormancy caused by the stratification pre-treatments. By germinating in darkness, non-dormant seeds were in effect removed from the incubating seed population, and thus prevented from undergoing further changes in dormancy. Including non-dormant seeds in germination counts would therefore obscure any changes in dormancy occurring in the remaining population, which would only be revealed by testing their germination in light. The number of seeds germinating in light was therefore expressed as a percentage of the total number of seeds that had not germinated during stratification.

2.6 Plant Growth

2.6.1 For growth rate experiments

To assess the effect of temperature on growth rates plants were grown at 8°C, 12°C, 15°C, 18°C, 20°C and 25°C in long days (16h light, 8h dark). To assess the effect of photoperiod on growth rates, plants were grown in 8, 10, 12, 14, and 16 hours light per 24 hour cycle, at 20°C. The required conditions were achieved using Sanyo MLR growth cabinets with light levels set to 70-80µmol m^2 s^{-1}, except in the case of short day conditions (8h light, 16h dark) where plants were grown in a growth room. Plants were checked regularly and watered to prevent the soil from drying out. Bolting date and the start of seed set (first mature seed) were recorded for each individual plant.

2.6.2 For production of seeds

Plants were grown in standard long-day conditions (16h light, 8h dark) at 20°C until bolting, at which point they were transferred to a cabinet operating at the required seed maturation temperature, with a long-day photoperiod. Where an alternating diurnal temperature regime was required, cabinets were set to provide the correct temperature during light and dark phases, which were each 12 hours in length. Seeds were harvested from plants when approximately 50% of siliques had dehisced.

To investigate the effect of a sustained change in temperature during seed maturation, all mature seeds were harvested before transferring plants and remaining immature seeds to a second cabinet operating at the required temperature, and long-day photoperiod. Mature seeds from subsequent harvests were pooled from 5 parent plants, and subjected to stratification and germination tests. In all cases, cabinets

were set to provide 70-80 µmol m^2 s^{-1}, and when required for dormancy experiments, freshly harvested seeds were used within 24 hours.

2.7 Field Experiments

2.7.1 Seed set timing

All field experiments were conducted in a walled garden within the University of York campus grounds. Seedlings for field experiments were germinated in growth cabinets and transplanted into seed compost as described above. Before transferring to the field site, seedlings were incubated at either 15°C or 8°C for 1 week to allow them to acclimatise to temperatures appropriate to the season, and avoid mortality due to transplant shock. Upon transfer to the field site, P40 trays were placed in outer trays with drainage holes, and stood on the soil surface. To compare growth rates at different times of year, fresh trays of seedlings were transferred to the field site at regular intervals between October 2011 and February 2014.

March 2012 was unusually dry, and plants were provided with a small amount of additional water. Slug pellets were applied where necessary, and fences around the perimeter excluded vertebrate herbivores. Plants were checked regularly, and dates for bolting and the start of seed set were recorded for each individual plant.

2.7.2 Seed collection for dormancy experiments

The dormancy of seeds matured at different times of year was investigated by harvesting seeds from plants grown at the field site, and subjecting them to stratification and germination tests. Due to natural dispersal, only a small number of

seeds could be collected from each parent plant. Seeds from parents belonging to the same batch were therefore pooled. Details of harvest dates and numbers of plants pooled from each batch are provided in Table 2.2. Stratification and germination was carried out as described above.

Table 2.2 Details of harvest dates, and parent plants harvested from field batches

Batch	Harvest date	No. of plants
A	30/05/12	6
B	30/05/12	6
C	30/05/12	20
F	1/08/12	16
G	21/8/12	14

2.7.3 Seedling emergence

Emergence timing of field and laboratory grown seeds was investigated following a method described previously by Footitt et al. (2011). Briefly, two pots were filled with sterile soil up to 3cm from the top. The soil was covered with 125µm nylon mesh to prevent seeds from becoming buried too deeply. Seeds were sown evenly over the mesh and covered to a depth of 2cm with sterile soil. Pots were then placed in holes dug at the field site to ensure soil surfaces were level. At regular intervals the soil within the pots was disturbed to expose buried seeds to light. Subsequent seedling emergence was recorded, and germinated seedlings were removed. The number of seedlings that emerged after each disturbance was expressed as a percentage of the total emergence by the end of the experiment.

Field grown seeds consisted of approximately 500 seeds in total, pooled from batches A, B and C which were harvested from the field site on 30th May 2012 (see

Table 2.2). Seeds had been stored at room temperature in sealed tubes for 2 weeks prior to the start date. It is reasonable to expect seeds dispersed naturally at this time would experience a period of dry after-ripening of this length before burial.

Lab grown seeds consisted of Col-0 seeds matured at 18°C in growth cabinets, as described previously. Approximately 500 seeds were obtained by pooling seeds from 5 parent plants, which had been stored in sealed tubes at room temperature for 6 weeks. The relatively warm seed maturation temperature, and longer period of dry after-ripening was intended to confer reduced dormancy, and enable a comparison between the emergence timing of field matured seeds.

2.8 Data Sources

2.8.1 Temperature loggers

Ground level temperature was measured at the field site using LogTag TRIX-8 data loggers, contained in protective enclosures (LogTag) to protect against water damage. Readings were taken every 10 minutes, and data was downloaded at monthly intervals.

2.8.2 Temperature and photoperiod data for model simulations

Temperature data consisting of daily mean, minimum and maximum temperatures spanning a period of at least 10 years (where available) was downloaded from weather station archives. For each calendar date, these values were averaged over the 10 year period to create an average annual cycle of mean, minimum and maximum daily temperatures. These were converted to estimates of hourly temperature by

assuming minimum daily temperatures between the hours of 21:00-02:00, maximum temperatures between 09:00-14:00 and mean temperature for the remaining hours.

Times of sunrise and sunset were also downloaded, and combined with temperature estimates to create the basis for photothermal and dormancy model simulations. Online sources for temperature and photoperiod data are provided in Table 2.3.

Table 2.3 Details of temperature and photoperiod data used for model simulations

Location of Weather station	Date range of temperature data	Location and year of sunrise and sunset data
Canada, Vancouver	2002-2011[a]	Vancouver, 2011 [c]
Cape Verde, Sal	2002-2011 [a]	Cape Verde, Praia, 20011 [c]
Finland, Oulu	2002-2011 [a]	Oulu, 2011 [c]
France, Dijon	2002-2011 [a]	Dijon, 2011 [c]
France, Nantes	2002-2011 [a]	Nantes, 2011 [c]
Germany, Landsberg	2002-2011 [a]	Landsberg, 2011 [d]
Italy, Catania	2002-2011 [a]	Catania, 2011 [c]
Italy, Naples	1995-2004 [a]	Naples, 2011 [c]
Lithuania, Vilnius	2002-2011 [a]	Vilnius,2011 [c]
Russia, Moscow	2002-2011 [a]	Moscow, 2011 [c]
Gran Canaria, Las Palmas	2002-2011 [a]	Las Palmas, 2011 [c]
Spain, Valencia	2002-2011 [a]	Valencia, 2011 [c]
UK, Edinburgh	2003-2011 [a]	Edinburgh, 2011 [c]
UK, York	1999-2011 [b]	York, 2011 [c]
USA, Colombia (Missouri)	2002-2011 [a]	Kansas City (Missouri), 2011 [c]

Data sources:
[a]. http://www.geodata.us/weather
[b]. http://weather.elec.york.ac.uk/archive.html
[c]. http://www.timeanddate.com/worldclock/sunrise.html
[d]. http://uk.weather.com/climate/sunRiseSunSet-Landsberg/Lech-GMXX1210

Chapter 3: Modelling Seed Dispersal Timing

3.1 Introduction

The timing of key developmental transitions can affect the expression of subsequent traits (Donohue, 2002). Seed development and dispersal timing are good examples of this. The season, and hence temperatures experienced while on the mother plant determine the level of primary dormancy in mature seeds, and the conditions after dispersal determine whether dormancy is lost, or if secondary dormancy is induced. The seasonal timing of seed dispersal therefore has a big effect on the germination timing of the next generation, which has already been shown to have a large impact on life history and fitness (Donohue et al., 2005c; Huang et al., 2010). Knowing when seed maturation and dispersal naturally occur will therefore be a key part of understanding the link between reproductive phenology and the control of seed dormancy by temperature.

The timing of seed set depends on flowering time. A model to predict bolting, the first visible sign of the floral transition, in *Arabidopsis* was developed by Wilczek et al. (2009). They used the concept of thermal time to measure the progression towards bolting in environmentally determined units. The model employs a photoperiod factor which modifies the rate of thermal unit accumulation according to day length, and facilitates accelerated flowering in long days. A vernalisation factor also reduces the rate of accumulation until plants are fully vernalised by chilling temperatures, thereby mimicking the action of FLC.

The work in this chapter was carried out with the aim of producing a model of seed development to extend the bolting model described by Wilczek et al. (2009). Three different models were developed and parameterised with laboratory data, and their performance was also assessed using field experiments. Simulations were then used to predict the timing of seed dispersal, the range of temperatures likely to be experienced during seed development, and also how these would be affected by altered germination or flowering time.

3.2 Results

3.2.1 The effect of temperature and photoperiod on seed maturation rates

In order to develop a model capable of predicting the timing of seed set in natural environments, developmental rates of seeds grown under controlled laboratory conditions were observed. Plants were grown in cabinets under a series of photoperiod and temperature regimes. The time in days from bolting to the production of the first fully matured seeds were recorded for each plant. The reciprocals of these measurements (1/days) were calculated to provide a measure of growth rates in each environment. These growth rates could also be interpreted as the daily fractional progression towards the production of mature seeds.

The effects of temperature and photoperiod on growth rates for Columbia (Col-0) and Landsberg erecta (L*er*) ecotypes are shown in Figure 3.1 and Figure 3.2 respectively. These results show a clear positive linear trend between growth rate and temperature, which is maintained across the full range of temperatures tested. The relationship between growth rates and photoperiod is also positive, but only up to 12h and 10h days for Col-0 and L*er* respectively. In days longer than these

thresholds growth rates reached a maximum, suggesting that short days have a rate limiting effect while in long days the limiting factor could be temperature. This was also supported by the increase in growth rate observed when plants were grown at 25°C compared to 20°C in 16h days (Figure 3.1). The proportional relationship between growth rate and temperature indicates a thermal time approach would be appropriate to model this process (Granier et al., 2002), and would also be applicable in a wide range of environments.

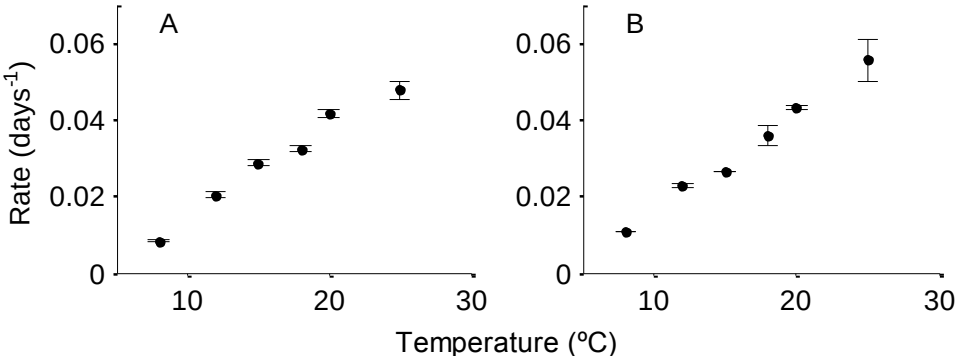

Figure 3.1 The effect of temperature on rates of seed development

Col-0 (A) and L*er* (B) plants were grown under long days (16h light, 8h dark) at constant temperature as indicated. Data points represent the mean and SE of at least 4 replicate seed batches per treatment.

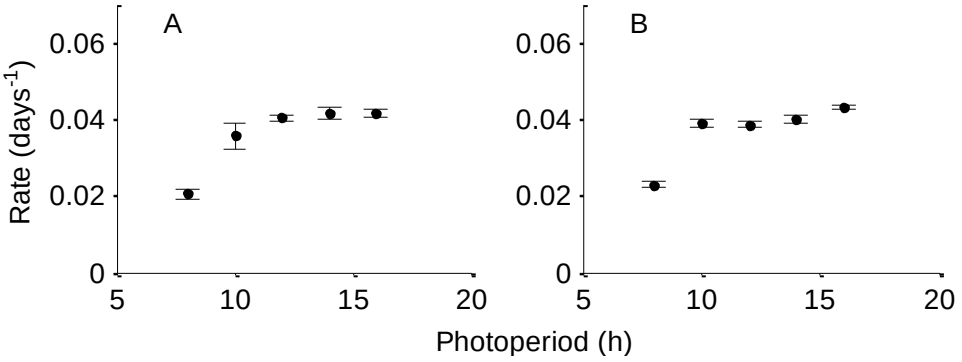

Figure 3.2 The effect of photoperiod on rates of seed development

Col-0 (A) and L*er* (B) plants were grown at constant 20ºC under different photoperiods by varying the number of daylight hours per day as shown. Data points represent the mean and SE of at least 4 replicate seed batches per treatment.

3.2.2 Model development

Thermal time models have a long history of being used to simulate plant growth and provide a way of predicting the timing of developmental transitions. Recent examples include flowering (Wilczek et al., 2009), germination (Alvarado and Bradford, 2002), and leaf emergence (Granier et al., 2002). The basic premise of a thermal time model is to integrate temperature information over time using a function such as the one below.

[1] $$T_h = \int_a^b f(x)\, dx$$

The function f describes the development in terms of the temperature at time x, which is then integrated from the point of bolting a, up to the point of seed maturity, b. This permits the calculation of T_h, a constant relating to the total thermal time required to achieve a switch in development, in this case the production of mature seeds.

In variable environments it is necessary to approximate this integral with a summation of the function over discrete time intervals, typically of one hour. The summation function becomes as follows:

[2] $$T_h = \sum_{t=1}^{n} \theta(t)$$

where $\theta(t)$ is a function to calculate the thermal time accumulated during the time interval t, and T_h is the cumulative total between bolting at t = 1, and seed maturity at $t = n$. If T_h is known in advance, this concept can be used to determine the value of n, and hence the time in hours required to produce mature seeds in any particular environment.

Most thermal time models assume that developmental rates are zero below a critical minimum temperature (T_b), and linearly proportional to temperature above this threshold. The thermal component is therefore calculated as the difference between T_b and the average temperature during the time interval t, or $T(t)$. This basic concept is used in each of the three models described below, however it was unclear from lab experiments what the most appropriate way to model seed maturation rates with photoperiod would be. Therefore, three slightly different approaches were tested.

Model 1: Photothermal time model

One simple way of incorporating the effects of photoperiod on seed growth rates was to only allow temperature during daylight hours to contribute to the thermal time total. At constant temperatures, this would create a linear relationship between the daily growth rate and the day length. This was in contrast to lab data, which showed

the growth rate reached a maximum in long days. However, it was anticipated that in natural environments long photoperiods would be associated with elevated summer temperatures. Therefore, temperature would not be limiting and growth rates in long days would continue to have a linear relationship with photoperiod. A similar method of using only day time temperatures was also used by Wilczek et al. (2009), who found that including temperatures at night led to a reduction in the model performance (Figure. S11 in Wilczek et al., 2009).

[3] $$\theta(t) = \begin{cases} (T(t) - T_b) \times L(t), T(t) \geq T_b \\ 0, T(t) < T_b \end{cases}$$

Equation [3] was used to quantify hourly thermal unit accumulation. To ensure only day time temperatures were used in the calculation of thermal time, $L(t)$ was used as a measure of daylight for the hour t. The value of $L(t)$ can range between 0 and 1, and represents the fraction of t (in hours) that occurs during daylight.

Model 2: Thermal time model

As a test of the assumptions of the first model, a second model was included that incorporated both day and night time temperatures into the thermal time calculations. As such, photoperiod information was ignored meaning that at constant temperature the growth rate with increasing photoperiod would be constant. This was similar to the behaviour of lab grown plants in long days, where increasing photoperiod had no effect on the growth rate. Equation [4] describes thermal time unit accumulation for this model.

[4] $$\theta(t) = \begin{cases} (T(t) - T_b), T(t) \geq T_b \\ 0, T(t) < T_b \end{cases}$$

Model 3: Modified photothermal time model

Data from laboratory experiments suggested that an improved fit might be achieved by applying a growth rate modifier to decelerate growth in short days, and limit growth to a maximum rate in long days. Justification for this approach also came from Wilczek et al. (2009), in which short- and long-day photoperiod thresholds were used to determine the rate of progression towards flowering. In the present model, the daylight component $L(t)$ employed in model 1 was replaced by a modifier $M(t)$ as shown in equation [5].

[5] $$\theta(t) = \begin{cases} (T(t) - T_b) \times M(t), T(t) \geq T_b \\ 0, T(t) < T_b \end{cases}$$

The value of the modifier was calculated using photoperiod thresholds $pmax$, and $pmin$ as follows:

[6] $$M(t) = \begin{cases} 1, & P(t) \geq P_{max} \\ \dfrac{P(t) - P_{min}}{P_{max} - P_{min}}, & P_{max} > P(t) > P_{min} \\ 0, & P(t) \leq P_{min} \end{cases}$$

where $P(t)$ is the photoperiod (number of daylight hours) of the day containing hour t, P_{max} is the critical long-day photoperiod, and P_{min} is the photoperiod at which the growth rate theoretically reaches zero. An illustration of the way in which the growth rate responds to photoperiod in each model is provided in Figure 3.3.

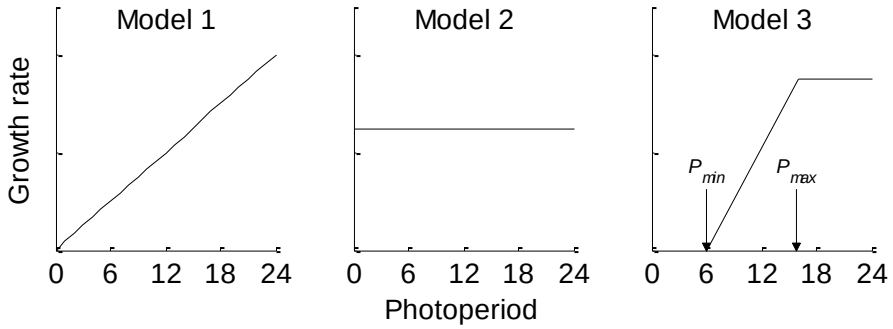

Figure 3.3 Hypothesised relationships between photoperiod and rates of seed development

Model 1; Growth rates increase proportionally with photoperiod. Model 2; Photoperiod has no effect on the growth rate. Model 3; Photoperiod thresholds are used to modify the growth rate between a maximum at P_{max} and a minimum at P_{min}.

3.2.3 Parameter estimation

Seed development times determined under laboratory conditions were used to estimate parameter values for each model using a non-linear least squares method. This method searches for parameter values which result in the smallest squared difference between observed data and model predictions. This was carried out using the *fit* function in the MATLAB curve fitting toolbox (Mathworks), using a trust region algorithm. Optimised parameter values estimated for both Col-0 and L*er* ecotypes are listed in Table 3.1.

In accordance with previous studies modelling plant growth responses (e.g. Batlla et al., 2009; Gualano and Benech-Arnold, 2009; Wilczek et al., 2009; Watt et al., 2011) the coefficient of determination (R^2) was used to evaluate model performance. This was calculated using equation [7], where obs represents the observed data and $pred$ represents values predicted by the model. The value of R^2 can also be interpreted as the fraction of the variance in the observed data that is explained by the model.

[7] $$R^2 = 1 - \frac{\sum(obs - pred)^2}{\sum(obs - \overline{obs})^2}$$

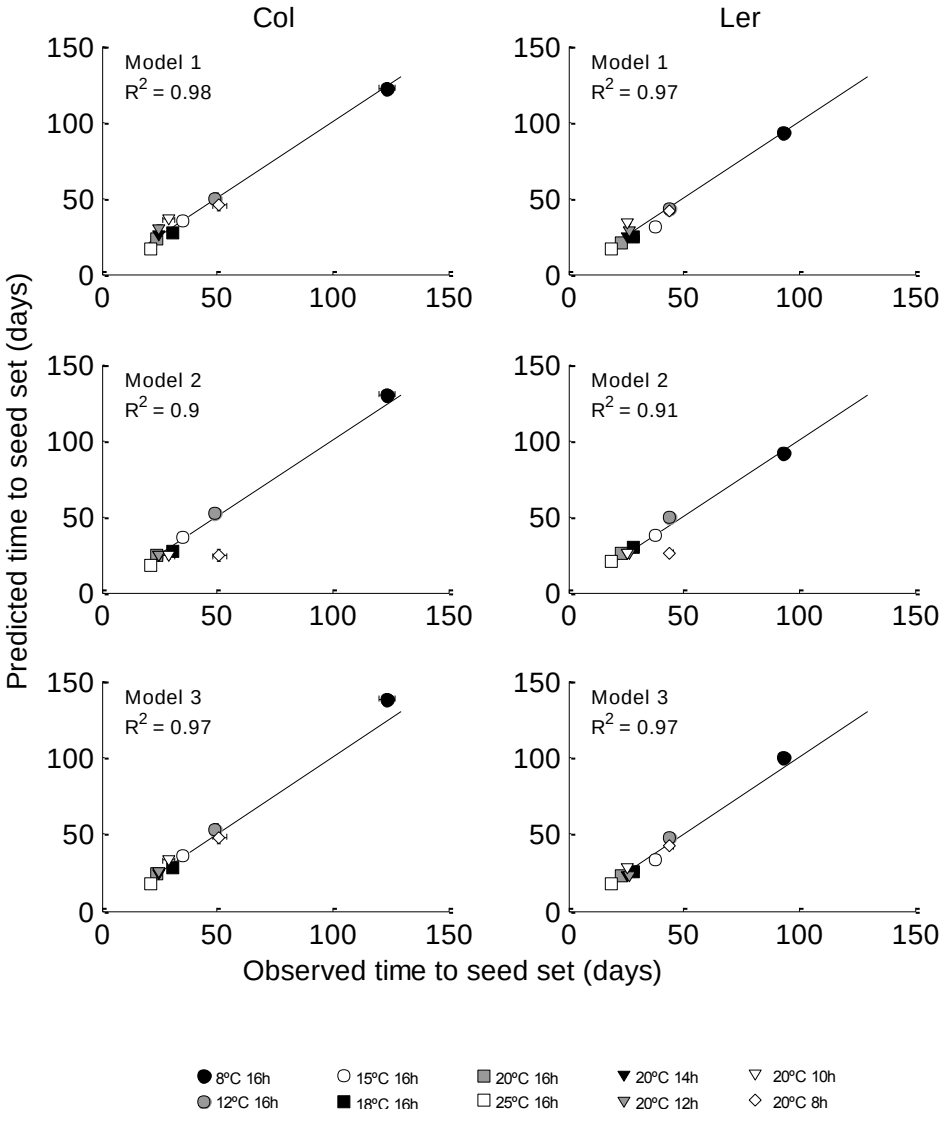

Figure 3.4 Observed and predicted time from bolting to seed set in laboratory conditions

Observed times to seed set from bolting in 10 different environments were used to train each model. The solid line indicates the position of points for a perfect model, and the fraction of the variance explained by each model is indicated by R^2 values Data points represent the mean and SE of at least 4 replicate seed batches per treatment.

Figure 3.4 shows seed maturation times predicted by each of the three seed set models plotted against observed values, and their respective R^2 scores. Since this data was used to train the models, they were expected to reproduce the data with good accuracy. This is true in all cases, and there is little to distinguish between them in terms of their performance. Models 1 and 3 perform marginally better, which was mainly due to model 2 underestimating seed maturation times in 8h photoperiods. However, it was presumed that this would not be relevant in predicting seed set times in natural environments since seeds are rarely produced during short days in winter. Furthermore, when the 8h data point was excluded from the analysis, the performance of model 2 was equivalent to the others. Therefore, in order to assess the ability of each model to predict seed set times in a natural environments, further validation of the models was carried out using data collected from field experiments.

Table 3.1 Fitted parameter values for three models of seed set timing

	Parameter symbol	Description	Value	
			Col-0	L*er*
Model 1	T_b	Base temperature (°C)	5.25	4.55
	T_h	Thermal time threshold (°C hours)	5370	5130
Model 2	T_b	Base temperature (°C)	5.35	3.25
	T_h	Thermal time threshold (°C hours)	8280	10332
Model 3	T_b	Base temperature (°C)	5.50	4.50
	P_{min}	Short day threshold (hours)	3.50	4.00
	P_{max}	Long-day threshold (hours)	12.50	11.50
	T_h	Thermal time threshold (°C hours)	8280	8400

3.2.4 Field experiments

In order to validate the three seed set models, Col-0 and L*er* plants were grown at a field site in York between October 2011 and July 2013. As with lab experiments, bolting and seed set dates were recorded for each individual plant. This data is summarised in Table 3.2 and Table 3.3 for Col-0 and L*er* respectively.

Herbivory occasionally led to plant mortality, particularly for cohorts bolting in late spring, despite precautions to exclude mollusc and vertebrate herbivores. Drought during spring 2012 also affected batches D and E, and a particularly harsh winter with snow late into 2013 also led to high mortality in batches I, J and K. However, in batches where plants survived to produce seeds, a general pattern of decreasing time to seed set could be observed for batches bolting later in the year, causing seed maturation to occur during increasingly warmer conditions. This trend then reversed for batches bolting in late summer, which caused seed maturation to occur during cooler autumn conditions (Figure 3.6).

Table 3.2 Summary of field observations of bolting and seed set timing for Col-0 in York, UK

Col	Transplant date	# bolted	Mean bolting date	SE of bolting date (days)	# reached seed set	Mean time to seed (days)	SE of time to seed (days)	Avg. first seed date
A	17/10/2011	6	11/02/2012	7.49	6	78.33	6.76	30/04/2012
B	08/11/2011	9	23/03/2012	0.78	6	65.33	1.69	28/05/2012
C	23/12/2011	20	06/03/2012	1.96	20	66.70	1.46	12/05/2012
D	09/03/2012	14	11/05/2012	1.42	2	50.50	0.50	01/07/2012
E	13/04/2012	7	24/05/2012	0.00	0	-	-	-
F	30/05/2012	16	20/06/2012	0.31	16	41.88	0.38	01/08/2012
G	28/06/2012	14	14/07/2012	0.73	14	30.14	0.73	14/08/2012
H	23/08/2012	10	17/09/2012	1.08	9	67.56	5.75	23/11/2012
I	25/09/2012	0	-	-	-	-	-	-
J	10/10/2012	0	-	-	-	-	-	-
K	08/10/2013	22	17/4/2013	2.13	8	51.13	4.59	07/06/2013
L	18/02/2013	15	12/05/2013	2.42	14	45	2.21	26/06/2013

Table 3.3 Summary of field observations of bolting and seed set timing for L*er* in York, UK

L*er*	Transplant date	# bolted	Mean bolting date	SE of bolting date (days)	# reached seed set	Mean time to seed (days)	SE of time to seed (days)	Avg. first seed date
A	17/10/2011	0	-	-	-	-	-	-
B	08/11/2011	5	31/01/2012	12.80	5	96.20	7.12	07/05/2012
C	23/12/2011	5	27/02/2012	1.00	5	82.40	3.01	19/05/2012
D	09/03/2012	5	17/05/2012	9.00	0	-	-	-
E	13/04/2012	11	22/05/2012	0.00	1	49.00	0.00	10/07/2012
F	30/05/2012	9	13/06/2012	0.78	9	36.22	0.78	20/07/2012
G	28/06/2012	0	-	-	-	-	-	-
H	23/08/2012	5	14/09/2012	0.00	5	62.80	0.49	15/11/2012
I	25/09/2012	0	-	-	-	-	-	-
J	10/10/2012	0	-	-	-	-	-	-
K	08/10/2013	0	-	-	-	-	-	-
L	18/02/2013	0	-	-	-	-	-	-

3.2.5 Environmental temperature measurement

In order to perform simulations, daily maximum, minimum and mean temperature data were retrieved from a nearby weather station. To calculate hourly thermal unit accumulation, the daily temperature data was used to estimate average hourly temperatures by assuming daily minimum temperatures between the hours of 21:00 and 02:00, daily maximum temperatures between the hours of 09:00 and 14:00, and daily mean temperature during the remaining hours. The weather station was situated approximately 300m from the field site and approximately 21m above ground level. For comparison ground level temperature loggers were also used at the field site from September 2012.

Figure 3.5A shows that the range of temperatures recorded at ground level was often greater than those recorded by the weather station, and this range also increased dramatically during the summer, particularly for daily maximum temperatures. A likely explanation is that heat absorbed by the ground was re-radiated, causing ground level air temperatures to reach as much as 28°C above those recorded by the weather station. Similarly, ground level temperatures just before dawn could be as much as 11ºC cooler than weather station measurements.

Despite occasionally large discrepancies between the measurement methods, these were generally short lived and there was good agreement between daily average temperature estimates (Figure 3.5B). Estimates using the two methods differed by less than 1 degree (0.8°C) on average. There were just two regions where the estimates were noticeably different, one in June and one in July. This was due to exceptionally sunny ‘heat wave’ conditions, leading to sustained elevated ground level temperatures which were sufficient to affect the mean daily temperature. However, it was reasoned that the most relevant periods for plant growth would be

spring and early summer where *Arabidopsis* most commonly flowers and sets seed. These weather extremes were also expected to be infrequent and short lived, and consequently have a minimal impact on simulation results. It was therefore concluded that hourly temperature estimates derived from weather station records would provide an acceptable estimate of temperature, and could be used as the basis for simulations in locations where ground level temperature data was not available.

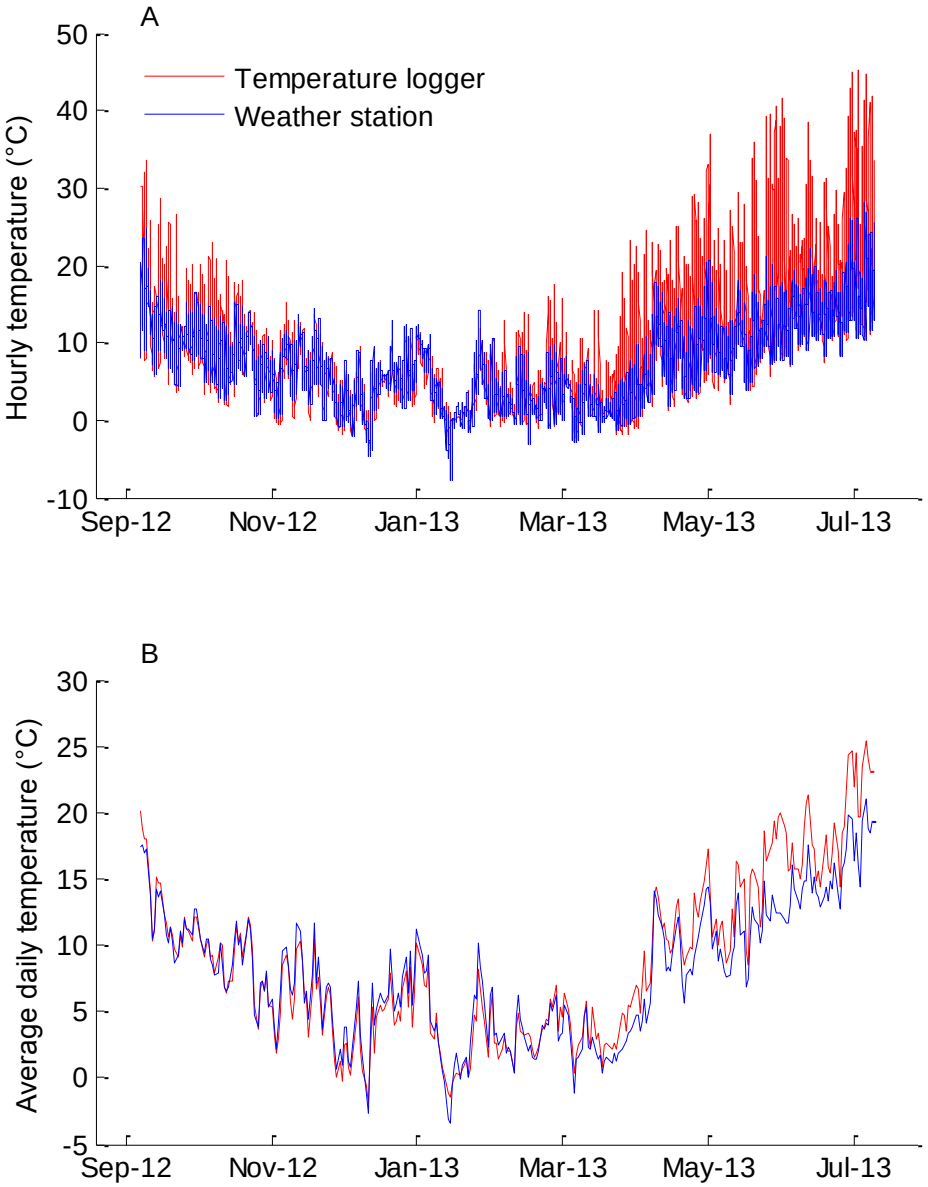

Figure 3.5 A comparison of temperatures recorded by a weather station, and by ground level data loggers

(A) Estimated hourly temperatures derived from weather station daily maximum, minimum and average temperatures (blue), and average hourly temperatures calculated from ground level readings taken every 10 minutes (red). (B) A comparison between average daily temperatures calculated from logger readings and extracted from the weather station archive.

3.2.6 Model validation

Figure 3.6 shows the predicted time required for Col-0 and L*er* to produce seeds when bolting was simulated on successive days. The models predicted that from January onwards, times would be progressively reduced for later bolting dates. This continued until late summer, where warm temperatures combined with long days would allow the most rapid seed production. A sudden sharp increase was predicted after this point, as later bolting would result in seed development taking place while temperatures were steadily decreasing in autumn and winter. Beyond this, predicted times to seed set began to decrease again, thereby repeating the annual cycle. All three models produced a similar annual pattern and differences between predictions were smallest for summer bolting dates, although could be quite large for bolting dates in autumn when times to seed set were longest.

Figure 3.6 also shows the observed times to seed set for batches of Col-0 and L*er* grown at the field site. The behaviour of plants was similar to the model predictions, with later bolting resulting in decreased time to seed set, up to a cut-off bolting date in late summer. Batch H bolted beyond this cut-off, and as predicted the time taken to produce seeds increased.

To compare model performance, plots of observed and predicted seed development times were produced, along with R^2 scores for each model (Figure 3.7). These plots revealed models were most successful in predicting seed set times in spring and summer, when the time required to produce seeds was lowest. Field observations for batches bolting at these times were close to the 1:1 line, indicating good model performance.

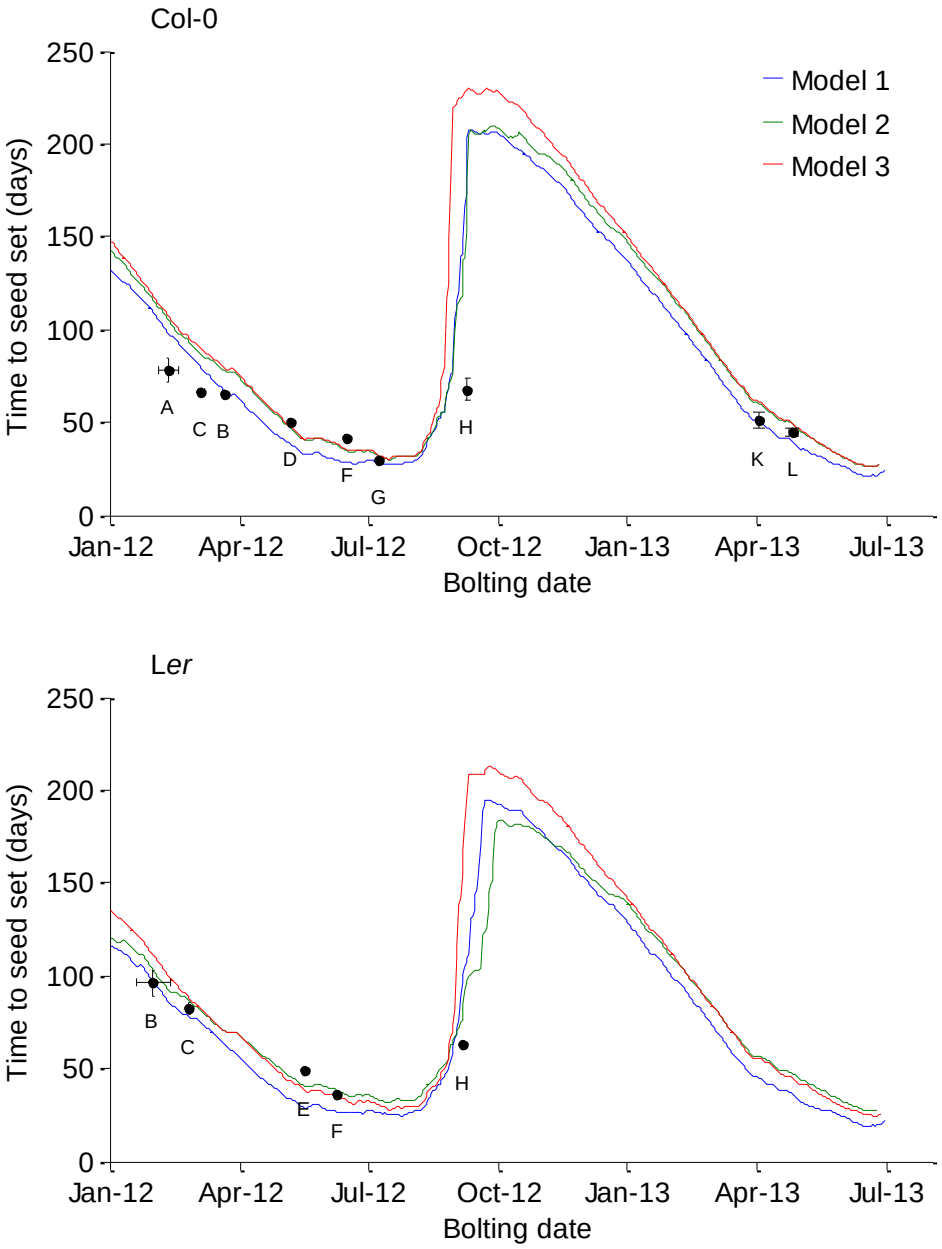

Figure 3.6 Model predictions and field observations of seed set timing

Three models were used to predict seed set timing for each bolting date between Jan 2012 and July 2013 (solid lines). Simulations were performed using hourly temperatures estimated from weather station records. Field observations are labelled with batch letters, and filled circles represent batch means. Standard errors for bolting dates (horizontal error bars) and seed set times (vertical error bars) are also shown.

However, models struggled to accurately predict seed set timing for batch H which bolted close to the predicted cut-off date in autumn. This was also a time of extreme sensitivity to bolting date, since small changes could lead to very large differences in seed set predictions, as illustrated by the steep gradient in Figure 3.6 for autumn bolting dates. This is also shown by vertical error bars in Figure 3.7, which were calculated using the mean bolting date ± one standard error to calculate the range of predictions for each batch. For batch H, the mean bolting date for Col-0 was 17th September ± 1.08 days (Table 3.2). This resulted in predictions of 203 ± 41 days (model 1), and 179 ± 69 days (model 2). Model 3 had already reached a relative plateau at the maximum possible seed development time by this bolting date, therefore the range of predictions was not as large.

An additional consideration is that batches for field experiments were germinated in the lab in order to transplant at the field site at the desired time. These bolting dates would therefore not necessarily be observed in natural populations where germination timing is under strict environmental regulation. Bolting in September would be considered unusual in this location, and for batch H this resulted in seed dispersal being delayed until the end of November and continuing into December and January. This would most likely result in a lower seed yield and reduced fitness compared to spring bolting varieties. Natural populations are therefore likely to avoid bolting at the end of summer in favour of a winter annual life history, where bolting is delayed until spring. For these reasons batch H was excluded from R^2 calculations, which instead focused on the more relevant spring and summer bolting batches.

The models that explained the greatest proportion of the variance for Col-0 and L*er* were model 1 (43%) and model 2 (93%) respectively. These models generally

provided good agreement with observed values, and mean absolute prediction errors were 8.5 days for Col-0 and 6 days for L*er*.

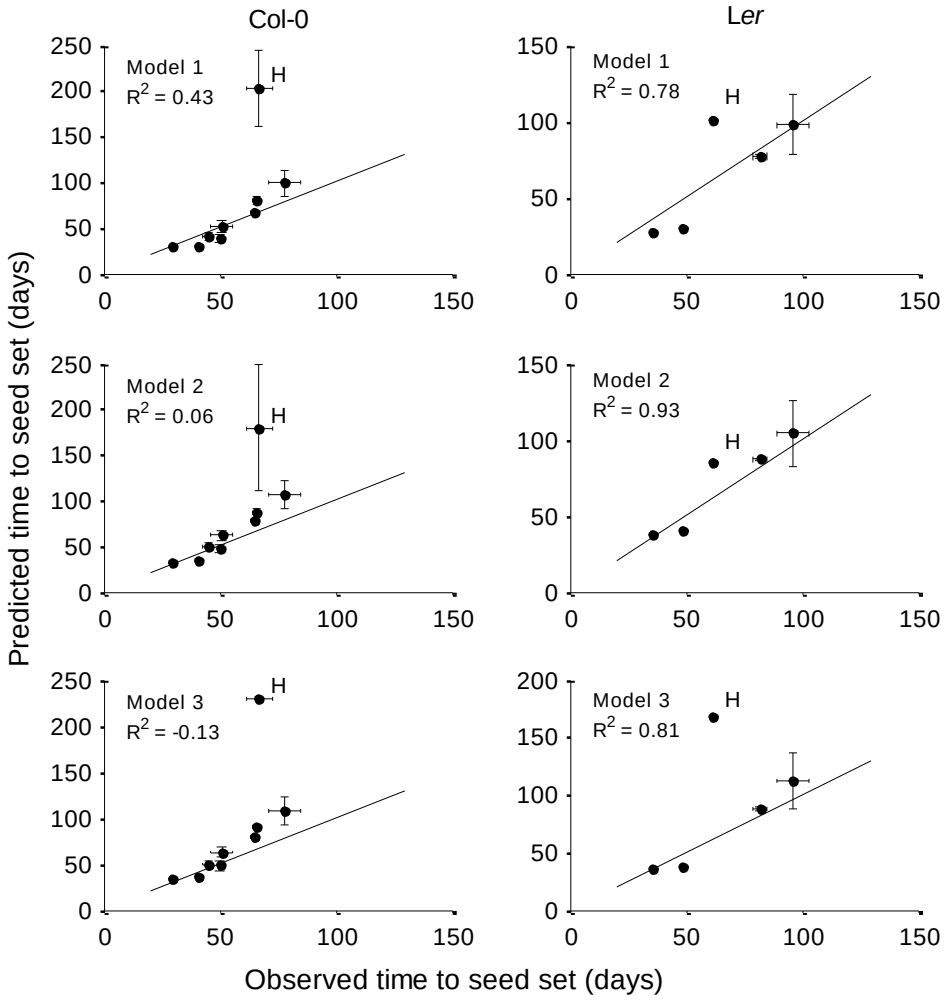

Figure 3.7 Observed and predicted time from bolting to seed set in field conditions

Times from bolting to seed set were predicted by three different models for Col-0 and L*er*, and compared to observations from field experiments. The solid line indicates the 1:1 line (the position of points for a perfect model). The fraction of the variance explained by each model is indicated by R^2 values (Batch H was excluded from R^2 calculations- see main text for explanations). Vertical error bars were calculated using mean bolting date $\pm$ standard error to calculate the range of predictions for each batch. Horizontal error bars indicate the standard error in time to seed set for each batch.

3.2.7 Sensitivity analysis

Sensitivity analysis was performed to assess the relative importance of each parameter on the model output. For each of the 3 seed development models, parameters were individually set to 105% and 95% of their original estimated value, while all other parameters were held constant. The predicted times required produce mature seeds for both Col-0 and L*er* were then calculated for each of the experimental field plantings, using the mean batch bolting date as the simulation start point.

The differences in predicted seed ripening times caused by a 10% change in parameter value were converted into a fraction of the original prediction, and divided by 0.1 to obtain the relative sensitivity. Equation [8] outlines the formula used for this calculation, where δN is the change in the model output caused by the 10% parameter change, and N is the prediction using original estimated parameters. Relative sensitivities were averaged across each of the field batches to calculate the overall sensitivity, which can be interpreted as the average fractional change in the model output per fractional change in parameter value.

[8] $$Relative\ sensitivity = \frac{\delta N/N}{0.1}$$

Table 3.4 lists the relative sensitivity calculated for each parameter in the case of Col-0 and L*er* models. This shows that in all cases the thresholds of the models (T_h) had the highest sensitivity, followed by the base temperature (T_b). Model 3 was relatively insensitive to changes in the photoperiod parameters ($P_{max}$and P_{min}), with P_{min} having the smallest overall effect.

Table 3.4 Relative sensitivity calculated for models of seed set in Col-0 and L*er*

	Parameter symbol	Description	Value	
			Col-0	L*er*
Model 1	T_b	Base temperature (°C)	0.66	0.64
	T_h	Thermal time threshold (°C hours)	1.0	1.0
Model 2	T_b	Base temperature (°C)	0.83	0.66
	T_h	Thermal time threshold (°C hours)	0.92	0.95
Model 3	T_b	Base temperature (°C)	0.51	0.85
	P_{min}	Short day threshold (hours)	0.0042	0.25
	P_{max}	Long-day threshold (hours)	0.074	0.66
	T_h	Thermal time threshold (°C hours)	0.58	0.89

3.2.8 Model simulations

Simulations and field experiments showed that bolting date has a large effect on the time required to produce seeds. This is because bolting date determines the temperature and photoperiod conditions that are experienced during seed development. It was also suggested that not all possible bolting dates would be relevant to *Arabidopsis* growing in the wild, since photoperiod and vernalisation requirements would favour bolting in spring or summer. Therefore, bolting dates were first predicted using the model described by Wilczek et al (2009). These predictions were used as input for the seed set model, which then calculated the date of seed dispersal and the temperature during seed maturation. The two models combined could therefore be used to examine the effect of germination date on seed set timing, and also to determine the range of seed maturation temperatures that are most relevant for primary dormancy establishment.

In addition to predicting plant growth in York, simulations were carried out using temperature data from a further 15 locations. These consisted of locations in Europe, Asia and North America, which were chosen to represent the distribution of

Arabidopsis ecotypes. Daily temperature records were collected from weather station archives, and the mean annual temperature cycle in each location was calculated using data spanning 10 years. This was also combined with times of sunrise and sunset in each location. Bolting and seed set dates were predicted as described above, and the maturation temperature was estimated as the mean temperature during the week prior to seed set. Output from Col-0 and L*er* models were similar, therefore only results from Col-0 are presented. Parameter values used were those defined for Col-0 in Wilczek et al. (2009), and seed set model 1 listed in Table 3.1.

Results of simulations using temperature data from York are presented in Figure 3.8. Germination in late summer resulted in bolting and seed set during autumn. However, germination from mid September onwards resulted in an over wintering life history, in which bolting was delayed until the following calendar year. This causes the discontinuous appearance of the graph in Figure 3.8A. The steep gradient in this region also indicates an area of extreme sensitivity to germination date. Similar findings were reported by Wilczek et al. (2009), who demonstrated high sensitivity to germination dates in late summer and early autumn in Norwich and Cologne. In contrast however, Figure 3.8 shows that germination dates ranging from September to March resulted in very little variation in predicted seed set dates, which tended to fall in mid to late May. This indicates that the sensitivity of bolting and seed set timing to germination date dramatically reduces in autumn and spring, after the critical window of high sensitivity in early autumn. This was not reported previously in Wilczek et al. (2009), who showed only the predicted time taken to reach bolting, rather than bolting date as a function of germination timing.

As a consequence of the low variation in seed set timing, predicted seed maturation temperatures were relatively constant at 12°C for germination dates spanning from

September to March (Figure 3.8B). As germination dates progressed into late spring and summer, generation times became shorter as warm temperatures and long days resulted in rapid flowering. Predicted seed maturation temperatures also increased until a cut-off in late summer. Germination at this time resulted in autumn bolting, when seed maturation could no longer be completed before the onset of cooler temperatures, causing a sharp drop in the predicted maturation temperatures.

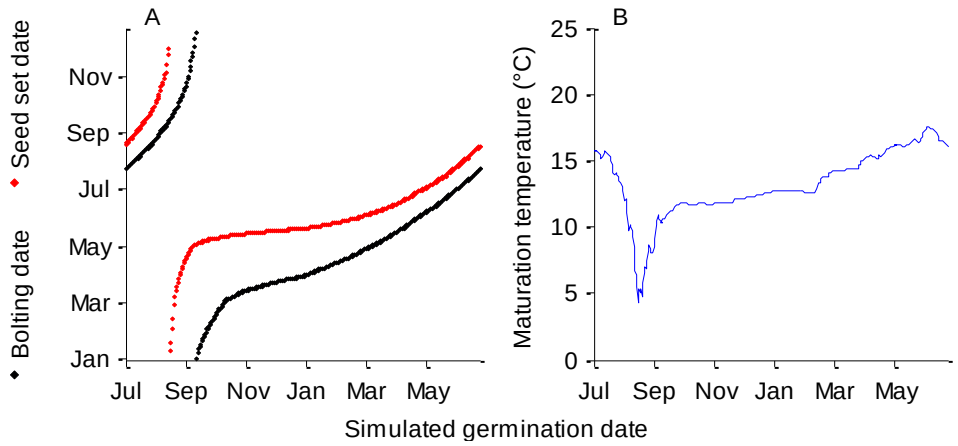

Figure 3.8 Simulations of bolting and seed set timing in York, UK

(A) Bolting date (black) and seed set dates (red) were predicted for Col-0 plants germinating on successive days. (B) Seed maturation temperatures were predicted from the mean temperature during the week prior to seed set.

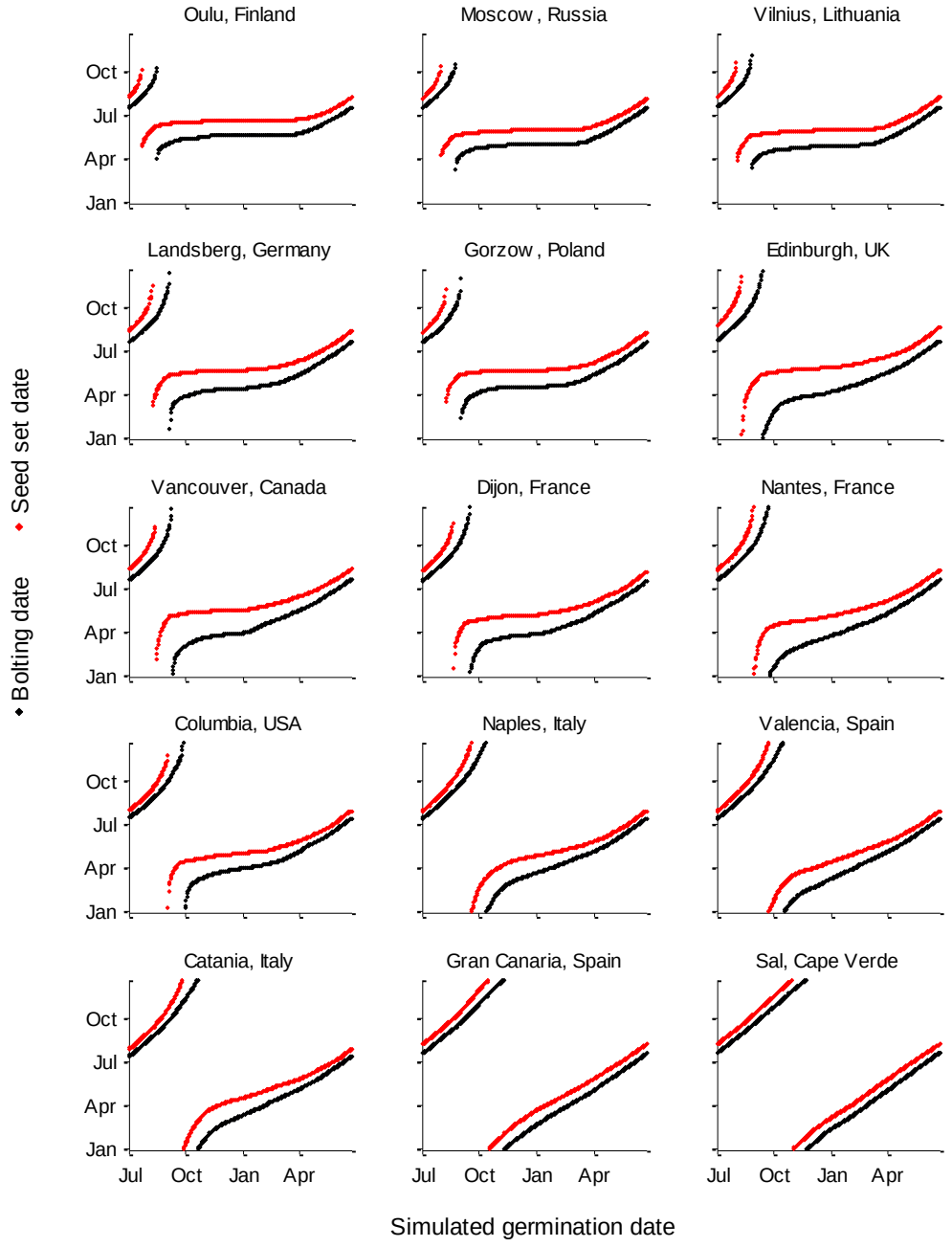

Figure 3.9 Simulations of bolting and seed set timing for in a range of locations

Bolting dates (black) and seed set dates (red) were predicted for Col-0 germinating on successive days in each location.

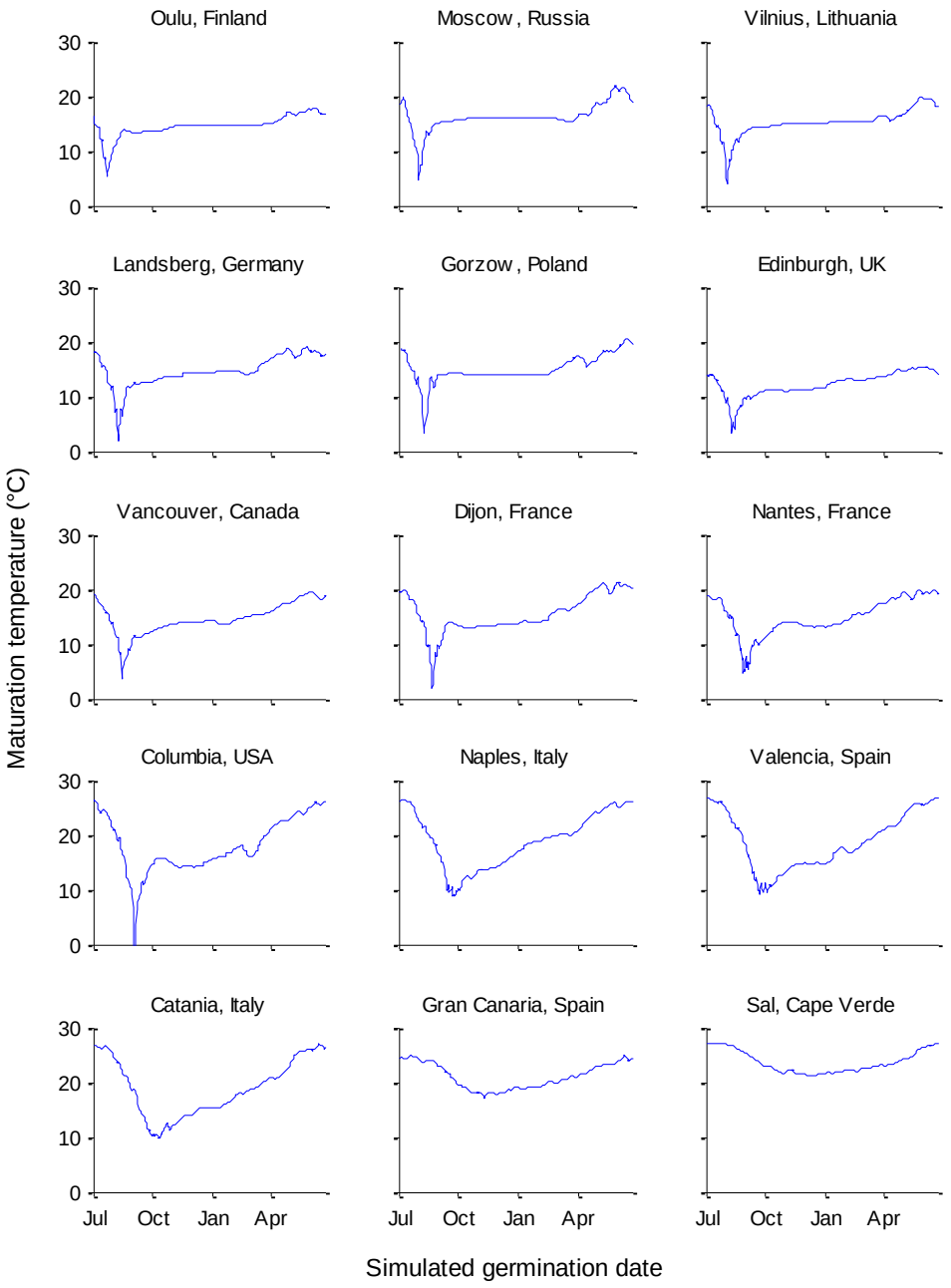

Figure 3.10 Simulations of seed maturation temperatures in a range of locations

Seed maturation temperatures were predicted from mean temperatures during the week prior to seed set. Seed set dates were predicted for each maternal germination date using combined bolting and seed set model simulations (see Figure 3.9).

Similar results were obtained from simulations in different locations (Figure 3.9 and Figure 3.10). A trend related to mean annual temperature was also observed. In general, the degree of synchronicity in bolting and seed set dates for autumn and spring germination was greater in cooler climates. This was because the switch to over wintering occurred earlier in autumn, and also because temperatures remained below T_b for longer in the spring. This meant all plants germinating within a wider time frame would not accumulate any photothermal units until the start of the growth season, and would therefore fulfil their photothermal requirements at the same time. In locations with increasing mean annual temperatures, winters were milder and synchronicity in over wintering and spring annual plants decreased. In equatorial climates such as Gran Canaria and Cape Verde, growth was not inhibited during winter leading to continuous rapid cycling.

3.2.9 Effects of altered flowering time

Climate change is resulting in altered flowering phenology in many plant species (Menzel et al., 2006b). Certain genetic mutations can also affect flowering time, such as those affecting genes in the photoperiod or vernalisation pathways. Therefore, to investigate potential effects of altered flowering time on seed set timing, the bolting model (Wilczek et al., 2009) was used to predict the normal bolting date of a typical winter annual germinating in November. This was then adjusted by ±40 days to examine the effect on the predicted seed set date (Figure 3.11).

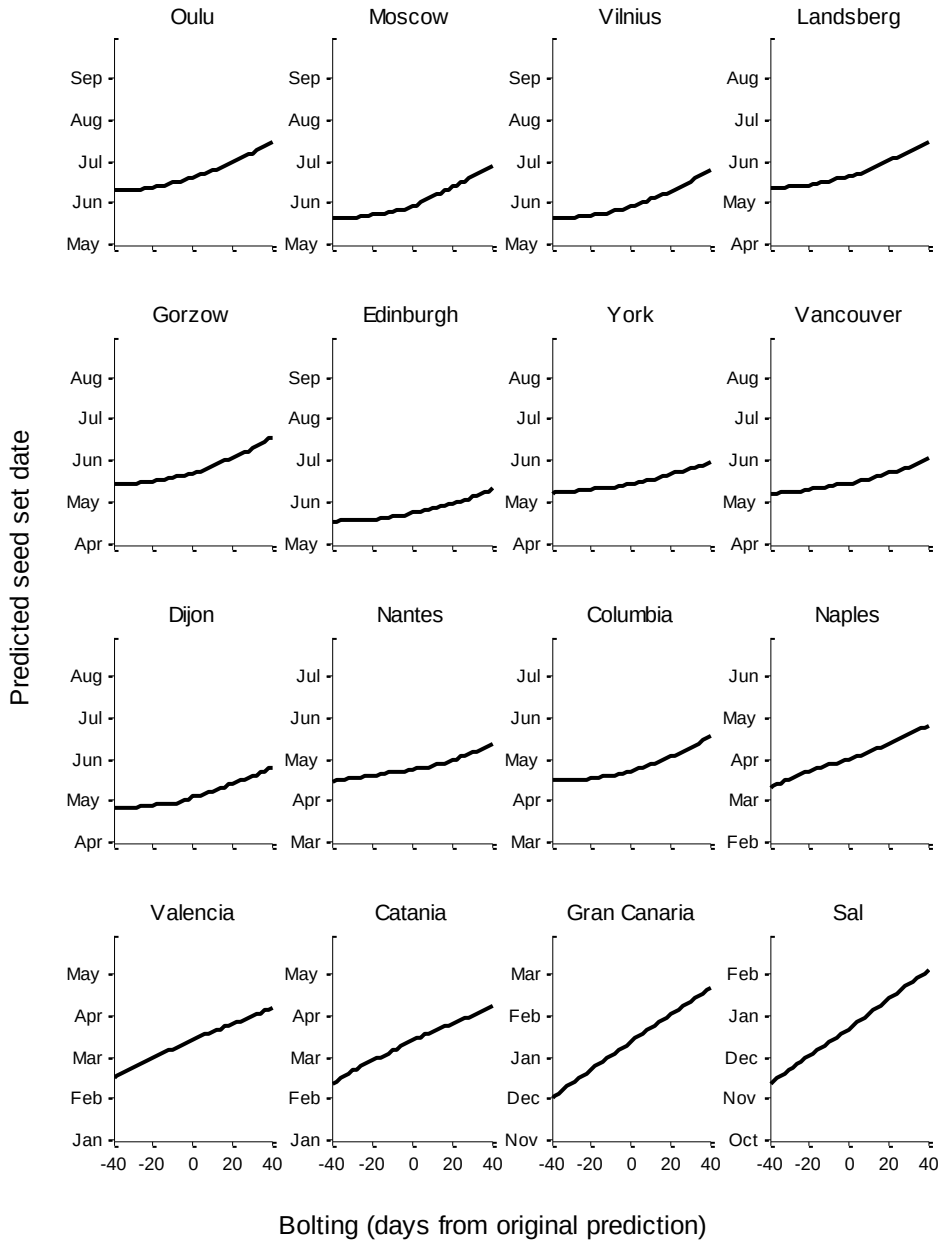

Figure 3.11 The effect of altered bolting date on seed set timing

Bolting date was predicted for a plant germinating on 1st November in each location. This prediction was then adjusted by ±40 days as shown, and used to predict seed set date.

Predictions in most locations were surprisingly stable despite large perturbations in bolting date. For example, adjusted bolting timing had the smallest overall effect in Edinburgh, where the range of seed set dates spanned just 25 days in May and July, despite simulated bolting dates spanning a total of 80 days. However, altered flowering time generally had a much larger effect in warmer climates. For example, the relationship between altered bolting and altered seed set timing was close to a 1:1 ratio in Cape Verde, where an 80 day difference in bolting dates led to an 85 day difference in seed set dates.

Notably, flowering early also had less of an effect on predicted seed set dates than flowering late, although only in locations with cold winters. This was because plants that bolted before spring temperatures increased above T_b would not accumulate any photothermal units until temperatures increased at the start of the growing season. Consequently, flowering early had little or no effect on the number of photothermal units accumulated by the start of the growing season, and therefore the predicted seed set date was not affected. Conversely, flowering later meant that plants missed out on photothermal units at the start of the growing season, meaning their requirements for seed production were not met until later in spring or summer.

3.3 Discussion

3.3.1 Temperature is the dominant environmental factor that determines seed set timing

In this chapter the growth rate characteristics of seeds maturing at different temperatures and photoperiods were analysed. This revealed a positive linear response between growth rate and temperature in the range 8-25°C. Previous studies

of leaf initiation, leaf expansion, and cell division in *Arabidopsis*, sunflower and maize suggest that linear growth rate responses to temperature are common in plants (Ben-Haj-Salah and Tardieu, 1995; Granier and Tardieu, 1998; Granier et al., 2002)

Increasing photoperiod also increased seed developmental rates, however this quickly reached a maximum at approximately 10 hours in L*er* and 12 hours in Col. One explanation for this behaviour is that as plants were grown at increasing photoperiods at 20°C, the temperature quickly became the dominant limiting factor. Therefore providing more hours of daylight per day had no additional effect since growth was already proceeding at the maximum rate permitted by the temperature. This theory was also supported by the data, which shows that growth rates had reached an apparent maximum in 16h days at 20°C (Figure 3.2). However, growth conditions of 16h days and 25°C resulted in an increase in the growth rate (Figure 3.1).

The linear relationship between growth rate and temperature suggested that a thermal time approach would be suitable to model seed development in *Arabidopsis*. Three models were developed based on data collected from laboratory experiments, each differed in the way photoperiod information was incorporated to determine the growth rate (Figure 3.3). Each model also performed similarly when predicting laboratory data, which was used to constrain the model. The fact that all three models also predicted similar behaviour when used to simulate seed set timing in the field also showed that altering the way photoperiod was used to predict growth rates had little effect. Interestingly, model 2 which disregarded photoperiod and used only temperature to predict seed set timing, was the most effective at predicting seed set timing in L*er* under field conditions. This shows that temperature alone can be an adequate predictor of seed set timing in some cases. Furthermore, sensitivity analysis

confirmed that predictions made by model 3 were less sensitive to changes in photoperiod parameters P_{min} and P_{max} than the temperature parameter T_b.

While temperature is clearly an important factor which determines how quickly seeds are produced, it was evident that when plants were grown at the field site other factors caused additional variation that could not be accounted for by the models. The best models for Col-0 (model 1) and L*er* (model 2), explained 43% and 93% of the variance in seed set timing, meaning that other unknown factors collectively accounted for 57% and 7% of the variance. These may have included environmental factors such as water availability, light intensity, and nutrient availability; as well as biotic factors such as herbivory and disease. It may also be the case that additional factors are more important in different locations and climates and even in different seasons. For example water availability might play a more important role in warmer climates, or in summer. Therefore, in order to increase confidence in the models and extrapolate them to other climates, this should be a subject of future investigation.

3.3.2 Predictions were unaffected by model choice

Figure 3.6 shows that the models were able to correctly predict the pattern of behaviour with changing bolting dates, and the differences between the three models were relatively minor. The main differences occurred for bolting dates that would have resulted in seeds maturing in short photoperiods. However, because this was never observed in the field, the choice of model made very little practical difference to predicting the observed plant behaviour.

Figure 3.7 also illustrates that the relationship between observed and predicted values were similar for the three models, however the R^2 values show large

differences, particularly for Col-0. This was because they were disproportionately affected by a small number of large prediction errors, namely predictions of batches A and H. Due to the sharp increase in the time to seed set for bolting dates in September, the timing of seed set in batch H was overestimated by as much as 163 days in Col-0 (model 3). This meant the total sum of the squared errors was greater than the total variability in the data, leading to negative R^2 values in all cases. Due the sensitivity of predictions around this bolting date, batch H was excluded from all R^2 calculations. However batch A, which bolted very early in the year also had a large affect, leading to a negative score for model 3 and a value close to zero for model 2 (Col-0).

R^2 values are routinely used in the biological literature to evaluate the performance of models (e.g. Batlla et al., 2009; Chantre et al., 2009; Wilczek et al., 2009; Watt et al., 2011). It provides an easy to understand metric from a simple comparison between observed and predicted values, and is usually called the coefficient of determination, but has also been referred to as the Nash-Sutcliffe efficiency (Nash and Sutcliffe, 1970). As defined in equation [7], R^2 values can range between 1 and $-\infty$; where 1 indicates a perfect model, and negative values indicate that the mean of the data points would be a better predictor. The use of R^2 is also re-enforced by various statistical packages such as Matlab, which typically report an R^2 value when fitting any statistical model to data. However there is a growing opinion that this measure may be inappropriate to evaluate non-linear models (Spiess and Neumeyer, 2010). In particular, the assumption that the variance of the errors is equal for all measurements (homoscedasticity) is violated in non-linear models. This is evident from Figure 3.7 which shows smaller deviations between observed and predicted values when the time to seed set is short, but increasing deviations as the time to

seed set increases. Because all errors are squared, these occasional large values can dominate the result of the R^2 calculation.

3.3.3 The length of seed development is sensitive to bolting date

Because of the dependence on temperature which varies substantially depending on the time of year, predictions of the time required to produce seeds could vary from as little as 21 to 207 days depending on the bolting date (Figure 3.6). This relationship with bolting date leads to a predictable annual pattern, where successive bolting dates early in the year lead to progressively decreasing seed maturation times, caused primarily by the effects of increasing temperatures on the growth rate. Bolting dates at the start of July result in the shortest seed maturation times, since seed development occurs during the warmest time of year. However, bolting in late summer leads to plants producing seeds in an environment that becomes progressively cooler, resulting in a rapid increase in predicted time to seed set. The longest seed development times were predicted for bolting dates in September, where seed maturation was not completed until the following spring.

The sudden transition from short to very long generation time was also reported by Wilczek et al. (2009), who observed a brief window of extreme sensitivity to germination timing in autumn causing a predicted transition from a rapid cycling to a winter annual habit. This phenomenon is a general feature of thermal time models in seasonal climates, since it is driven by a reduction in the availability of thermal units when temperatures drop in autumn and winter. This transitional period is significant for plants because it represents a boundary where initiating flowering could result in a serious reduction in fitness if seed production could not be completed. Flowering

in late autumn has been observed for *Arabidopsis* in some locations (Thompson, 1994; Griffith et al., 2004), however a recent study reported that plants sown in Norwich, UK that bolted in September and October suffered from high mortality and reduced fitness (Fournier-Level et al., 2013). It would therefore be expected that plants that were well adapted to a particular climate would avoid bolting at this time and instead opt for an overwintering life history.

3.3.4 Seed set conditions are conserved for spring and winter annuals

The length of seed maturation depends strongly on the flowering time due to the dependence of the growth rate on temperature. Flowering time is under strict environmental regulation via several well characterised genetic pathways (Srikanth and Schmid, 2011; Andres and Coupland, 2012), which ensure flowering is initiated in the appropriate season. In this chapter, a model which simulates the action of the vernalisation and photoperiod pathways to predict flowering time (Wilczek et al., 2009) was used in conjunction with a model of seed set timing in order to limit the bolting dates used as input to those that would be most likely to occur in natural populations, and also to simultaneously examine the effect of germination date on seed set timing.

The result of these combined simulations showed that for Col-0 growing in York (Figure 3.8) a surprisingly wide range of germination dates, specifically from late autumn to early spring, led to similar dates of seed set in May. This conservation in seed set timing meant that seed maturation was also occurring under similar environmental conditions, and the temperature during the week prior to seed maturity averaged at approximately 12°C. This seed maturation temperature is

relatively cold, and would most likely result in seeds with high primary dormancy in Col-0 (Penfield and Springthorpe, 2012). This dormancy is likely to be lost through after-ripening during summer, and seeds would then be able to germinate the following autumn or spring. Therefore the models predict a stable winter/spring annual life history that is robust across a wide range of germination dates. Additionally, this pattern of behaviour was consistent across several sites (Figure 3.9), and only began to break down in warmer climates where milder winters led to continued growth and a predicted rapid cycling behaviour all year round.

As well as predicting winter and spring annual behaviour, the combined models also predict rapid cycling for late spring and summer germinants, where flowering and seed set occur in the same growing season. This would also result in seeds with reduced primary dormancy due to increased seed maturation temperatures. However, there is also a critical cut-off for late summer germination dates where plants would again revert to a winter annual habit, which was also predicted in Wilczek et al. (2009).

3.3.5 Seed set timing is resistant to perturbations in flowering time

Although the time (in days) required to produce mature seeds after bolting is highly sensitive to the bolting date (Figure 3.6), the dynamics of later bolting combined with a shortening of seed maturation results in a bunching up of seed set dates. This was not shown specifically, however can be seen in Figure 3.8 where germination during September results in predicted bolting dates spanning from December through to March. However, this large range of bolting dates results in a relatively small range of predicted seed set dates in May. Therefore, despite sensitivity of the length

(in days) of seed development to flowering time, the timing (calendar date) of seed set is surprisingly insensitive to flowering time, at least during part of the year and especially for winter annuals.

This was also demonstrated in different locations by simulating a typical winter annual, and adjusting the predicted bolting date (Figure 3.11). Provided winter temperatures were sufficiently cold ($< T_b$), early bolting had little effect on predicted seed set dates, although late flowering did result in later seed set. However, robustness of seed set timing against changes in flowering time, and also against changes in germination timing could be a mechanism which stabilises plant phenology against unpredictable environmental fluctuations, such as those caused by unseasonal weather conditions.

3.3.6 Conclusions

The results in this chapter provide an insight into the interacting roles of phenology and environment in life histories of *Arabidopsis*. Combining a previously published model of flowering time in the Col-0 ecotype (Wilczek et al., 2009) with a novel model of seed set revealed conserved seed set timing and seed maturation environments for winter and spring annuals. This was robust against changes in germination and flowering time across a number of different locations. A rapid cycling habit was predicted for Col-0 growing in warmer climates and for plants germinating in summer. However, plants germinating after an autumn cut-off date were predicted to revert back to a winter annual habit in temperate climates.

Chapter 4: Modelling Seed Dormancy and Germination

4.1 Introduction

The previous chapter highlighted the importance of germination timing to both flowering and seed dispersal timing. Several other studies have also shown that germination timing is a key factor that determines life history (Donohue et al., 2005a; Galloway and Burgess, 2009; Wilczek et al., 2009). Understanding how plants regulate the timing of germination in response to seasonal cues is therefore central to understanding life history phenology as a whole.

The key to germination timing is seed dormancy, which dictates the ability of seeds to respond to favourable germination conditions. Temperature plays a major role in the establishment of primary dormancy during seed maturation, with cooler temperatures generally resulting in seeds that are more dormant at maturity (Schmuths et al., 2006; Donohue et al., 2008; Chiang et al., 2009; Donohue et al., 2012). Temperature also affects dormancy after seed dispersal, and chilling or cold stratification is commonly used to promote germination in dormant seeds. However, prolonged chilling has also been shown to induce dormancy in some *Arabidopsis* ecotypes (Finch-Savage et al., 2007; Penfield and Springthorpe, 2012). The effects of temperature on dormancy are therefore not straightforward, as the same temperature can be dormancy breaking in some circumstances, and dormancy inducing in others. Since both maternal and post-dispersal temperatures affect seed

dormancy, reproductive timing and seasonal changes in temperature must also interact to determine germination behaviour, although this is not well understood.

A number of models have already been suggested which attempt to link dormancy alleviation with environmental factors. Many of these are based on the thermal time concept, in which changes in dormancy are associated with accumulated low temperature chilling time, or high temperature after-ripening time (e.g. Batlla and Benech-Arnold, 2003; Batlla and Benech-Arnold, 2005; Chantre et al., 2009). Another common approach is to use threshold models, in which population parameters such as base water potential are altered by perceived changes in dormancy (Bradford and Somasco, 1994; Bradford, 2002). Thermal time and threshold models have also been successfully combined, and used to reproduce changes in sensitivity to different germination stimuli as a result of dormancy loss or induction (Chantre et al., 2009; Chantre et al., 2010). However these models can become complex, requiring distinct parameter sets for specific treatments, *i.e.* different temperature ranges or light treatments. Current dormancy models also tend to assume initial levels of primary dormancy are fixed, and are unable to account for maternal effects. The work in this chapter was therefore aimed at developing a model which incorporates the effects of both pre- and post-maturation temperature on seed dormancy, which could be used to predict germination frequencies. The model was then used to predict germination behaviour in seeds dispersed at different times of year, and in different locations and climates.

4.2 Results

4.2.1 The effect of temperature on seed dormancy

To quantify the effect of temperature on dormancy, a series of experiments were carried out using seeds from the Columbia ecotype (Col-0) matured at different temperatures, and then given different stratification treatments. These treatments were carried out in darkness to simulate burial, and consisted of incubation on agar plates at constant temperatures for varying lengths of time. Dormancy was then measured by transferring seeds to germination conditions (22°C; 16h light), and counting numbers of germinated and un-germinated seeds after 7 days. Some seeds germinated in darkness during the stratification treatments, and by doing so they were prevented from undergoing further changes in dormancy. In effect this meant they did not receive the full stratification treatment, and could not be considered the same as seeds which germinated only after the full treatment plus incubation in light. Therefore, dark germinated seeds were not included in the development of this model, but are considered separately in the next chapter.

Figure 4.1 shows the number of seeds germinating at the end of the germination assay as a percentage of the total number of seeds (excluding those that germinated in the dark). These results reveal a clear trend of reduced primary dormancy with increasing maturation temperature. Seeds matured at 12°C were the most dormant, and very few germinated after stratification. In seeds matured at 13°C and 14°C some dormancy was broken after 1-2 week stratification treatments, however their germination did not exceed 25% and secondary dormancy was induced in seeds stratified for longer than 2 weeks. Primary dormancy was reduced further in seeds matured at 15°C and above, which germinated to almost 100% after stratification at 4°C. Dormancy was lowest overall in seeds matured at 18°C, and germination in

freshly harvested seeds was approximately 40%. As a result of reduced dormancy, many seeds matured at 18°C germinated during incubation in the dark. Since dark germinating seeds were excluded from this analysis, sample sizes were reduced causing the variation and standard error of these measurements to be increased.

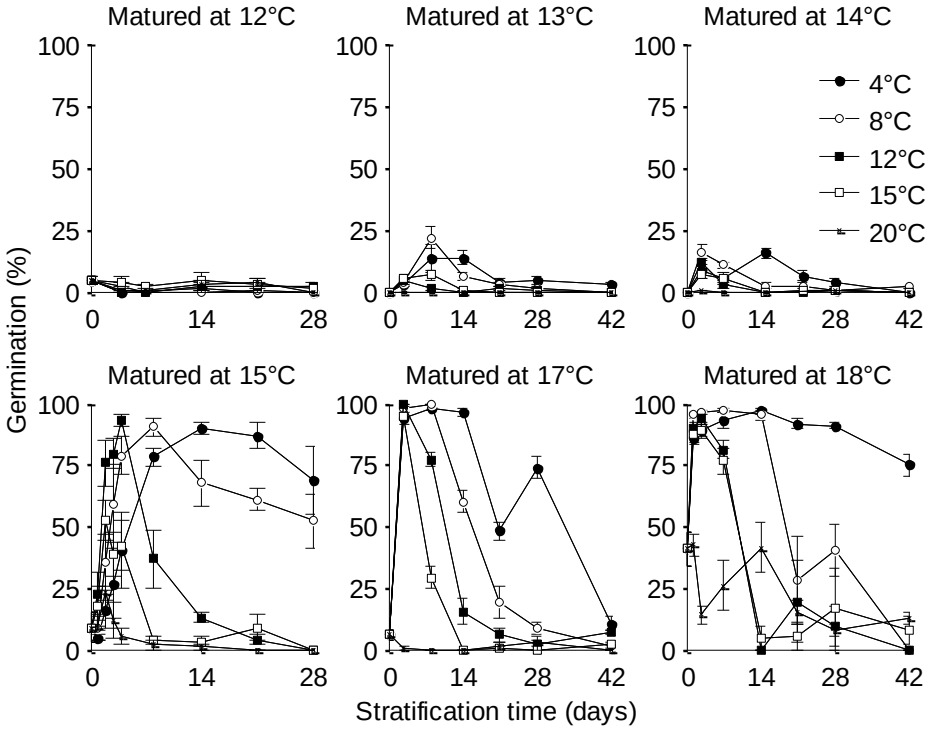

Figure 4.1 The effect of maturation and stratification temperature on germination in light

Col-0 seeds matured at 12°C, 13°C, 14°C, 15°C, 17°C, and 18°C were imbibed on water agar, and stratified in darkness at constant temperature (indicated by symbols). Seeds were transferred to germination conditions (22°C; 16h light) after the stratification time shown, and germinated seeds were counted after 7 days. Seeds which germinated in darkness were excluded, and germination in light was expressed as a percentage of the remaining total. Data represent the mean and SE of 5 replicate seed batches.

The stratification temperature also affected the rate of dormancy loss, which was most visible in seeds matured at 15°C. Surprisingly, low temperature stratification resulted in a slower rate of dormancy loss, and a later peak in the germination compared to warmer temperatures. This did not appear to be the case for seeds

matured at 17°C and 18°C; although loss of dormancy occurred rapidly, and therefore the effect of stratification temperature may not have been visible at this sampling resolution. Stratification at increasingly warmer temperatures also resulted in increased rates of secondary dormancy induction, generally resulting in earlier peaks and subsequent decreases in the germination. This trend suggests that the consistently poor germination observed after stratification at 20°C was due to rapid secondary dormancy induction.

4.2.2 Model development

Totterdell and Roberts (1979) originally proposed that dormancy loss and dormancy induction may be occurring simultaneously in seed populations. This notion was later used by Batlla et al. (2009) to model germination percentages in the annual weed *Polygonum aviculare*. In their model, rates of dormancy loss and induction were independently controlled by temperature, and the combined effect of both processes determined the final germination percentage. Population-based approaches have also been successful in describing variation in germination rates, as well as how this can be affected by changes in dormancy (e.g. Alvarado and Bradford, 2002). Ideas from these two approaches were therefore combined in an attempt to model dormancy and germination in *Arabidopsis*.

Assuming a population of seeds contains a mixture of dormant and non-dormant individuals, any random seed will either germinate or will be dormant given favourable conditions. Dormancy and germination are therefore mutually exclusive. This relationship is defined in equation [9], where $P(G)$ is the probability of germination, and $P(D)$ is the probability of dormancy.

[9] $$P(G) = 1 - P(D)$$

Assuming that primary and secondary dormancy are independent processes and that a seed can be rendered dormant by either process, the general disjunction rule can be used to define the total probability of dormancy using the individual probabilities of primary and secondary dormancy, $P(D_p)$ and $P(D_s)$, as shown in equation [10].

[10] $$P(D) = P(D_p \cup D_s) = P(D_p) + P(D_s) - P(D_p)P(D_s)$$

It is well known that populations of seeds do not germinate synchronously, but generally have some kind of distribution in their germination timing. Population based threshold models (e.g. Bradford, 2005) typically attribute this variation to parameters such as base water potential (ψ_b), which are thought to be normally distributed within populations. In any particular environment, only the fraction of seeds with ψ_b vales exceeding the environmental water potential (ψ) are thought to be non-dormant, and therefore capable of germination.

This idea can also be applied more generally to seed dormancy. Assuming dormancy depth is normally distributed within a population, one can imagine a threshold value below which a seed is no longer dormant. Assuming that treatments such as chilling can change the mean dormancy of the population, applying these treatments would shift the entire dormancy distribution of the population. Such a shift would result in a proportion of seeds becoming non-dormant as their dormancy is shifted to below the threshold. If the mean dormancy changes at a constant rate, the proportion of non-dormant seeds over time would follow a regular cumulative distribution curve. If the seeds were also provided with enough light and moisture to enable germination as

soon as dormancy was lost, then a typical cumulative germination curve should be observed with increasing incubation time. However, this description only applies to the loss of primary dormancy. If seeds were prevented from germinating, for example by incubating in darkness, then secondary dormancy would eventually be induced.

It is often reported that maximum germination percentages are reduced at elevated germination temperatures (e.g. Windauer et al., 2012). This can easily be explained if dormancy loss and induction are occurring simultaneously. In such cases, dormancy induction would be accelerated by the increased temperature, and so some of the population would enter secondary dormancy before losing primary dormancy. A portion of the population would therefore remain dormant, even when incubated in continuous light. The idea of normally distributed dormancy was therefore applied to both primary and secondary dormancy individually (Figure 4.2). It was assumed that freshly harvested seed populations have initially high mean values of primary dormancy (Figure 4.2A) and low mean values of secondary dormancy (Figure 4.2C). Over time the mean primary dormancy would decrease, resulting in a cumulative reduction in the percentage of primary dormant seeds (Figure 4.2B), while the opposite trend was presumed to occur for secondary dormancy (Figure 4.2D).

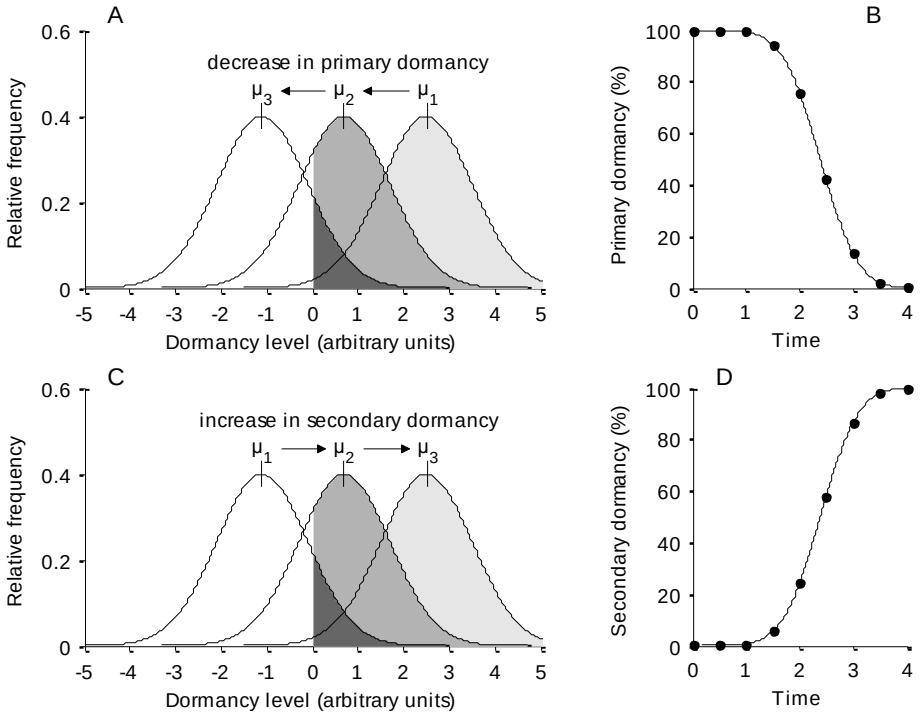

Figure 4.2 An illustration of primary and secondary dormancy frequencies within a seed population

Theoretical normal distributions of primary (A) and secondary (C) dormancy are shown, with fractions of dormant seeds (those seeds having dormancy above a threshold) indicated by shading. As the mean dormancy (μ) of the population changes, the percentage of the population that is primary (B) or secondary (D) dormant follows a cumulative decrease or increase over time.

Logistic functions were chosen to reproduce the desired s-shaped cumulative distribution curves. This approach avoided the need to directly quantify population parameters such as the mean and standard deviation in dormancy values. The logistic functions used to define probabilities of primary and secondary dormancy over time are given in equations [11] and [12]; where x is the stratification time in days; R_p and R_s are the rates of primary dormancy loss and secondary dormancy induction; and A_p and A_s are offset parameters.

[11] $$P(D_p) = \frac{1}{1 + e^{R_p(x+A_p)}}$$

[12] $$P(D_s) = 1 - \left[\frac{1}{1 + e^{R_s(x+A_s)}}\right]$$

Offset parameters were required because a standard logistic curve passes through x = 0 at y = 0.5, however for the purposes of this model the curves were repositioned so that $P(D_p)$ crosses x = 0 at y = 0.99, and $P(D_s)$ crosses x = 0 at y = 0.01. That is to say, in freshly harvested seeds 99% were presumed to have primary dormancy and 1% secondary dormancy. Due to the asymptotic nature of the logistic function it was not possible to set these initial values to 0% or 100% as this would result in an offset of $-\infty$. These values were therefore chosen as a reasonable approximation.

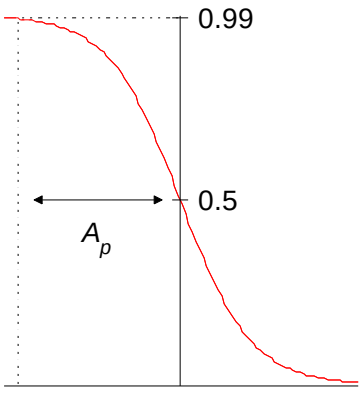

Figure 4.3 An illustration of the offset parameter for primary dormancy

The red line indicates a standard logistic curve passing through (0,0.5). The offset in x required to ensure the curve passes through (0,0.99) is shown by A_p.

The magnitude of the two offset parameters required to position the curves correctly can be found by rearranging equations [11] and [12] making the offset the subject, and then substituting values of $x = 0$ and either $P(D_p) = 0.01$ or $P(D_s) = 0.99$. This

results in equations [13] and [14], which indicate the offset is inversely proportional to the rate parameters.

[13] $$A_p = \frac{\ln(0.01)}{R_p}$$

[14] $$A_s = \frac{\ln(0.01)}{R_s}$$

Combining equations [9]-[14] results in a framework for a population based model of germination probability over time, which encompasses both primary and secondary dormancy dynamics. An example of how these individual probabilities can change over time is provided in Figure 4.3. This framework is conceptually simple, and requires only the rates of primary dormancy loss and secondary dormancy induction (R_p and R_s) to be determined for different temperature treatments. A list of parameter symbols and meanings is also provided in Table 4.1.

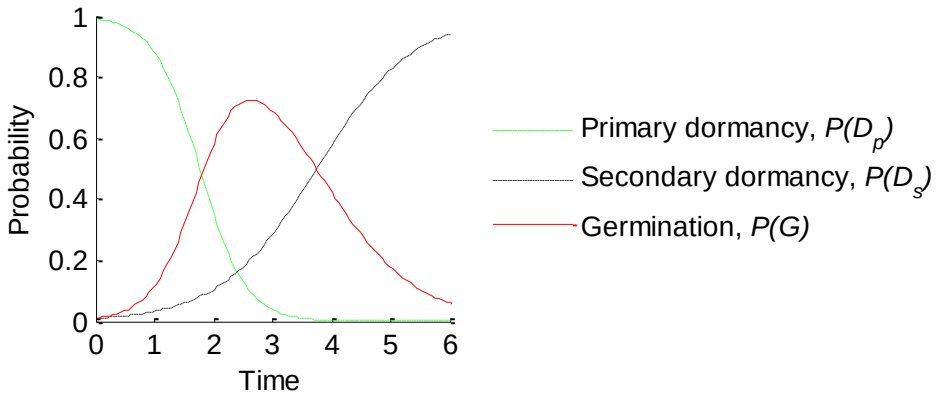

Figure 4.4 An illustration of the dormancy model

Probabilities of primary dormancy (green), secondary dormancy (black) and germination (red) at different times after the start of stratification are shown.

Table 4.1 A list of symbols used in the dormancy model

Symbol	Meaning
$P(G)$	The probability, or relative frequency of germination; defined in equation [9]
$P(D)$	The total probability, or relative frequency of dormancy within a population; defined in equation [10]
$P(D_p)$	The probability, or relative frequency of primary dormancy; defined in equation [11]
$P(D_s)$	The probability, or relative frequency of secondary dormancy; defined in equation [12]
R_p	The rate parameter for primary dormancy loss; defined in equations [18] and [20]
A_p	The offset parameter for primary dormancy; defined in equation [13]
R_s	The rate of secondary dormancy induction; defined in equation [19]
A_s	The offset parameter for secondary dormancy; defined in equation [14]
T_i	Stratification or imbibition temperature (°C)
T_m	Seed maturation temperature (°C)
x	Stratification time (days since imbibition)
Lower case parameters; a, b and c	Parameters whose values were determined by fitting the models to the training data
e	The exponential constant

To determine optimised values of R_p and R_s at different temperatures, the *fit* function in MALTLAB curve fitting toolbox (Mathworks) was used with a trust-region algorithm to fit the model to the training data shown in Figure 4.1. This data consisted of 30 individual germination time series experiments, each having a different combination of maturation and stratification temperature. Fitting of R_p and R_s values was performed individually for each time series, and the resulting parameter values were plotted in order to determine if any relationship with either stratification or maturation temperature could be discerned.

The data presented in Figure 4.1 also suggested that the rate of secondary dormancy induction was increased at warmer temperatures. This observation was used to inform the model parameterisation by constraining R_s within boundaries that increased with stratification temperature, while R_p was allowed to vary within fixed

boundaries. These conditions led to R_p values which were relatively constant for a given stratification temperature, but which increased with maturation temperature (Figure 4.5). It was therefore concluded that primary dormancy should be modelled using only maturation temperature, and secondary dormancy should be modelled using only the stratification temperature.

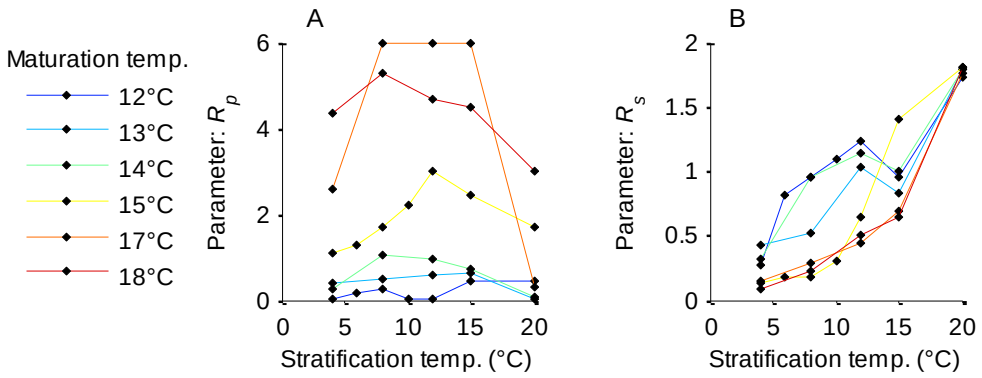

Figure 4.5 The effect of temperature on fitted dormancy rate parameters

(A) The rate of primary dormancy loss (R_p) and (B) the rate of secondary dormancy induction (R_s) plotted with stratification temperature. Maturation temperatures are also shown by colour coding. Parameter values were determined for each combination of maturation and stratification temperature by fitting the model to data from individual germination time series experiments. Values of R_s were also constrained within boundaries that increased with stratification temperature, while R_p was constrained within wider, fixed boundaries. These conditions revealed that R_p was largely unaffected by stratification temperature, but had a positive relationship with maturation temperature.

Linear, logistic and exponential functions were used to model R_p and R_s with maturation and stratification temperature respectively. The general forms of these functions are given in equations [15] – [17]; where R is the rate being modelled (either R_p or R_s); T is the temperature, either stratification or maturation temperature as appropriate; and a, b, and c are parameters whose values were varied to obtain an optimised fit.

[15] $$R = aT + b$$

[16] $$R = \frac{a}{1 + e^{-b(T-c)}}$$

[17] $$R = ae^{bT}$$

4.2.3 Parameter estimation

Each of the 9 possible pair wise combinations of linear, logistic, and exponential functions for modelling R_p and R_s were tested with the training data. Each combination was parameterised using all the training data simultaneously to obtain the global best fit, using the same fitting method described in the previous section. The goodness of fit in each case was measured using the coefficient of determination (equation [7]), values of which are shown in Table 4.2 for each model.

The highest scoring models were the logistic-exponential and linear-exponential models, which were both able to reproduce the training data with equal accuracy (R^2 = 0.83). The extra complexity of the logistic-exponential model, caused by the additional parameter compared to the linear-exponential model, did not provide any benefit in terms of predictive power. Therefore, the simpler linear-exponential model was chosen as the optimum model. Equations for R_p and R_s for this model are given in equations [18] and [19], where T_m is the maturation temperature and T_i is the stratification or imbibition temperature.

[18] $$R_p = 1.5605\,T_m - 21.7861$$

[19] $$R_s = 0.0545\,e^{\,0.1799\,T_i}$$

Table 4.2 Results from fitting 9 possible models to the germination training data

R_p model	R_s model	Number of Parameters	Total R^2
Exponential	Exponential	4	0.71
Exponential	Logistic	5	0.62
Exponential	Linear	4	0.64
Logistic	Exponential	5	0.83
Logistic	Logistic	6	0.82
Logistic	Linear	5	0.75
Linear	Exponential	4	0.83
Linear	Logistic	5	0.81
Linear	Linear	4	0.73

Figure 4.7 shows the model outputs plotted with the training data, including predictions of primary dormancy, secondary dormancy and germination probability. Each subplot also indicates the R^2 for the individual time series. Where seeds were very dormant, R^2 scores do not give a good representation of the model fit. Negative R^2 scores can arise when the variance in the data is smaller than the prediction errors, and in very dormant populations where the germination is consistently very low, the variance is also very small. However, visual inspection of the fits show that the model predicts the germination very well overall. The only major exception occurs where seeds were matured at 18°C and incubated at 20°C, which resulted in an unusual zigzag germination pattern. This could simply be an anomaly in the data, or could be evidence of an unusual behaviour under warm maturation and warm

stratification conditions. Figure 4.6 also shows the distribution of observed values plotted against predicted values from which the total R^2 was calculated. This shows a clear correlation between the model predictions and observed germination, which indicates that the model performs well overall.

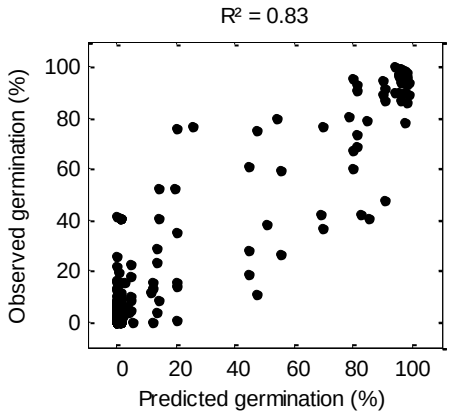

Figure 4.6 The relationship between observed and predicted germination frequencies

The observed germination values were all the data points used to train the model, also shown in Figure 4.1. Predicted values for each data point were generated by the model. A good fit of the model to the training data is revealed if there is a good 1:1 relationship between observed and predicted values.

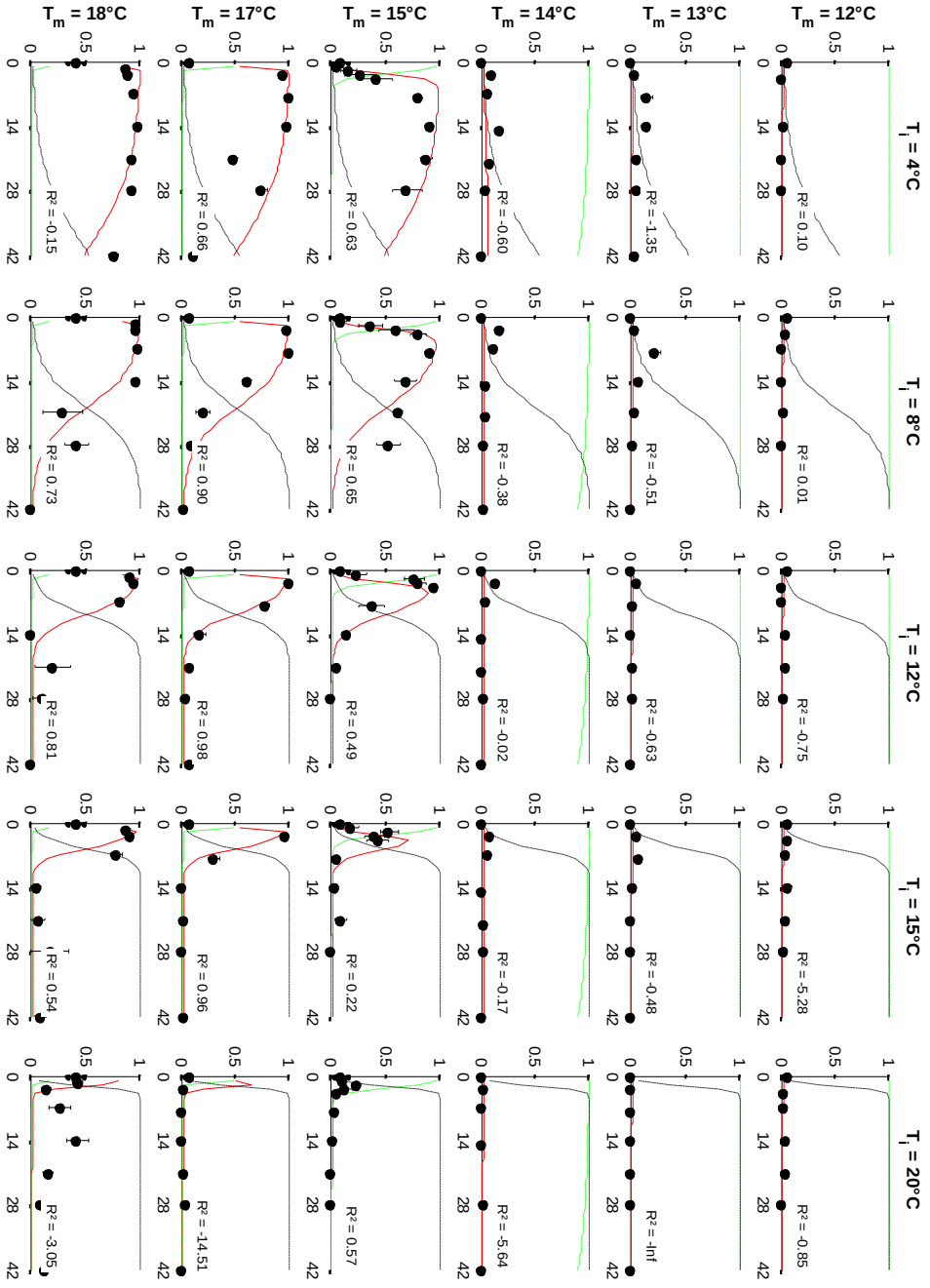

Figure 4.7 Predictions of the dormancy model showing individual fits with the training data

Training data consisted of 30 individual time series experiments at 6 maturation temperatures (rows) and 5 stratification temperatures (columns). Predicted probabilities of primary dormancy (green), secondary dormancy (black), and germination (red) are shown along with the mean and SE of observed germination (filled circles). Individual R^2 are shown for each time series.

4.2.4 Model validation

As a test of the model, stratification experiments were repeated with seeds matured at 2 additional temperatures; 16°C and 20°C. The fitted model was then used to predict the germination. Figure 4.8 shows model predictions for seeds matured at 16°C, along with the germination data and R^2 values. The germination behaviour was similar to that observed previously for seeds matured at 15°C and 17°C. In general primary dormancy was broken within the first week of stratification, and secondary dormancy was induced progressively earlier at warmer temperatures. After stratification at 20°C however, dormancy was not broken and germination remained close to zero, although the model predicted an increase in germination after a stratification period of 1-3 days. Because the variance in the observed germination was very low at this temperature, this resulted in a large negative R^2 value. However, the accuracy of predictions was generally good, and the model explained 88% of the total variance.

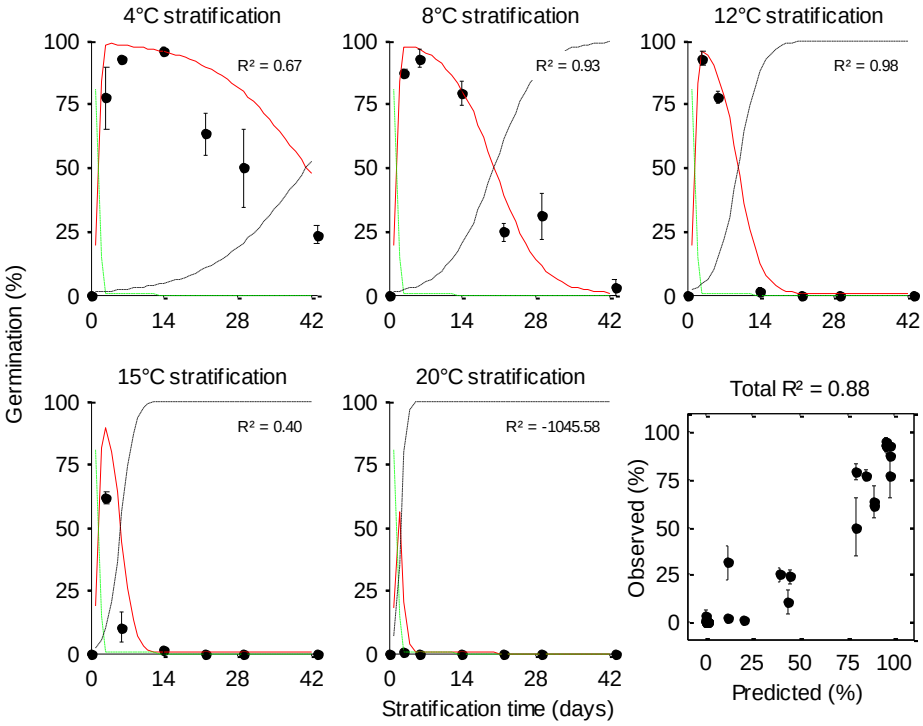

Figure 4.8 Model predictions and observed germination of seeds matured at 16°C

Predicted primary dormancy (green) secondary dormancy (black) and germination (red) percentages are shown as coloured lines. Filled circles represent the mean and SE of 5 replicate seed batches. Individual R^2 are shown for each time series, and the total $R^2 = 0.88$.

Figure 4.9 shows model predictions and germination of seeds matured at 20°C. In contrast to seeds matured at 18°C or below these seeds had very little dormancy, with 87% germinating without requiring any stratification. Because of this low initial dormancy, many seeds germinated in darkness during the stratification treatments; in some cases as many as 100%. Since dark germinating seeds were excluded, the time series data from stratification treatments at 8-15°C were truncated. The prediction that germination would decrease as a result of secondary dormancy induction was therefore untested in some cases. The model did however predict a reduction in germination after stratification at 20°C, although germination unexpectedly increased again after 4 weeks. At 4°C stratification, the model also predicted a gradual reduction in the germination due to secondary dormancy, although the

observed germination remained consistently high. These errors, combined with low total variance in the data resulted in negative R^2 scores at all temperatures, with an overall score of -1.41.

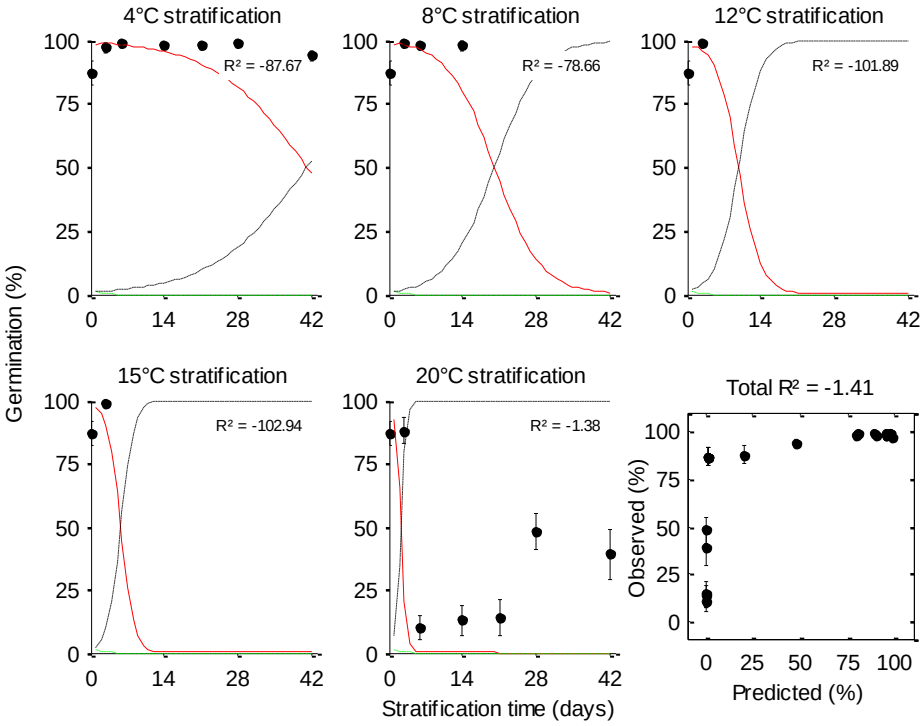

Figure 4.9 Model predictions and observed germination of seeds matured at 20°C

Predicted primary dormancy (green) secondary dormancy (black) and germination (red) percentages are shown as coloured lines. Filled circles represent the mean and SE of 5 replicate seed batches. Individual R^2 are shown for each time series, and the total R^2 = -1.41.

4.2.5 Simulations in variable environments

In order to investigate the model behaviour in natural environments it was necessary to make some adjustments to the model implementation. Using a linear function to calculate the rate of primary dormancy loss theoretically means that zero and negative rates would be possible. Equation [18] can be rearranged to calculate that negative rates would occur at maturation temperatures of <13.96°C. A negative rate

would mean the logistic primary dormancy curve would change direction. Therefore, instead of predicting a loss of primary dormancy over time, the model would predict an increase. In practice this means seeds would be permanently dormant, which fits with the observations during lab experiments (Figure 4.1). However, a rate of zero would result in an indeterminate value of primary dormancy due to a division by zero. This could cause problems when running simulations with temperature data from field experiments, where maturation temperatures of exactly 13.96°C could potentially arise.

To solve this problem an additional condition was imposed on R_p such that it could only assume positive non-zero values. To implement, this required setting a minimum value for R_p, which was chosen to be 10^{-4}. The modified formulation of R_p is shown in equation [20].

[20] $$R_p = \begin{cases} 1.5605\,T_m - 21.7861, & T_m > 13.96°C \\ 10^{-4}, & T_m \leq 13.96°C \end{cases}$$

This modification had no effect on predictions of seed behaviour in lab experiments. The minimum value for R_p was sufficiently small to ensure that primary dormancy loss was predicted to occur extremely slowly when the maturation temperature was 13°C or below. Therefore secondary dormancy was always induced before primary dormancy was lost, ensuring the model correctly predicted that seeds remained dormant.

The maturation temperature for a population of seeds is fixed at the point of maturity. Primary dormancy is therefore unaffected by changes temperature after maturity, and $P(D_p)$ can be calculated for any point in time using equations [11],

[13] and [20]. This is not the case for secondary dormancy however, which is only dependent on the temperature after maturity. In a continuously changing environment it is therefore necessary to use discrete time intervals, and use the average temperature during each interval to calculate changes in $P(D_s)$.

The rate of change, or the gradient of $P(D_s)$ also continuously changes, and depends on the total stratification time x. For instance, in a freshly harvested seed population initial increases in $P(D_s)$ would be slow, as only the most dormant few individuals would gain dormancy (Figure 4.2D). However, increases in $P(D_s)$ would continuously accelerate over time, until the point where half the population had gained secondary dormancy. Beyond this point, $P(D_s)$ would increase progressively slower, until the last few seeds with the lowest initial dormancy finally became dormant.

It is not possible however, to use the total stratification time to determine the rate of $P(D_s)$ increase during any time interval. This is because the increase in $P(D_s)$ is also affected by temperature, and therefore time spent at different temperatures would result in different amounts of dormancy being gained. For example stratification for 1 day at 20°C might result in half the population becoming dormant, but at 4°C only a small fraction might become dormant in the same period. This is because secondary dormancy is induced more slowly at cooler temperatures, and therefore a longer stratification period would be required to reach an equivalent dormancy state. It is therefore necessary to convert between equivalent stratification times at different temperatures in order to correctly calculate changes in dormancy during each time interval.

It is possible to convert stratification times at a different temperature if initial values of $P(D_s)$, and T_i are known. This conversion can therefore be used to calculate the new $P(D_s)$ at each time step in sequence, using known values from the previous time step. In general, at the end of any time step t; x_t is the stratification time, and $x_t{}'$ is its equivalent value after a change in temperature. Equation [21] shows the calculation of $x_t{}'$ where; $P(D_s)_t$ is the known probability of secondary dormancy at the end of interval t; and $(R_s)_{t+1}$ $(A_s)_{t+1}$ are the rate and offset parameters calculated using the temperature from the next interval, i.e. interval $t+1$.

[21] $$x_t{}' = \frac{\ln\left(\frac{1}{1 - P(D_s)_t} - 1\right)}{(R_s)_{t+1}} - (A_s)_{t+1}$$

The value of x at the end of the second interval can then be calculated by adding the length of an interval (Δx) onto $x_t{}'$ (equation [22]). The secondary dormancy at the end of the new time interval can then be calculated with equation [12].

[22] $$x_{t+1} = x_t{}' + \Delta x$$

The concept is illustrated in Figure 4.10, where the dotted line indicates the adjustment in x required to maintain equivalent dormancy after a change in temperature.

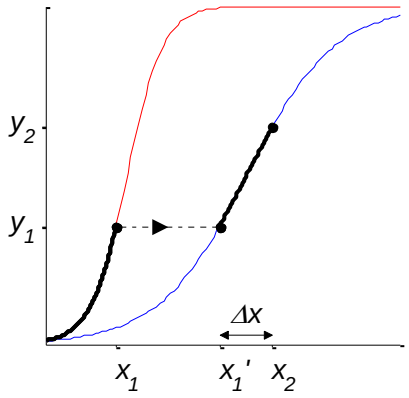

Figure 4.10 An illustration of the frequency of secondary dormancy after a change in temperature

Frequency curves at two different constant temperatures are illustrated by red and blue lines, and the frequency of secondary dormancy in a population switching between the two temperatures is traced by solid black lines. After incubating at the first temperature until x_1, the dormancy frequency is y_1. After a change in temperature the adjusted value of x that maintains equivalent dormancy is shown by x_1'. The length of a time step is shown by Δx, after which the value of x becomes x_2 and the dormancy is y_2.

4.2.6 Model Simulations

Temperature data collected from weather stations was used to simulate seed set on each successive day of the year. Primary dormancy, secondary dormancy and predicted germination probabilities were calculated for up to 60 days after the seed set date. The mean temperature during the week immediately prior to seed set was used as the maturation temperature for primary dormancy calculations, and the secondary dormancy was calculated in time steps of 1 day using the daily mean temperature. The simulation output from York is shown in Figure 4.11 and Figure 4.12. These figures show that seeds set on the majority of dates are predicted to be highly dormant due to maturation temperatures below 14°C (dark areas in Figure 4.11A). Seeds dispersed during most of the year are therefore likely to remain dormant within the soil seed bank, until favourable conditions are able to break secondary dormancy.

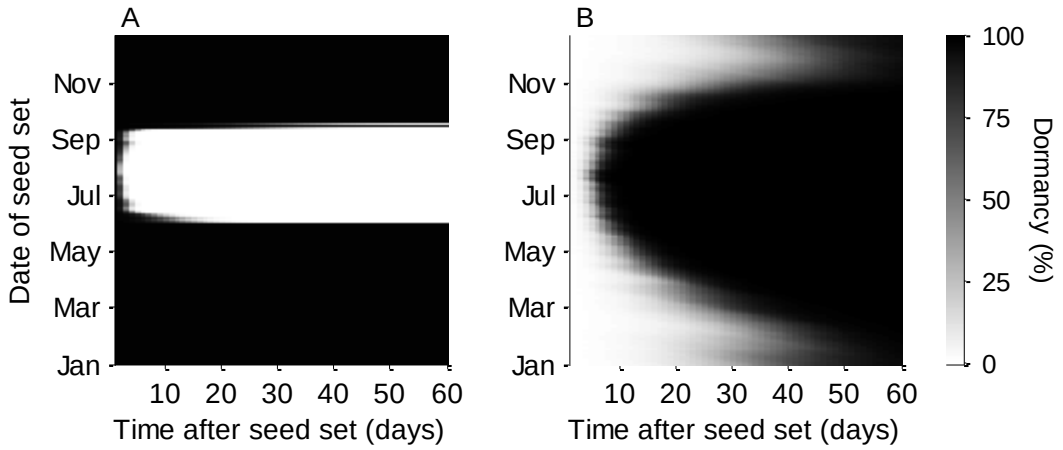

Figure 4.11 Simulations of primary and secondary dormancy in seeds dispersed on different dates in York, UK

Seed set was simulated on each sucessive day of the year. Percentages of primary (A) and secondary dormant (B) seeds on each day after the seed set date (for up to 60 days) are indicated by shading.

However, seed set dates ranging from approximately June to September, result in maturation temperatures above 14°C (light areas in Figure 4.11A). Seeds produced on these dates are predicted to have reduced primary dormancy which is lost quickly after dispersal. The elevated mean daily temperatures on these dates also result in a predicted increase in the rate of secondary dormancy induction (Figure 4.11B), however the corresponding reduction in primary dormancy is sufficient to allow a high germination probabilities for a short period after seed set (dark areas in Figure 4.12A). This is illustrated more clearly in Figure 4.12D, where the predicted peak germination is plotted for each seed set date. This reveals a clear window in summer where the peak germination reaches high levels. Strikingly, the cut off between dates that allow germination and those that do not is very sudden. This switch is caused by the change in primary dormancy when maturation temperatures exceed 14°C, which was also observed in lab experiments (Figure 4.1).

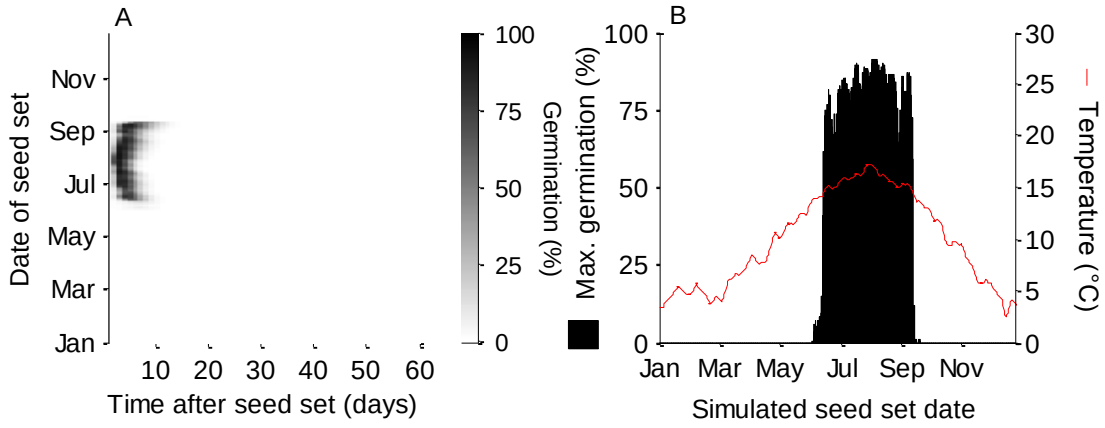

Figure 4.12 Simulations of germination in seeds dispersed on different dates in York, UK

Seed set was simulated on each sucessive day of the year. Germination percentages were calculated for each day after the seed set date (A), using the individual probabilities of primary and secondary dormancy (see Figure 4.11). Shading indicates the likelihood of germination. (B) Peak germination percentages are plotted for each seed set date (black bars), along with the maturation temperature (solid red line).

Simulations were also performed using temperature data from a range of locations, in order to assess the effect of different climates on the predicted dormancy and germination behaviour (Figure 4.13). Periods of reduced primary dormancy were predicted in all locations when maturation temperatures exceeded 14°C. In most cases this caused predicted germination levels to peak around 75% for seeds dispersed in summer. However, in some locations a reduction in germination during the warmest part of the summer was predicted. This was because elevated temperatures caused secondary dormancy induction which was rapid enough to counteract the reduced primary dormancy caused by elevated maturation temperatures.

To investigate this further, artificial climate data was generated by incrementing mean daily temperatures between a minimum winter temperature on the 1st January,

and a maximum summer temperature on the 1st of July. Simulations using different combinations of minimum winter and maximum summer temperatures showed that a period of reduced primary dormancy, and therefore high levels of germination would always occur whenever the mean daily temperature exceeded 14°C (results not shown). However, secondary dormancy induction increasingly suppressed maximum germination levels when temperatures exceeded 20°C, to the extent that germination was reduced to 50% at 25°C, and zero at 29°C.

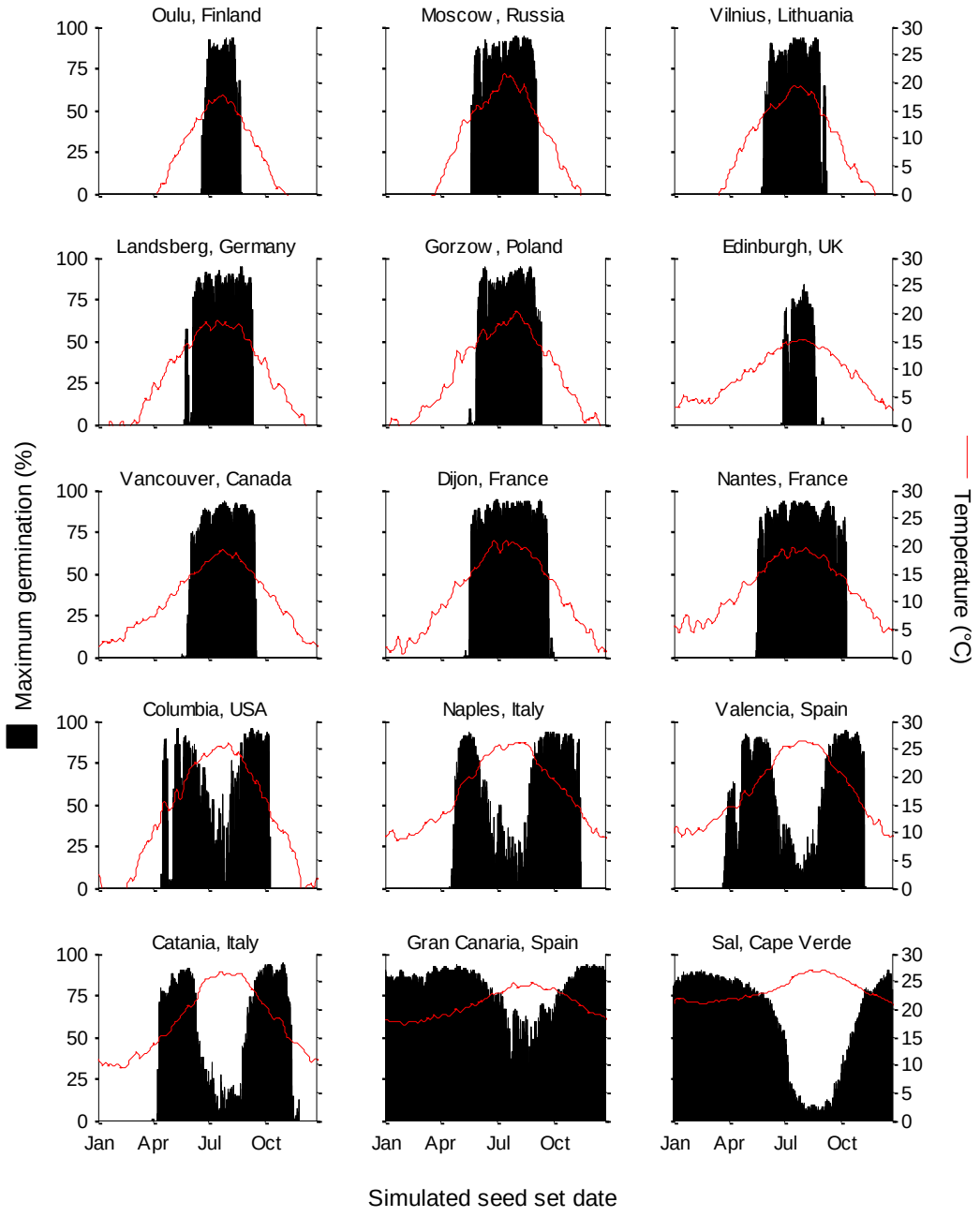

Figure 4.13 Simulations of germination in seeds dispersed on different dates in a range of locations

Seed set was simulated on each successive day in each location, and the peak germination was plotted for each seed set date (black bars). The mean weekly temperature, used as an estimate of the maturation temperature, is also shown (solid red line). In general the predicted germination exceeded 50% when mean daily temperatures were between 14°C and 25°C.

4.3 Discussion

4.3.1 Temperature affects dormancy rather than seed viability

A number of previous studies have shown imbibed *Arabidopsis* seeds undergoing a loss of primary dormancy followed by an induction of secondary dormancy (Derkx and Karssen, 1993b; Toorop et al., 2005). These studies also found that the process of secondary dormancy induction was increased at warmer temperatures, but importantly could still be broken by chilling. The viability of Col-0 and L*er* seeds imbibed for long periods in darkness at 20°C was also confirmed by stratification at 4°C, which induced high levels of germination (Figure 6.9 and Figure 6.10). Additionally, seeds with high initial dormancy due to low maturation temperatures become capable of responding to stratification after a period of dry after-ripening (data not shown). Together these observations show that temperature mainly affects germination by altering dormancy, rather than seed viability.

4.3.2 A new perspective on seed dormancy

The results presented in this chapter challenge some common assumptions about seed dormancy. Firstly, it is often implied that a prerequisite for secondary dormancy induction is primary dormancy loss, and a transition through a period of non-dormancy (Hilhorst, 1998). Currently, the easiest and most common way of measuring dormancy is indirectly; by measuring the germination in favourable conditions. However, since both primary and secondary dormancy result in a lack of germination, it is difficult to distinguish the two states. It is however much easier to identify secondary dormancy following primary dormancy loss, since this would result in a readily observable increase followed by a subsequent decrease in

germination. This is perhaps why this assumption is so widely held, however in 1979 it was suggested that the germination of *Rumex* species after varying durations of stratification at different temperatures could best be explained if loss of primary dormancy and induction of secondary dormancy were occurring simultaneously (Totterdell and Roberts, 1979). This idea was subsequently used to explain the germination behaviour in a diverse range of species, including for example *Polygonum aviculare* (Batlla et al., 2009). *Orobanche* spp. (Kebreab and Murdoch, 1999), *Picea sitchensis* (Jones et al., 1997), as well as *Arabidopsis thaliana* (Derkx and Karssen, 1993b). The mathematical model presented in this chapter illustrates that this behaviour can be predicted very effectively using three simple assumptions; primary dormancy loss and secondary dormancy induction occur simultaneously; both process are dependent on different aspects of environmental temperature; and dormancy states are normally distributed within seed populations.

The fact that similar models have been used to explain this kind of germination behaviour in diverse range of species indicates a potential common mechanism. Furthermore, many additional studies report reductions in total germination following stratification at supra-optimal temperatures (e.g. Windauer et al., 2012; Wang et al., 2013). In light of the current work, it is likely that this reduction is due to accelerated secondary dormancy induction, rather than an incomplete loss of primary dormancy. Therefore, in order to facilitate an improved understanding of seed dormancy it may be necessary to clarify the definition of secondary dormancy. Several prominent reviews use definitions which imply primary dormancy loss and secondary dormancy induction must occur sequentially, (Hilhorst, 1998; Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006), however Bewley and Black state that secondary dormancy induction can occur in mature primary dormant

seeds if conditions are unfavourable for germination (Bewley and Black, 1994; Bewley, 1997). Khan and Karssen, 1980, and more recently Penfield and King, 2009, also define secondary dormancy as states which are induced after seed maturity, a definition which is supported by the work presented here.

Secondly, it is commonly stated that dormancy is broken most effectively by cold temperatures. While this may be true in some circumstances, this work suggests that in fact brief warm imbibition may be just as effective at breaking primary dormancy. This has also been observed in *Arabidopsis* previously. For example, one study reports primary dormancy loss in the L*er* ecotype after stratification at 25°C for 70 hours (Toorop et al., 2005), and similarly Derkx and Karssen, 1993b, describe an experiment in which dormancy was lost after just 10 hours stratification at 30°C, but was completely re-induced after 2 days. This also illustrates that whilst warm temperatures quickly break primary dormancy, they also cause accelerated induction of secondary dormancy.

Furthermore, secondary dormancy is often associated with seasonal dormancy cycles, and consequently changes in secondary dormancy are often presumed to occur slowly (Finch-Savage and Leubner-Metzger, 2006). These results disagree with this viewpoint however, and illustrate that secondary dormancy may be induced very rapidly in some circumstances. Therefore, rather than cold temperatures being more effective at breaking dormancy, it may be more accurate to consider that cold temperatures inhibit secondary dormancy induction, thus ensuring that seeds remain in a non-dormant state for longer.

Finally, the model simulations reveal behaviour that strongly resembles thermoinhibition. This is the inhibition of germination at supra-optimal temperatures,

sometimes also called thermodormancy (Negm et al., 1972). This phenomenon has been studied since at least the 1920's, where Borthwick and Robbins, 1928, showed that germination of lettuce was inhibited at elevated, but sub lethal temperatures. Evenari, 1952, later described this state at 'heat dormancy' and showed that it could be broken to allow germination by removing fruit coats or treatment with O_2, CO_2 or thio-urea. More recent studies have also shown that thermoinhibition occurs as a result of a simultaneous increase in ABA content and sensitivity (Tamura et al., 2006; Leymarie et al., 2008; Toh et al., 2008). Transcriptomic analysis has also highlighted similarities between after ripened seeds imbibed at high temperatures and dormant seeds imbibed at room temperature (Cadman et al., 2006), suggesting a possible common dormancy mechanism. Some authors have even gone as far as suggesting that thermoinhibition and secondary dormancy are one and the same process (Leymarie et al., 2008; Leymarie et al., 2009). This viewpoint is also supported by the model presented in this chapter, in which high temperatures results in rapid secondary dormancy induction and reduction in the maximum predicted germination.

Given the relative lack of understanding of secondary dormancy, and also the potential importance of secondary dormancy in determining the seasonal timing of germination, the field of dormancy research would benefit from wider acknowledgement that thermoinhibition and secondary dormancy may be caused by the same process. Furthermore, induction of secondary dormancy by short high temperature pre-treatments could provide a convenient method for studying secondary dormancy more easily.

4.3.3 General model discussion

The nature of the model raises some interesting points for discussion. Firstly, the models which were best able to reproduce the training data each featured an exponential increase in the rate of secondary dormancy induction with stratification temperature (Table 4.2). This agrees with metabolic theory in ecology, where biological reaction rates are presumed to follow an Arrhenius temperature relationship (Brown et al., 2004). However, the stratification temperature was found to have a much smaller effect on the rate of primary dormancy loss, which was instead mostly determined by the maturation temperature.

In the optimum model the rate of primary dormancy loss, R_p, is calculated as a linear function of maturation temperature. This entails a sudden switch from negative R_p (or 10^{-4} using equation [20]) to positive R_p when the maturation temperature reaches ~14°C. In the case of secondary dormancy however, R_s has an exponential relationship with the stratification temperature. As a consequence secondary dormancy induction occurs more quickly than primary dormancy loss when prevailing temperatures are greater than 20°C, leading to a prediction of thermoinhibition. This behaviour also caused a predicted reduction in the germination of seeds dispersed in summer in the hottest locations (Figure 4.13).

However, there is some uncertainty regarding this prediction due to the fact that elevated maturation temperatures resulted in increased germination in darkness (Figure 4.9). It is therefore uncertain whether seeds matured at high temperatures would be able to delay germination for long enough to enter secondary dormancy. This would mean seeds dispersed in summer would be forced to germinate during the hottest part of the year. However, there was also some evidence that stratification

at 20°C reduced dark germination and allowed secondary dormancy induction in seeds matured at 20°C, although this appeared to be only temporary (Figure 4.9). This suggests incubation and stratification at equally high temperature may be sufficient to induce secondary dormancy and inhibit germination. If this is the case and the model predictions are correct, then the combined effect of primary and secondary dormancy provide an effective mechanism to restrict the germination of newly dispersed seeds to within a specific temperature range. Testing the behaviour of seeds matured and stratified at temperatures above 20°C would therefore be a good test of the model, and would also inform predictions relating to seeds dispersed in summer.

A final point to note is that this model was parameterised using germination data the Col-0 ecotype, which is generally considered to have low dormancy. In general, lower dormancy is associated with high latitude, lower mean annual temperatures, and high summer precipitation (Kronholm et al., 2012), therefore presumably the temperature range in which germination is predicted to occur is one which is favourable for seedling establishment in these environments. It may also be possible to use the methods described in this chapter to parameterise the model for different ecotypes, and examine whether different temperature sensitivities and altered dormancy lead to different predicted behaviour, which could be advantageous in different climates.

4.3.4 Conclusions

Germination behaviour after different maturation and stratification temperature treatments can be explained by simultaneous primary dormancy loss and secondary

dormancy induction. Modelling these processes suggested that the rate of primary dormancy loss has a linear relationship with the maturation temperature, whereas the rate of secondary dormancy induction has an exponential relationship with stratification temperature. Using this model to simulate seed set at different times of year suggested that maturation temperatures below 14°C would prohibit germination of newly dispersed seeds during most of the year. However, seeds dispersed during summer would be likely to germinate within a short time period, resulting in rapid cycling behaviour. The model also predicts a reduction in germination frequencies when mean daily temperatures exceed 20ºC. Thus, primary and secondary dormancy may function to limit germination to within a specific temperature range. However, this prediction also depends on seeds becoming buried and remaining dormant in darkness for long enough to enter secondary dormancy, which may not be possible at high maturation temperatures when germination in darkness is more common.

Chapter 5: Modelling Germination in the Dark

5.1 Introduction

Germination in *Arabidopsis* requires light (Baskin and Baskin, 1983; Cadman et al., 2006; Finch-Savage et al., 2007), which stimulates the expression of phytochrome-activated genes for GA biosynthesis (Yamaguchi et al., 1998). Temperature induced changes in seed dormancy have also been shown to alter seed sensitivity to GA; seeds with higher dormancy generally require larger doses of GA in order to germinate, but this requirement can be lowered by cold stratification (Derkx and Karssen, 1993b). Loss of dormancy is also accompanied by an increase in light sensitivity, causing altered fluence response curves (Cone and Spruit, 1983), and in some cases seeds can become extremely sensitive to very low fluences of light. This very low fluence response (VLFR) can lead to germination being triggered in complete darkness as seeds become sensitive to the low levels of endogenous Pfr present in most seed batches (Cone et al., 1985a).

In the previous chapter, the effects of maturation and stratification temperature on seed dormancy were quantified and used to model the likelihood of germination in light. However, some germination in darkness was also observed due to seeds acquiring the VLFR during stratification. To date, few studies have attempted to quantify the relationship between dark germination and environmental temperature, and the ecological relevance of this response is not well understood. The work in this chapter was therefore aimed at developing a model to predict the likelihood of seeds

acquiring the VLFR during stratification, and hence the probability of germination in darkness. The model was then used to investigate the likelihood and ecological significance of dark germination in different locations and climates.

5.2 Results

5.2.1 The effect of temperature on germination during dark stratification

To quantify the effect of temperature on germination in the dark, freshly harvested seeds matured at 6 different temperatures were imbibed on agar plates and immediately wrapped in foil to exclude light. Plates were then incubated at 5 different temperatures for up to 6 weeks. The germination in darkness was sampled at regular intervals by removing a plate from each incubating population, and counting germinated and ungerminated seeds. The dark germination was then expressed as a percentage of the total number of seeds (Figure 5.1).

There was a clear positive relationship between the maturation temperature and dark germination, which was almost exclusively limited to seeds matured at 17°C and 18°C. The stratification temperature also affected the amount of dark germination observed, which was most common between 8°C and 15°C. Increasing the maturation temperature to 18°C increased this range, although dark germination at 4°C and 20°C was still less common, and was also delayed in comparison to the other stratification treatments. There was also a tendency for seeds to germinate earlier with increasing stratification temperature, although this was not true at 20°C.

A cumulative germination curve was expected from these experiments, however Figure 5.1 shows the germination decreasing in some cases. The most likely explanation for this is seedling mortality. After prolonged stratification in darkness eitiolated seedlings became increasingly pale and degraded, making them difficult to identify. The number of germinated seeds sampled after long incubation periods, and also at warmer temperatures where seedlings degraded more quickly, were therefore likely to have been underestimated.

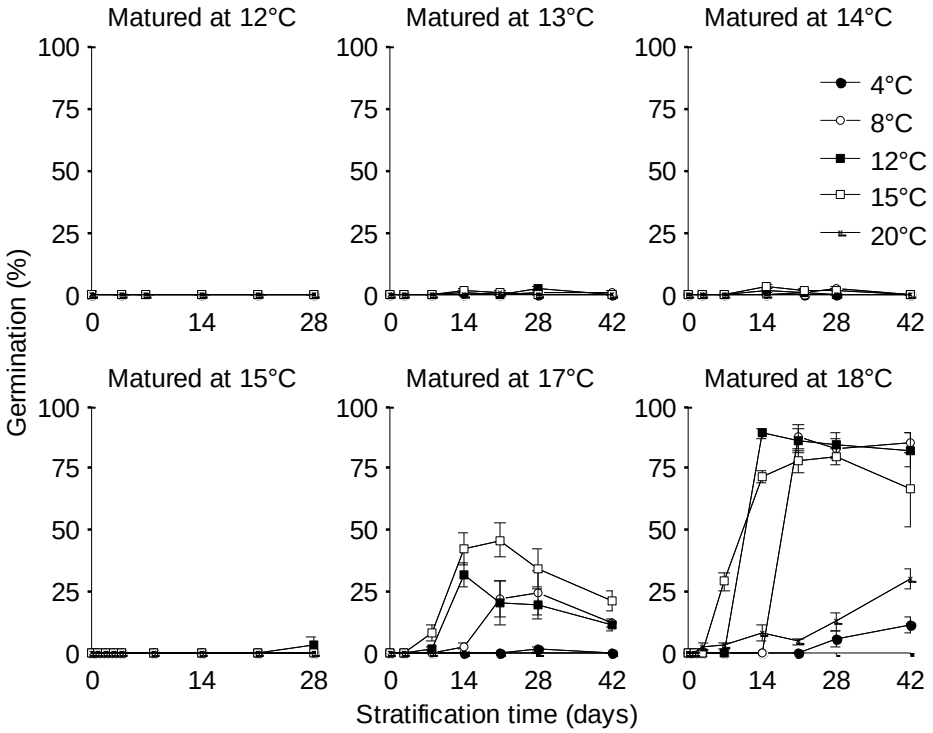

Figure 5.1 The effect of maturation and stratification temperature on germination in darkness

Col-0 seeds matured at 12°C, 13°C, 14°C, 15°C, 17°C, and 18°C were imbibed on water agar, and incubated in darkness at constant temperature (indicated by symbols). Germinated seeds were counted after the stratification time shown, and expressed as a percentage of the total seed count. Data represent the mean and SE or 5 replicate seed batches.

5.2.2 Model development

Model 1: A logistic model of dark germination probability

A logistic function was used to model the cumulative probability of dark germination $P(G_d)$ over time x, using equation [23]. Parameters M, R and A represent the maximum probability of dark germination, the rate of increase and the offset respectively. An example illustration of this model is provided in Figure 5.2.

[23] $$P(G_d) = \frac{M}{1 + e^{-R(x+A)}}$$

The offset parameter A determines the position of the curve along the x-axis. To ensure the predicted dark germination at the start of the experiment was 1%, A was calculated by rearranging equation [23], and substituting $P(G_d)$ for 0.01 and x for 0. This results in equation [24], and also ensures that only parameters M and R require additional modelling with temperature.

[24] $$A = \frac{\ln(M/0.01 - 1)}{-R}$$

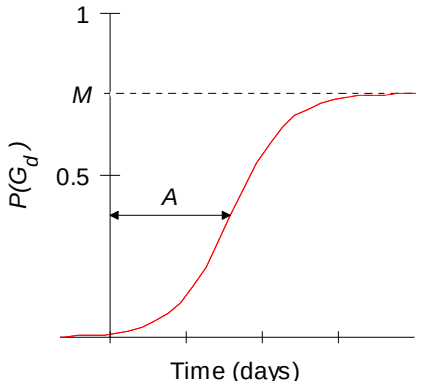

Figure 5.2 An illustration of Model 1: A logistic model of dark germination

The red line indicates the logistic function representing the probability of dark germination, $P(G_d)$ over time. Parameter M determines the maximum or final dark germination probability, parameter A indicates the offset required to position the curve. The rate of increase is also determined by parameter R (not shown).

The training data shown in Figure 5.1 was split into 30 individual germination time series, one for each unique combination of maturation and stratification temperature. The model was then fitted to each data series individually in order to determine optimised values for M and R at each temperature combination. The fitted values of M and R were then plotted against the temperature to determine if any trends were visible (Figure 5.3). Using the parameter values shown in Figure 5.3, the logistic model was able to reproduce the training data in Figure 5.1 with good accuracy (R^2 = 0.96).

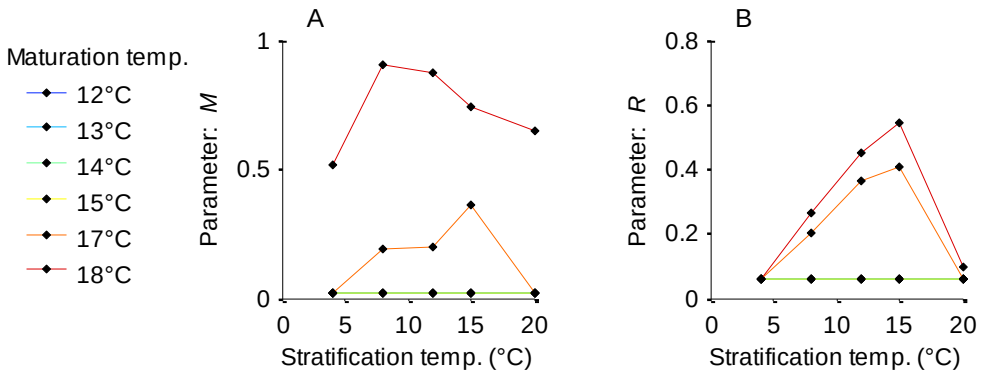

Figure 5.3 Effects of temperature on fitted model 1 parameter values

(A) Parameter M, the maximum probability of dark germination; and (B) Parameter R, the rate of increase in the dark germination probability plotted with stratification temperature. Maturation temperatures are also indicated by colour coding. Parameter values were determined for each temperature combination by fitting the model to data from individual germination time series experiments.

The value of parameter M (Figure 5.3A) peaked between stratification temperatures of 8°C and 15°C, and the height of the peak was affected by the maturation temperature. Very few seeds germinated in darkness if the maturation temperature was 15°C or below, and M did not increase above the lower boundary used by the fitting algorithm.

Equation [25] was formulated to model the combined effect of the stratification temperature (T_i) and maturation temperature (T_m) on M.

[25] $$M = -a(T_i - b)^2 + e^{c(T_m - d)}$$

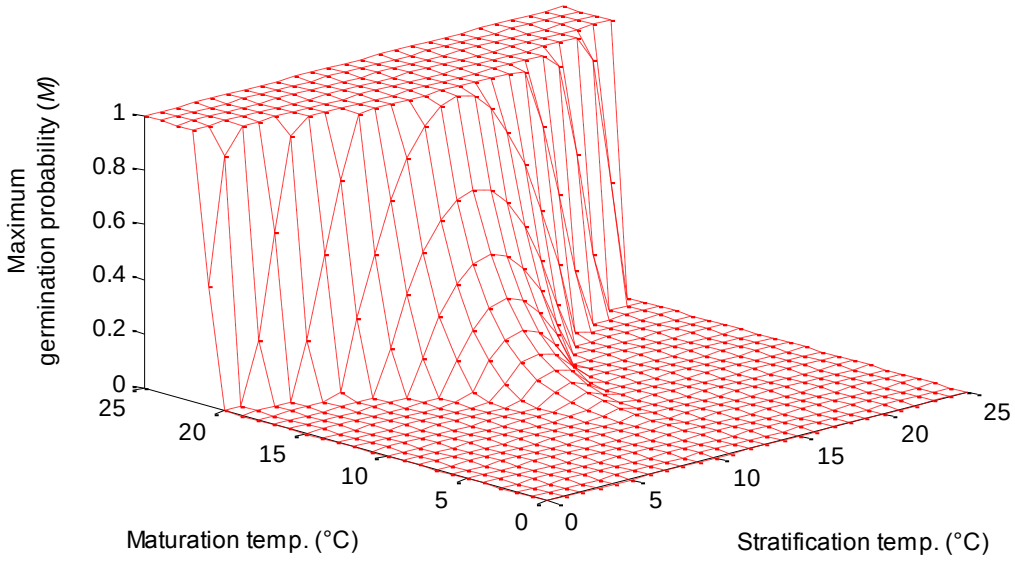

Figure 5.4 An illustration of parameter M, the maximum probability of germination in darkness

The curve was produced with equation [25], using a=0.02, b=12, c=0.4 and d=18. The value of M was also restricted to $0.02 < M \leq 1$.

This function can be plotted in 3 dimensions, with the stratification and maturation temperature on the x- and y- axes, and the value of M on the z-axis (Figure 5.4). The squared term in equation [25] creates a negative quadratic curve with respect to the stratification temperature. Parameter b determines the position of the maximum, and therefore represents the optimum stratification temperature. Parameter a determines the width of the curve, or the degree to which the probability of dark germination decreases at stratification temperatures above and below the optimum. The

maximum value for a negative quadratic of this nature is zero, however the addition of the exponential component in equation [25] shifts the quadratic curve upwards, depending on the maturation temperature. The maturation temperature therefore determines the maximum value of M, which occurs at the optimum stratification temperature. Parameters c and d determine the steepness and the position of the exponential with respect to the maturation temperature. In conjunction with the width of the quadratic, determined by parameter a, the maturation temperature also determines the range of stratification temperatures where dark germination should occur, i.e. where M is greater than zero. In addition, since M represents a probability and cannot exceed 1, and also to avoid complex numbers during the calculation of the offset, M was restricted by limits of $0.02 < M \leq 1$.

Using this model, the maximum amount of dark germination is determined by a combination of the stratification and the maturation temperature. However, the time required for the probability of dark germination to reach M is determined by parameter R. Figure 5.3B shows that R generally increased with stratification temperature. However, when the value of M was low, R also decreased. This was because when there was no germination R had no effect and the fitting algorithm therefore arbitrarily chose the lower boundary value.

For simplicity, a linear equation [26] was chosen to model R with stratification temperature. Parameters f and g are the gradient and the y-intercept. R was also restricted to values >0 to avoid negative rates.

[26] $$R = fT_i + g$$

Model 2: A model of primary and secondary dormancy frequencies in darkness

In the previous chapter, a model was described that predicted the probability of germination in the light based on predicted frequencies of primary and secondary dormancy in seed populations. The results shown in Figure 5.1 also suggest roles for both primary and secondary dormancy in dark germination. For example, there was a clear positive correlation between the maturation temperature and the amount of dark germination, which would be expected if increasing the maturation temperature reduced the level of primary dormancy. Dark germination was also suppressed at 20°C, even in seeds matured at 18°C. This could potentially be explained by increased rates of secondary dormancy induction at warm stratification temperatures, causing germination to be inhibited.

It was hypothesised that the same processes leading to dormancy or germination in light would also apply in darkness. Furthermore, a framework able to explain germination behaviour in both light and dark would indicate a good explanatory model. Therefore, the same basic model used to simulate germination in light was also used here. Formulas for calculating the probability of primary and secondary dormancy, $P(D_p)$ and $P(D_s)$, are given in equations [11-14] in the previous chapter, where x is the stratification time in days, R_p and R_s are the rates of primary dormancy loss and secondary dormancy induction, and A_p and A_s are offset parameters. These probabilities were then used to calculate the overall probability of dormancy $P(D)$, and hence germination $P(G)$ in equations [9 and 10], however $P(G)$ in this case refers to germination in darkness, or $P(G_d)$.

A modification to the basic model was necessary due to differences between seeds which required light to germinate, and seeds which germinated in darkness. By

definition light requiring seeds do not germinate unless exposed to light, and so could enter secondary dormancy during stratification in darkness. In the light model, this caused the probability of germination to decrease after prolonged stratification in darkness. However, seeds which lost their light requirement due to gaining the VLFR were able to germinate in darkness. This germination was cumulative, and increasing the probability of secondary dormancy would not affect seeds that had already germinated. It was therefore necessary to prevent secondary dormancy from causing the germination probability from decreasing. However, it would still be possible for some seeds to enter secondary dormancy before gaining the VLFR. The role of secondary dormancy in this model is therefore to determine the maximum level of dark germination, rather than causing the probability of germination to decrease (Figure 5.5).

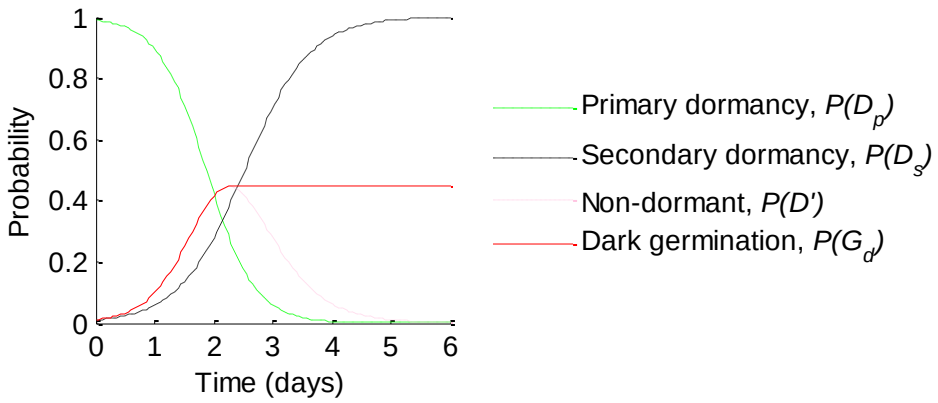

Figure 5.5 An illustration of Model 2: A model of primary and secondary dormancy frequencies

Probabilities of primary and secondary dormancy were modelled over time using logistic functions. These were then used to calculate the total probability of dormancy, and hence germination in the dark. Although the probability of a seed being non-dormant decreased due to secondary dormancy induction after prolonged stratification, germination in the dark is cumulative. The probaility of dark germination was therefore prevented from decreasing.

Initial fitting of R_p and R_s was performed for each combination of maturation and stratification temperature by fitting the model to individual germination time series. These fitted parameter values were then plotted with temperature to determine whether any trends could be seen (Figure 5.6). Values of R_s were also constrained within boundaries that increased with stratification temperature, since warmer temperatures are known to increase the rate of secondary dormancy induction (Totterdell and Roberts, 1979; Derkx and Karssen, 1993b; Toorop et al., 2005).

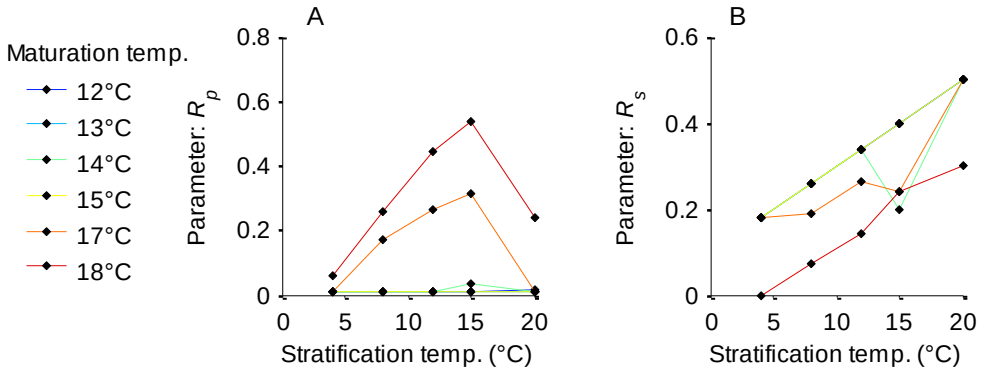

Figure 5.6 Effects of temperature on fitted model 2 parameter values

(A) Parameter R_p, the rate of primary dormancy loss; (B) parameter R_s, the rate of secondary dormancy induction plotted with stratification temperature. Maturation temperatures are also indicated by colour coding. Parameter values were determined for each combination of maturation and stratification temperature by fitting the model to individual data series. Values of R_s were also constrained within boundaries that increased with stratification temperature.

Using the R_p and R_s values shown in Figure 5.6, the model of primary and secondary dormancy could reproduce the training data in Figure 5.1 with good accuracy (R^2=0.96). R_p values peaked at stratification temperatures of around 15°C, but the height of the peak was affected by the maturation temperature (Figure 5.6A). In this respect, R_p behaved similarly to the maximum germination parameter (M) from the previous model, therefore a similar equation was used [27].

[27] $$R_p = -a(T_i - b)^2 + e^{c(T_m - d)}$$

Additionally, the value of R_p was limited to values >0, since a rate of zero would result in an indeterminate value during the calculation of the offset, A_p. Unlike M from the previous model, R_p does not represent a probability and so has no upper limit.

To maintain consistency with the model of germination in light, an exponential function was used to model R_s with stratification temperature [28].

[28] $$R_s = fe^{gT_i}$$

Model 3: A model of primary and secondary dormancy with a thermal time model of germination

Model 2 was designed to demonstrate that simultaneous temperature-dependent primary dormancy loss and secondary dormancy induction can explain the germination behaviour of imbibed seeds when they are incubated in continuous darkness, as well as when they are placed in light. However, one significant difference between model 2 described above, and the model in light described in the previous chapter is the effect of the stratification temperature on the rate of primary dormancy loss, R_p. The main factor affecting R_p in the light was the maturation temperature (Figure 4.5A). A small effect of stratification temperature on R_p in the light was observed, however this was negligible and ignoring it still allowed the model to predict the germination with good accuracy (Figure 4.6). In contrast however, the stratification temperature had a much larger effect on R_p in the dark,

which was evident by the earlier germination of seeds stratified at warmer temperatures (Figure 5.1). This was also reflected during the initial fitting of parameters R and R_p in models 1 and 2 (Figure 5.3B and Figure 5.6A), which were clearly affected by the stratification temperature.

Rather than suggesting that temperature affected rates of primary dormancy loss differently in light and darkness, it was hypothesised that the effect of stratification temperature in the dark may have been due to differences in the germination rate caused by temperature. During the collection of the training data, seeds germinated in the dark at temperatures ranging from 4°C to 20°C, whereas all germination in the light occurred after transferring seeds to 22°C. Germination in the dark therefore took place over a much wider range of temperatures than germination in light, potentially leading to large differences in germination rates.

It is generally acknowledged that like many other physiological processes, the rate of germination has a positive linear relationship with temperature in the sub-optimal range (Hegarty, 1973; Roberts, 1988). This relationship has been used as the basis for modelling germination rates in many species using the concept of thermal time (Garcia-Huidobro et al., 1982; Washitani and Takenaka, 1984; Washitani, 1985), and later hydrothermal time (Gummerson, 1986; Alvarado and Bradford, 2002; Bradford, 2002). Models such as these generally state that the total thermal time (TT) required to complete germination, measured in °C days, can be calculated by multiplying the difference in °C between the environmental or stratification temperature (T_i) and the minimum temperature required for germination (T_b), by the number of days taken to germinate (t_G). This relationship can therefore be rearranged to calculate t_G for any given temperature [29], which can be considered to be the time difference between

the moment a seed loses dormancy, and completion of germination (i.e. protrusion of the radicle). The probability of germination in the dark at time t, is therefore equal to the probability that a seed was not dormant, $P(D')$, at time t-t_G [30].

[29] $$t_G = TT/(T_i - T_b)$$

[30] $$P(G_d)_t = P(D')_{t-t_G}$$

[31] $$P(D') = 1 - P(D)$$

$P(D')$, the probability of non-dormancy, was calculated using equation [31]; where $P(D)$ is the probability of dormancy, which was calculated using equation [10]. This used individual probabilities of primary and secondary dormancy, which were themselves modelled over time using equations [11] and [12]. Parameters R_p and R_s represent the rates of primary dormancy loss and secondary dormancy induction, and A_p and A_s are offset parameters, calculated using equations [13] and [14]. In order to maintain consistency with the model of germination in the light, linear and exponential functions were also used to model R_p and R_s in the dark (equations [18] and [19]) however values for parameters a, b, c, and d were determined specifically for dark germination by fitting with the dark germination data.

Additionally, germination in the dark is cumulative and so cannot decrease once it has reached a maximum. Therefore, while the probability of non-dormancy can decrease as a result of secondary dormancy induction, the germination was prevented

from decreasing. This model is illustrated in Figure 5.7, and a full list of symbols used in all three models is provided in Table 5.1.

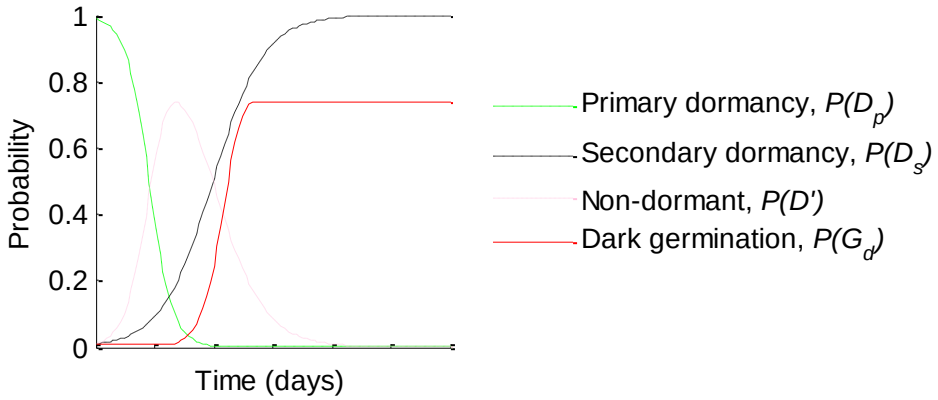

Figure 5.7 An illustration of Model 3: A model of primary and secondary dormancy with a thermal time model of germination

Individual probabilities of primary and secondary dormancy were modelled over time using logistic functions. These were then used to calculate the total probability of a non-dormant seed. However, germination of non-dormant seeds was delayed depending on the temperature, which was presumed to alter the germination rate. This delay was calculated using a simple thermal time model. Although the probability of a non-dormant seed decreased due to secondary dormancy induction after prolonged stratification, germination in the dark is cumulative. Therefore, the probaility of dark germination was prevented from decreasing once the maximum was reached.

Table 5.1 A list of symbols used in dark germination models

Symbol	Meaning	Applies to model
$P(D_p)$	The probability, or relative frequency of primary dormancy	2 and 3
R_p	The rate parameter for primary dormancy loss	2 and 3
A_p	The offset parameter for the primary dormancy equation	2 and 3
$P(D_s)$	The probability, or relative frequency of secondary dormancy	2 and 3
R_s	The rate parameter for secondary dormancy induction	2 and 3
A_s	The offset parameter for the secondary dormancy equation	2 and 3
T_i	Stratification, or imbibition temperature (°C)	All
T_m	Seed maturation temperature (°C)	All
$P(D)$	The total probability or relative frequency of dormancy within a population	2 and 3
$P(G_d)$	The probability of germination in the dark	All
$P(D')$	The probability a seed is not dormant, or 1- $P(D)$	2 and 3
x	Stratification time (days since imbibition)	All
TT	Thermal time required for germination (°C days)	3
T_b	The base germination temperature, i.e. the minimum temperature required for germination	3
t_G	Time required for germination (days)	3
Lower case parameters; a, b, c, d, f, g	Parameters whose values were determined by fitting the models to the training data	All
e	The exponential constant	All
M	Maximum dark germination	1
R	Rate of dark germination	1
A	The offset for the dark germination equation	1

5.2.3 Parameter estimation

Parameter values were estimated by fitting each model to all the training data simultaneously to obtain the global best fit. This was done using Matlab (Mathwoks) *fit* function as described previously. Fitted parameter values are listed in Table 5.2.

Table 5.2 Fitted parameter values for dark germination models

Model 1		Model 2		Model 3	
Parameter	Value	Parameter	Value	Parameter	Value
a	2.36 x 10^{-2}	a	7.00 x 10^{-3}	a	1.07
b	10.6	b	13.5	b	-18.0
c	0.972	c	0.610	c	6.10 x10^{-2}
d	17.9	d	19.0	d	0.141
f	3.00x10^{-2}	f	0.101	T_b	1.64
g	2.22x10^{-14}	g	7.90 x10^{-2}	TT	94.3

Overall, the three models reproduced the training data well, and were able to explain a large proportion of the total variance in dark germination (Figure 5.8). The performance of the three models was also similar, with R^2 values ranging from 0.83 to 0.89. The best model overall was model 3, the dormancy model combined with a thermal time model of germination. Fits of each of the three models with the training data are shown in Figure 5.9. Only plots from maturation temperature of 15°C and above are shown, because below 15 °C dark germination was minimal and model predictions were close to zero in all cases.

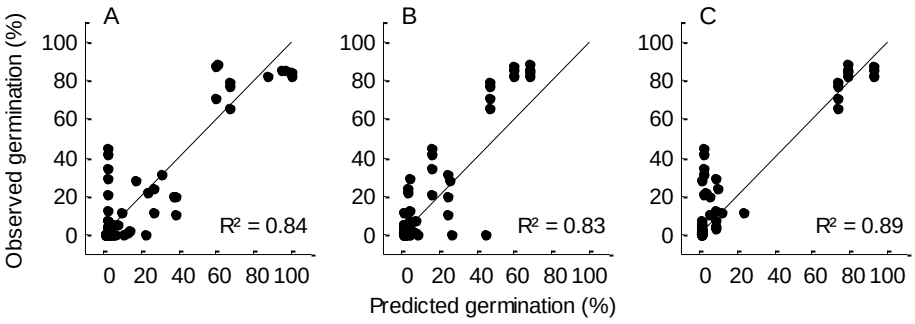

Figure 5.8 The relationship between observed and predicted dark germination frequencies

The observed germination data was used to train the three dark germination models; (A) Model 1, (B) model 2 and (C) model 3. Predicted values were then generated by each model. A good fit with the training data is revealed if points are close to the 1:1 line (solid black line).

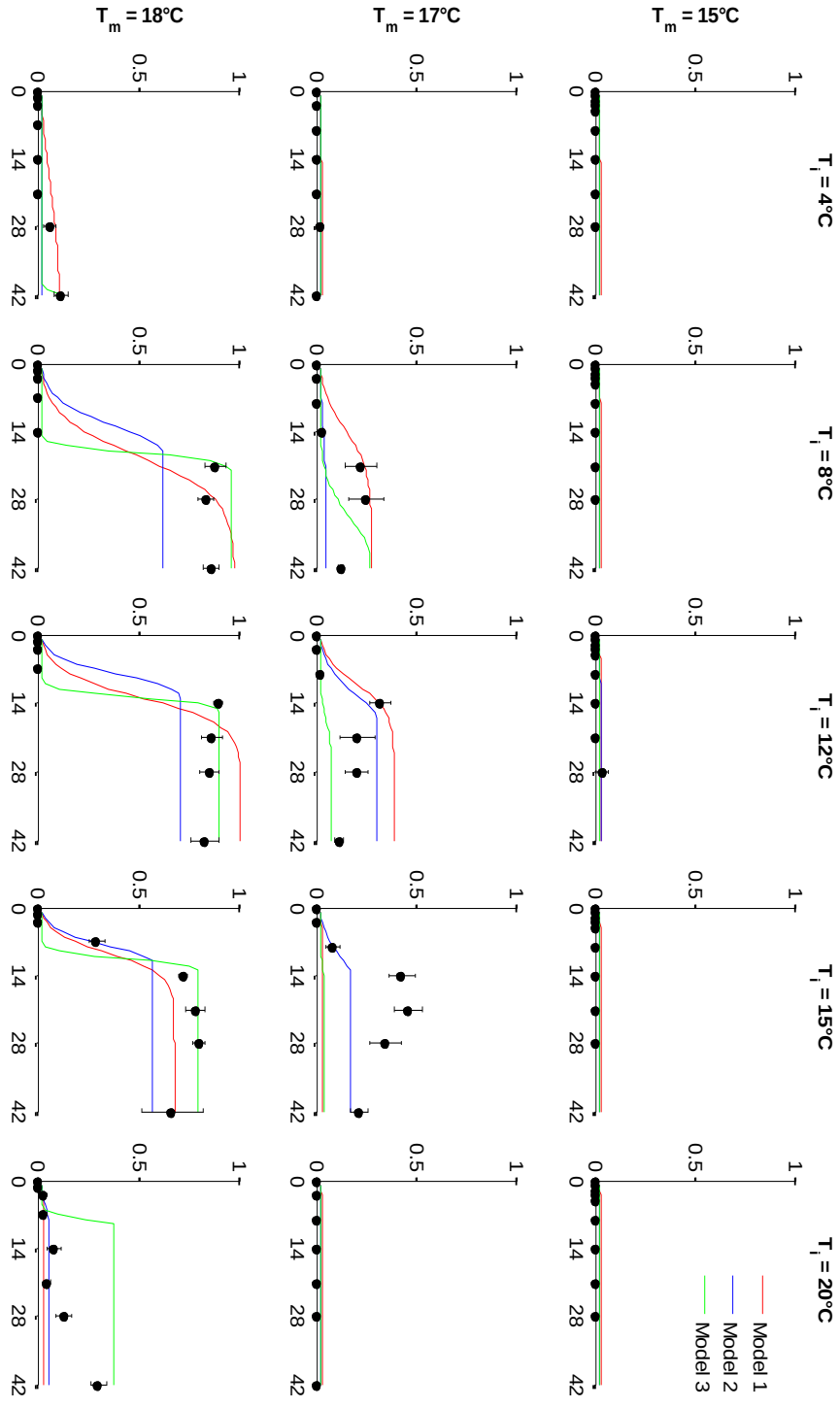

Figure 5.9 Predictions of the three dark germination models showing individual fits with the training data

The training data consisted of 30 individual time series experiments at 6 maturation temperatures (rows; not all are shown) and incubated in darkness at 5 stratification temperatures (columns). Probabilities of dark germination predicted by model 1 (red), model 2 (blue) and model 3 (green) are indicated by solid lines, along with mean and SE of observed dark germination (black circles).

For model 1, the fitting algorithm was unable to find a solution which accurately reproduced the maximum germination (M) at all stratification temperatures. This might suggest that the true model is not symmetrical at sub- and supra-optimal temperatures. Parameter R determined when the maximum germination was reached, which was fairly accurate in most cases. However the predicted increase was too slow, and tended to result in an over-estimation of dark germination at earlier time points.

Model 2 tended to underestimate the maximum germination, which in this case was determined by the interaction of primary and secondary dormancy. This suggests that either the rate of primary dormancy loss was underestimated, or the rate of secondary dormancy induction was overestimated. However if R_p was increased, dark germination would be predicted to occur earlier than it was observed. A linear model of R_s was also substituted for the exponential model in an attempt to reduce estimates of R_s. However, this also resulted in a worse fit with the training data (R^2=0.69; data not shown).

Model 3 resulted in the smallest overall prediction errors, and was able to reproduce the abrupt change from almost zero germination to high germination in seeds matured at 18°C. This was because primary dormancy loss was predicted to occur rapidly due to the relatively warm maturation temperature, however germination was delayed due to the effect of the stratification temperature.

5.2.4 Model validation

Aside from fitting with the training data, each model was also evaluated using additional data that was not used for parameterization. This data was collected using

seeds matured at two further temperatures; 16°C and 20°C, and stratified at 5 different temperatures as before. These maturation temperatures were chosen firstly because 20°C is higher than any maturation temperature used for parameterisation of the models. This additional data should therefore provide a good test of model predictions. Secondly, a decrease in the maturation temperature from 17°C to 15°C was sufficient to reduce the dark germination to zero (Figure 5.1). Consequently, the sensitivity to the maturation temperature was expected to be high at around 16°C, therefore also providing a good test of the model.

Results of dark germination after maturation at 16°C, as well as predictions from each model are shown in Figure 5.10. Dark germination was limited to 8°C and 12°C stratification temperatures. This narrower range of permissive germination temperature is consistent with an increase in initial dormancy levels, compared with seeds matured at higher temperatures. However, all three models underestimated germination at these stratification temperatures. All three models did however correctly predict minimal germination at the 3 other stratification temperatures. Although, due to the low overall variance this was not reflected in R^2 scores which were 0.08, -0.05 and -0.13 for models 1, 2 and 3 respectively. Model 1 therefore performed marginally better than the other two, largely because predictions for the 12°C stratification data were closest to observed values.

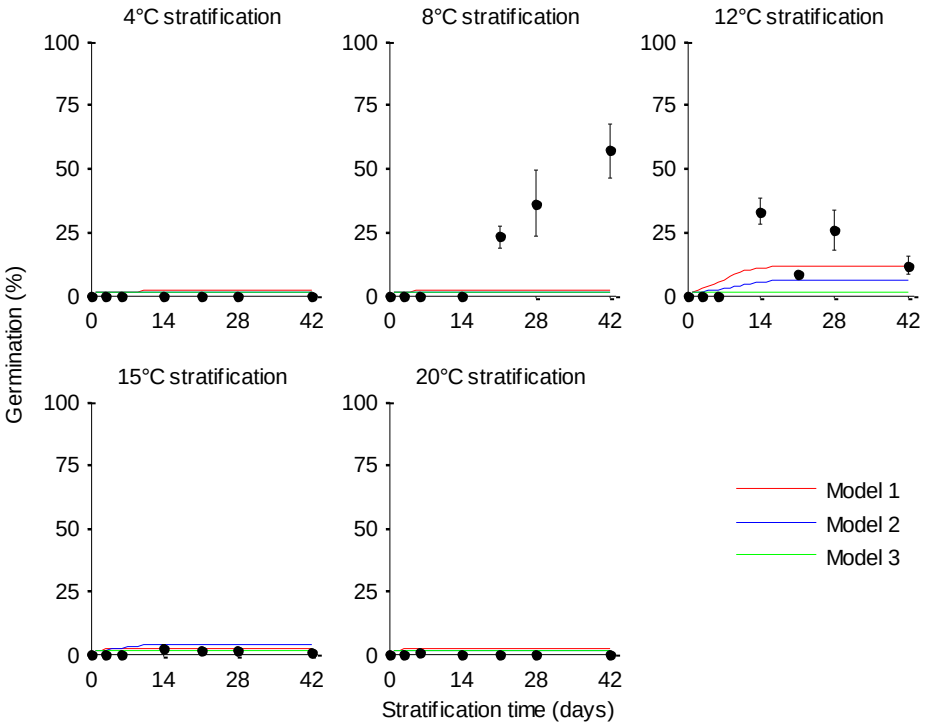

Figure 5.10 Model predictions and observed dark germination in seeds matured at 16°C

Predictions made by model 1 (red), 2 (blue) and 3 (green) are shown by solid lines. Filled circles represent the mean and SE of 3 replicate seed batches. R^2 values for models 1,2 and 3 were 0.08, -0.05 and -0.13 respectively.

When seeds were matured at 20°C, high levels of dark germination were observed after stratification at 4°C, 8°C, 12°C, and 15°C (Figure 5.11). This occurred after 6 weeks at 4°C, 3 weeks at 8°C and after only 1 week at 12°C and 15°C. The timing of germination was therefore related to the stratification temperature, with seeds stratified at low temperatures requiring longer to germinate. Fewer seeds germinated during dark stratification at 20°C, and a maximum of 24% was observed after 4 weeks.

Each model predicted high levels of dark germination at all stratification temperatures. This led to inaccuracy for seeds stratified at 20°C, which were overestimated by all three models. Models 1 and 2 also incurred large errors by incorrectly predicting the timing of germination. Model 1 predicted a very gradual increase in the germination frequency, while model 2 predicted a very rapid increase which occurred soon after imbibition. In reality the increase in germination was rapid, however the timing of this increase was dependant on the stratification temperature. In this respect model 3 provided the best predictions, and this was reflected by the highest R^2 score (0.70). R^2 scores for model 1 and 2 were 0.34 and -0.32 respectively.

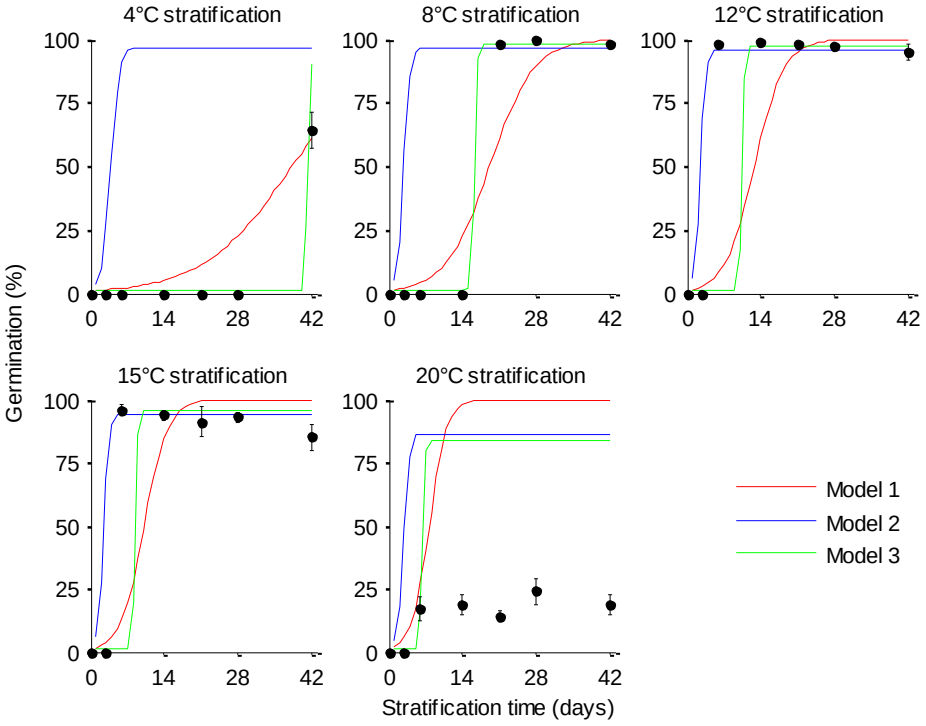

Figure 5.11 Model predictions and observed dark germination in seeds matured at 20°C

Predictions made by model 1 (red), 2 (blue) and 3 (green) are shown by solid lines. Filled circles represent the mean and SE of 3 replicate seed batches. R^2 values for models 1,2 and 3 were 0.34, -0.32 and 0.70 respectively.

In summary, none of the models were able to accurately predict the behaviour of seeds from both additional maturation temperatures. All of the models produced similar predictions for seeds matured at 16°C, and all underestimated the germination at 8°C and 12°C to a similar degree. There was therefore not much to distinguish the models based on this data. However, model 3 provided the best predictions for seeds matured at 20°C as a result of predicting the timing of germination with greater accuracy than either of the other two models. Consequently model 3 had the greatest overall performance, and was able to explain 72% of the total variance over the two maturation temperatures. Model 3 was therefore chosen as the preferred model.

5.2.5 Simulations in variable environments

In the previous chapter, some adjustments were required in order to run simulations in fluctuating temperature environments. Because the current model also uses the same basic mechanism to model dormancy, the same adjustments were also required. Briefly, these included setting a minimum value of R_p to ensure zero and negative rates were avoided, and converting the continuous model of secondary dormancy into a discrete model, in which the average daily temperature was used to calculate the change secondary dormancy in daily intervals. An extra step was also added to the secondary dormancy model, which involved the calculation of equivalent stratification times at different temperatures. This was necessary to maintain the estimate of the population's dormancy after a change in temperature, since the change in dormancy occurring during each time step depends on the initial dormancy

at the start of the time step. This was described in section 4.2.5 with equations [21] and [22], and the same method was also used here.

The thermal time model was also converted to a discrete model. The thermal time accumulated each day $\theta(t)$ was calculated using equation [33], until the cumulative total reached the threshold (TT). The number of days required was then used to calculate the timing of germination.

[33] $$\theta(t) = \begin{cases} (T_i(t) - T_b), T_i(t) \geq T_b \\ 0, T_i(t) < T_b \end{cases}$$

5.2.6 Model predictions

To investigate how dark germination might be affected by seed set timing, and by climates in different locations, simulations were performed using temperature data collected from a range of weather stations. Figure 5.12 and Figure 5.13 were generated by running simulations using temperatures in York UK, and are presented here to demonstrate the typical output and behaviour of the model.

The rate of primary dormancy loss, was calculated using a linear function which increased with maturation temperature. This meant primary dormancy loss was negligible until the maturation temperature exceeded 16.9°C. The maturation temperature was estimated by calculating the mean temperature during the week prior to the simulated seed set date, and for the majority of the year maturation temperatures were below this threshold. The model therefore predicted that seeds set on most dates would have sufficient primary dormancy to prohibit germination in the dark (black areas in Figure 5.12A). However, maturation temperatures did exceed

16.9°C on an small number of dates, which resulted in loss of primary dormancy within a few days (white area in Figure 5.12A).

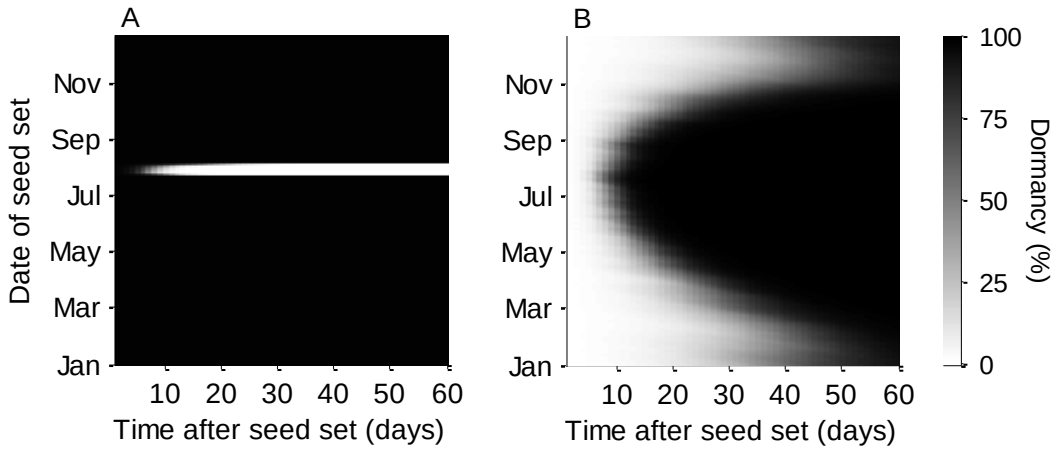

Figure 5.12 Simulations of dark dormancy in seeds dispersed on different dates in York, UK

Seed set was simulated on each sucessive day of the year. Percentages of primary (A) and secondary dormant (B) seeds on each day after the seed set date (for up to 60 days) are indicated by shading.

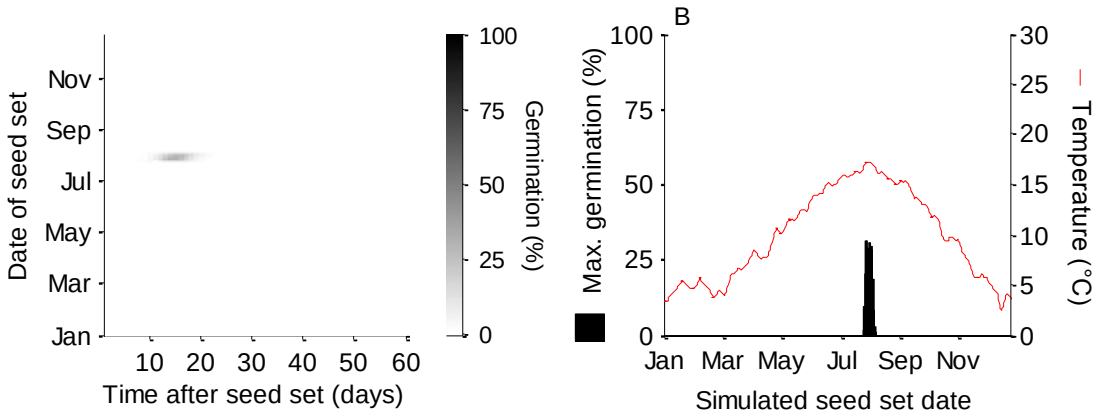

Figure 5.13 Simulations of dark germination in seeds dispersed on different dates in York, UK

Seed set was simulated on each sucessive day of the year. Dark germination percentages were calculated for each day after the seed set date (A), using the individual probabilities of primary and secondary dormancy in the dark (see Figure 5.12). Shading indicates the likelihood of dark germination. (B) Maximum dark germination percentages are plotted for each seed set date (black bars), along with the maturation temperature (solid red line).

The rate of secondary dormancy induction was calculated with an exponential function that increased with environmental temperature. Rates of secondary dormancy induction were therefore greatest in the summer when the temperature was highest (Figure 5.12B). This opposes the reduced primary dormancy of seeds set in the summer, and limits dark germination to a maximum of 32% (Figure 5.13). There was also a delay in the predicted timing of germination due to the effect of temperature on the germination rate, and although primary dormancy was lost within a few days of dispersal (Figure 5.12A), germination was predicted to occur approximately 7 days later (Figure 5.13A).

A similar pattern of behaviour was also observed when the simulations were repeated using temperature data from other locations (Figure 5.14). Again, dark germination was only predicted when the maturation temperature (i.e. the mean weekly temperature) exceeded 16.9°C. This meant that in most locations, particularly in northern latitudes which tended to have cooler climates, dark germination was limited to seeds set on a small number of dates during the hottest part of the year. As an extreme example, no dark germination was predicted in Edinburgh during any part of the year because mean weekly temperatures never exceeded 16.9°C (max. mean daily temp. = 16. 1°C). At the opposite extreme, the mean weekly temperature in Cape Verde never dropped below this threshold. The model therefore predicted that high proportions of seeds would germinate in darkness irrespective of the seed set date.

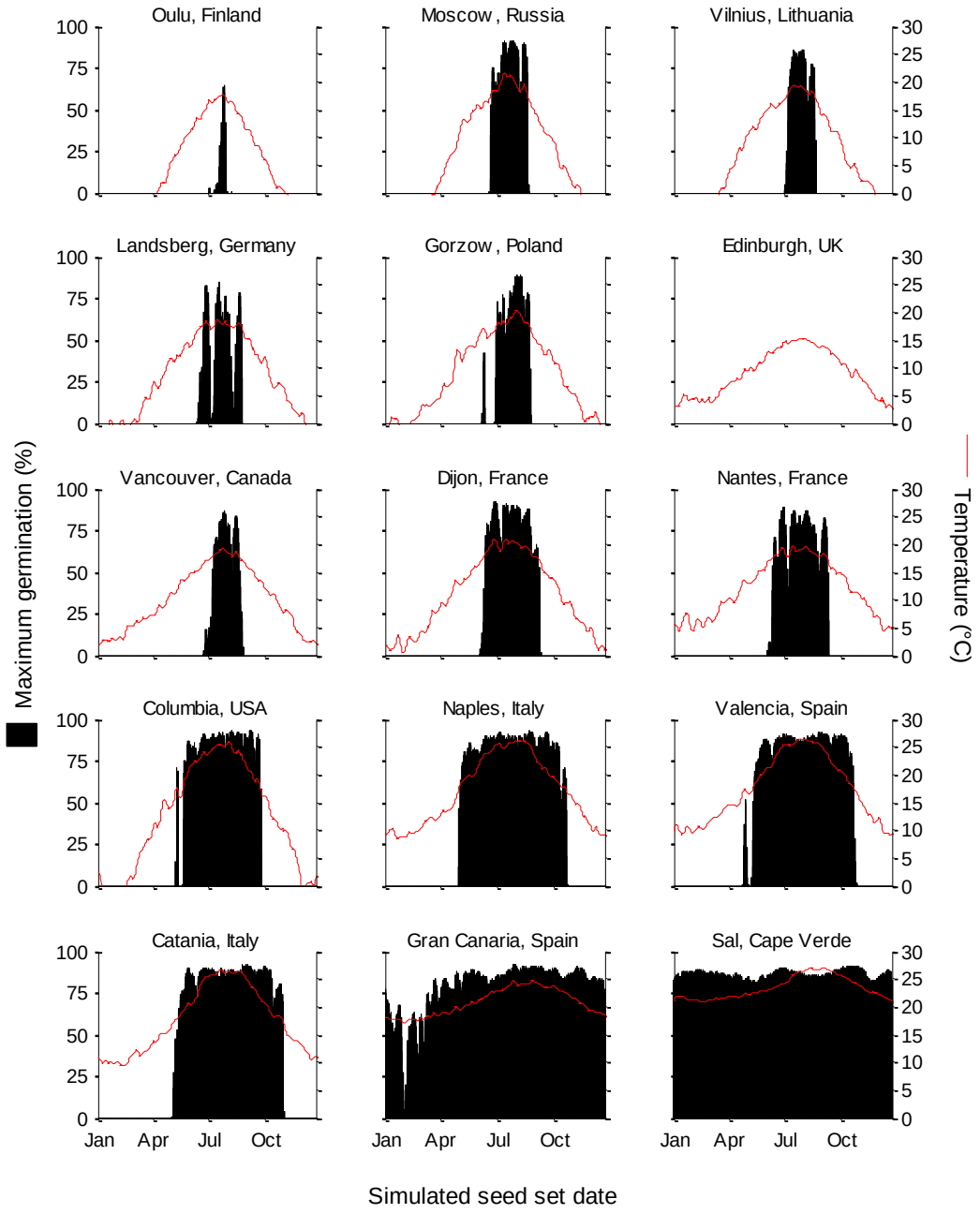

Figure 5.14 Simulations of dark germination in seeds dispersed on different dates in a range of locations

Seed set was simulated on each date in each location, and the maximum dark germination probability was plotted for each seed set date (black bars). The mean weekly temperature, used as an estimate of the maturation temperature, is also shown (solid red line). In general, the predicted dark germination exceeded 50% when mean daily temperatures were between 18°C and 31°C.

Figure 5.14 also illustrates the model's high sensitivity to the maturation temperatures around the threshold of 16.9°C. The temperature data used in the simulations was averaged over 10 years, which had the effect of decreasing the amount of random temperature fluctuations, and resulted in a relatively smooth annual temperature cycle. However, smaller fluctuations were still present in the averaged temperature data and these were still capable of having a large effect on the predicted germination, causing the jagged appearance of the graphs in Figure 5.14. Because of this sensitivity, variation from year to year is expected to be large, particularly when mean daily temperatures are close to the 16.9°C threshold. The germination on any given date could be therefore very difficult to predict in advance without accurate temperature forecasts.

In the previous chapter, it was predicted that in hot climates such as Cape Verde, germination in the light would be suppressed during the summer (Figure 4.13). This was because temperatures above 25°C caused increased secondary dormancy induction which was sufficient to counteract the reduced primary dormancy caused by warmer maturation temperatures, resulting in thermoinhibition. In contrast, the model of dark germination predicted that a much higher temperature would be required to suppress the VLFR in warm matured Col seeds. Additional simulations using a range of artificially generated climate data suggested that the probability of dark germination would be greater than 50% in temperatures ranging from 18°C to 31°C, and the temperature would have to exceed 36°C to reduce dark germination to zero. In Cape Verde, the mean daily temperature ranged from 21°C to 27°C, which is well within the range predicted to result in high levels of dark germination.

5.3 Discussion

5.3.1 Effects of temperature on germination in darkness.

The results in this chapter have shown that temperature has a large effect on germination in darkness in *Arabidopsis*. Virtually no germination in darkness occurred if seeds were matured at 15°C or below, but this increased to almost 100% if the maturation temperature was increased to 18°C or above. Dark germination was also most common in seeds stratified at temperatures between 8°C and 15°C, and was reduced or suppressed completely at temperatures outside of this range. However, increasing the maturation temperature to 18°C or 20°C did increase this range, showing that both pre- and post-maturation temperatures play equally important roles in determining whether dark germination takes place, and these effects are likely to be linked to seed dormancy.

These results are consistent with previous observations of dark germination in lettuce seeds (Blaauw-Jansen and Blaauw, 1975; Small et al., 1979), which originally led to the study of fluence response curves in *Arabidopsis* (Cone and Spruit, 1983; Cone et al., 1985a) and other species (Kendrick and Cone, 1985; Takaki et al., 1985; Rethy et al., 1987). Pre-treatment of seeds at low temperature was also found to reduce dormancy, and increase proportions of seeds germinating in darkness (VanDer Woude and Toole, 1980). This may also be because dark reversion of Pfr is temperature dependent (Taylorson and Hendricks, 1969; Schäfer and Schmidt, 1974). Pre-existing Pfr would therefore remain present for longer periods during stratification at low temperatures, while seeds simultaneously gain sensitivity via the VLFR, as dormancy is lost. Conversely, Pfr would revert to Pr in darkness more quickly at elevated temperatures, counteracting any increase in sensitivity to Pfr occurring due to dormancy loss. Accelerated dark reversion of Pfr to Pr may explain

why dark germination was reduced at 20°C, compared to lower stratification temperatures (e.g. Figure 5.11). Batch effects have also been documented (Cone and Spruit, 1983), which are likely to be caused by different seed maturation conditions resulting differences in primary dormancy. The work described in this chapter is however the first attempt to quantify the effects of both seed maturation and post-harvest stratification temperatures on and dark germination.

5.3.2 Dark germination can be explained by the effects of temperature on dormancy and germination rates

Three models were developed and parameterised using data collected under laboratory conditions, and each was able to reproduce the training data with similar accuracy (Figure 5.8). Modes were also validated with data from seeds matured at two additional temperatures; 16°C and 20°C. All three models generally underestimated dark germination of seeds matured at 16°C (Figure 5.10). This may be due high sensitivity to maturation temperature within the range 15°C to 17°C, which resulted in an abrupt change in behaviour (Figure 5.1). Predicting the correct behaviour at this maturation temperature would therefore benefit from more data in order to determine parameters more accurately.

However, the behaviour of seeds matured at 20°C was predicted most accurately by model 3 (Figure 5.11). This model simulated the effects of temperature on primary dormancy loss and secondary dormancy induction in the same way as the model of germination in light, with an added thermal time component to explain the effect of stratification temperature on the germination rate. This model outperformed the other two particularly well at low temperatures, which led to a greater delay in the

germination. This suggests that temperature has the same effect on dormancy regardless of whether seeds are exposed to light, and also adds support to the idea that simultaneous processes of dormancy loss and induction are acting within imbibed seeds (Totterdell and Roberts, 1979; Batlla et al., 2009).

5.3.3 Contrasting light and dark germination models

The model used to predict germination in darkness discussed in this chapter, and the model discussed in the previous chapter used to predict germination after exposure to light, both use the same basic framework. Both models estimate frequencies of primary and secondary dormancy to calculate the probability of germination, and primary and secondary dormancy frequencies are estimated with functions based on logistic equations. Parameters a-d, which govern the rates of primary dormancy loss and secondary dormancy induction in both models, are therefore performing the same function and can be directly compared. As may be expected, parameters values in both models are within the same order of magnitude, however differences between the two parameter sets are sufficient to alter the temperature sensitivities of the two models.

The linear relationship between the rate of primary dormancy loss (R_p) and the maturation temperature (T_m) created a threshold, below which R_p became negligible (Figure 5.15A). This meant the level of primary dormancy would be too high to allow germination before induction of secondary dormancy. The slight differences in values of a and b meant that models predicted germination in light only when T_m > 13.9°C, and when T_m > 16.9°C in the dark. This also demonstrates the model's

flexibility, and by adjusting parameters a and b it should be possible to simulate ecotypes with different dormancy and temperature sensitivities.

The relationship between the rate of secondary dormancy induction (R_s) and the stratification temperature (T_i) was exponential in both light and dark models (Figure 5.15B). Differences in parameters c and d meant that predicted rates of secondary dormancy induction were lower in the dark model than in the light model. Furthermore, differences between the two models increased substantially at higher temperatures. Consequently, the light model predicted that secondary dormancy induction would be sufficient to counteract reduced primary dormancy, and completely suppress germination at temperatures above 29°C. However, in the dark model the temperature needed to reach 36°C to achieve the same effect. This implies that secondary dormancy induction is less effective at suppressing germination in seeds with lower initial dormancy, and which are likely to germinate in the dark. It's possible for example, that the balance of hormone concentrations and sensitivities becomes so heavily weighted towards the promotion of germination, that a more substantial change in temperature is required to tip the balance back towards dormancy induction. Unfortunately however, the dark model was parameterised with relatively sparse data, which affects the accuracy and confidence in the estimated parameter values. It is also worth noting that germination of seeds matured at 20°C and incubated at 20°C was over estimated by the model (Figure 5.11). It is therefore possible that the prediction that temperatures of 36°C are required to suppress dark germination (in seeds also matured at 36°C) is an overestimate, and further experiments would be required to test this.

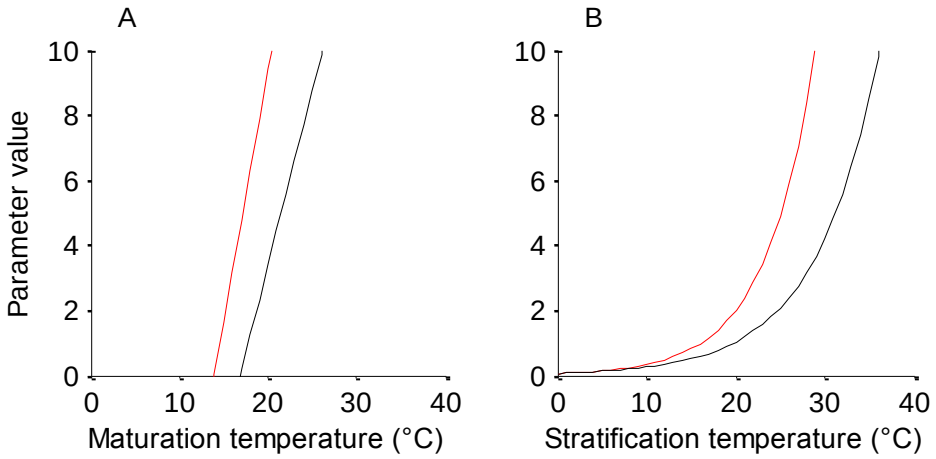

Figure 5.15 A comparison of dormancy rate parameters between light and dark models

(A) The rate of primary dormancy loss (R_p) with maturation temperature; (B) The rate of secondary dormancy induction (R_s) with stratification temperature. Parameter values in light and dark models are shown in red and black respectively.

5.3.4 Ecological implications of dark germination

In the previous chapter, simulations predicted that dormancy induction would completely inhibit germination of light requiring seeds when average daily temperatures reached 29°C. This resulted in inhibition of germination for seeds produced in summer in hot climates such as Cape Verde. However, simulations in this chapter predicted a high probability of germination in darkness when average temperatures were above 17°C, and also that virtually all seeds would lack the light requirement if maturation temperatures exceeded 20°C (Figure 5.14). Furthermore, the reduced primary dormancy caused by elevated maturation temperatures would not be counteracted by increased secondary dormancy induction until mean daily temperatures reached 36°C. This substantially increased the upper temperature limit for germination, and suggests that in locations such as Cape Verde, dormancy in Col seeds could be almost totally abolished. However, as mentioned previously there was some uncertainty in these predictions, due to the lack of data for parameterization for the dark model. Lab experiments also suggested temperatures might begin to inhibit

dark germination at 20°C (Figure 5.11). However, Figure 4.9 also showed that seeds matured at 20°C are less able to remain dormant for long periods at high temperature.

A lack of dormancy could have significant consequences, because plants would have very little control over the timing of their germination. In Cape Verde for example the model predicts that around 75% of seeds produced at any time of year would germinate within a short time of their dispersal. This would lead to continuous rapid cycling behaviour without any seasonal control. This may be disadvantageous, particularly since germination in summer can be lethal due to drought (Donohue et al., 2005c; Huang et al., 2010). A correlation has also been found between dry summers and increased seed dormancy (Kronholm et al., 2012; Wagmann et al., 2012), suggesting that higher dormancy is required to avoid germination in summer. It therefore raises the question whether Col would be able to survive in hot climates such as Cape Verde without acquiring increased dormancy adaptations, and whether ecotypes with similar low dormancy would be adversely affected by climate change. This may be an even larger problem for slower growing species which may be less able to adapt quickly.

5.3.5 Conclusions

Three models were developed to explain the effects of temperature on germination in darkness. The best model used the effects of temperature on primary and secondary dormancy, with an added thermal time component to predict the germination timing. This adds further support to the mechanism of simultaneous dormancy loss and induction that was originally suggested by Totterdell and Roberts, 1979. Despite

some problems due to lack of data for parameterization, this model provided an insight into the probable effects of seed dispersal at different times of year, as well as responses to climates in different geographical locations. Increasingly warmer climates were associated with substantially reduced dormancy of seeds dispersed in summer, potentially leading to a lack of control over germination timing.

Chapter 6: Additional Effects of Temperature on Primary and Secondary Dormancy

6.1 Introduction

To a large extent, the seasonal timing of germination relies on the regulation of dormancy by temperature (Probert, 2000), with other seasonal factors such as photoperiod playing a lesser role (Munir et al., 2001). It is well known that the environment during seed development affects primary dormancy levels in mature seeds (Fenner, 1991), and low temperatures are generally associated with higher ABA content (Kendall et al., 2011), increased *DOG1* expression (Chiang et al., 2011), and increased dormancy at maturity (Schmuths et al., 2006; Donohue et al., 2008; Kendall et al., 2011).

It has also been shown that primary dormancy is initiated during the early maturation phase of seed development (Raz et al., 2001), and reaches a maximum in ripe seeds (Karssen et al., 1983). However, it is not known whether temperature continues to play a role during the whole process of seed maturation, or whether final dormancy levels are determined at some point prior to full maturity. In natural environments, fluctuations in diurnal and even day-to-day temperatures can be substantial. In order to make predictions about seed behaviour in the field it is therefore essential to determine how, and importantly *when* temperature during seed maturation contributes to final levels of primary dormancy. The dormancy model simulations described so far in this thesis have been carried out using the assumption that primary dormancy is set by temperatures during the week prior to seed maturity. The

first half of this chapter was therefore aimed at testing this assumption, with analysis of seeds grown in the field, and also laboratory experiments designed to test how temperature at different points during seed development affects the levels of primary dormancy in mature seeds.

The second part of this chapter was aimed towards understanding secondary dormancy, which is generally less well studied and understood than primary dormancy. Secondary dormancy cycling is known to occur in buried seeds, and is thought to be regulated by seasonal temperature cycles (Baskin and Baskin, 1985). In agreement with this hypothesis, evidence for seasonal gene expression patterns in buried seeds, which correlates with seasonal temperature has recently been found (Footitt et al., 2011; Footitt et al., 2013). Results in previous chapters have also highlighted the potential importance of secondary dormancy. With the exception of seeds dispersed in summer, predicted levels of primary dormancy were sufficient to prevent germination until induction of secondary dormancy; thereby forcing seeds into the soil seed bank (e.g. Figure 4.11). Environmental regulation of secondary dormancy would therefore become the most important factor that determines the seasonal emergence timing of these seeds. However, the specific conditions that bring about alleviation of secondary dormancy are virtually unknown. Laboratory experiments aimed at discovering such conditions were therefore carried out, and the results were compared to seedling emergence experiments carried out in the field.

Many seasonally regulated processes such as flowering are controlled by the circadian clock (de Montaigu et al., 2010), and there is also evidence that flowering and germination share some regulatory elements (Chiang et al., 2009). An additional aim was therefore to investigate links with flowering and circadian pathways by

examining the behaviour of flowering pathway and circadian clock mutant seeds in selected dormancy experiments.

6.2 Results

6.2.1 Effects of alternating diurnal temperatures on primary dormancy

To test whether temperatures during daylight hours or at night have different effects on dormancy, wild type Col-0 and L*er* ecotypes, and flowering pathway mutant *ft-1* seeds were matured in three different temperature regimes; warm days and cold nights (20°C/12°C), cold days and warm nights (12°C/20°C), or at constant temperature (15°C/15°C). Lengths of day and night phases were 12 hours in all cases. Upon harvesting, seeds were stratified in constant darkness at 4°C for varying lengths of time, and their dormancy was assayed at intervals by measuring their germination in light at 22°C (Figure 6.1).

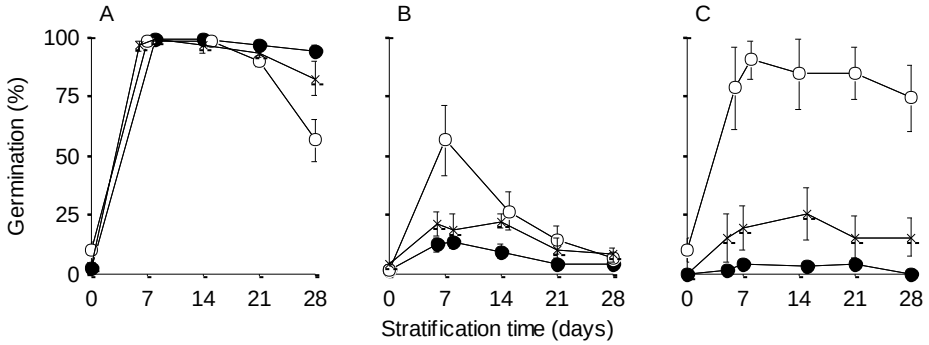

Figure 6.1 Effects of alternating diurnal temperatures during seed maturation

Seeds were matured in 3 day/night temperature regimes; 12°C/20°C (filled circles), 20°C/12°C (open circles), and 15°C/15°C (crosses). Germination in the light was assayed after stratification at 4°C for the time indicated. (A) Col-0; (B) L*er*; (C) *ft-1* in L*er* background. Data represents the mean and SE of 5 replicate seed batches.

In both Col-0 and L*er* ecotypes, the dormancy of seeds matured in alternating temperatures was similar to that of seeds matured at constant 15°C, rather than resembling seeds matured at constant 12°C or 20°C (e.g. Figure 4.1 and Figure 4.9). This shows that developing seeds respond to a combination of day and night time temperatures, rather than either day or night temperature alone. However, there were some differences depending on whether warm or cold phases occurred during the day or at night.

If day and night temperatures contribute equally to the regulation of dormancy, there should have been no difference between seeds matured in the two alternating temperature treatments because warm and cold phases were given in equal lengths. However, Col-0 seeds were slightly more dormant if they were matured in cold nights than if they were matured in cold days. Cold maturation temperatures are associated with increased dormancy, which therefore suggests that the temperature at night has a dominant effect on the primary dormancy of Col-0. In contrast, the opposite behaviour was observed in L*er* seeds, which were more dormant following maturation in cold days rather than cold nights. This suggests dormancy in the L*er* ecotype may be more sensitive to temperatures during the day.

Interestingly, the *ft-1* mutant had similar dormancy to the L*er* background when matured at constant temperature, however differences in dormancy caused by the two alternating maturation temperature treatments were greatly enhanced. As such, the dormancy of *ft-1* seeds was even more reflective of the day time temperature during maturation, with night time temperature apparently having very little effect. This experiment therefore showed that in addition to its role in the photoperiodic control of flowering, *FT* must also have a role in regulating primary dormancy in relation to the temperature at particular times of day. Specifically, these results

suggest that *FT* in the L*er* ecotype either inhibits the response to temperatures during the day, or promotes the response to temperatures at night.

6.2.2 Analysis of maturation temperature and dormancy in field grown seed batches

To investigate the effects of maturation temperature in seeds grown in a natural setting, seeds were collected from Col-0 plants grown at a field site within York University campus. Temperature records corresponding to the seed maturation period were also collected from the campus weather station. A time line showing growth and harvest dates for each seed batch, alongside mean daily temperatures is provided in Figure 6.2.

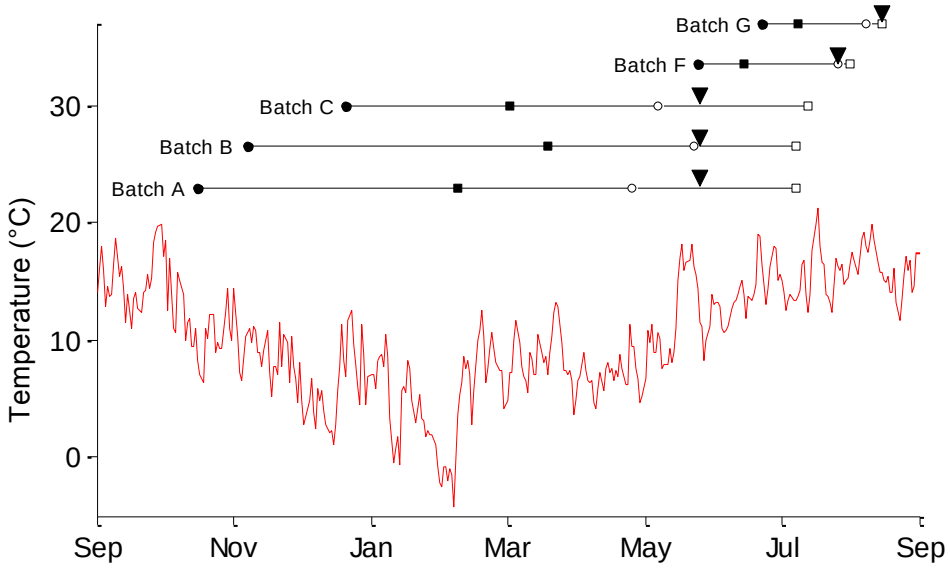

Figure 6.2 Harvest dates and mean daily temperatures during the growth of field grown plants

Mean daily temperatures (red) spanning September 2011 to September 2012 were gathered from weather station records. Timelines showing transplant date (filled circles), bolting date (filled squares), start of seed set (open circles), harvest date (arrow heads) and the end of seed set (open squares) are shown for each plant batch.

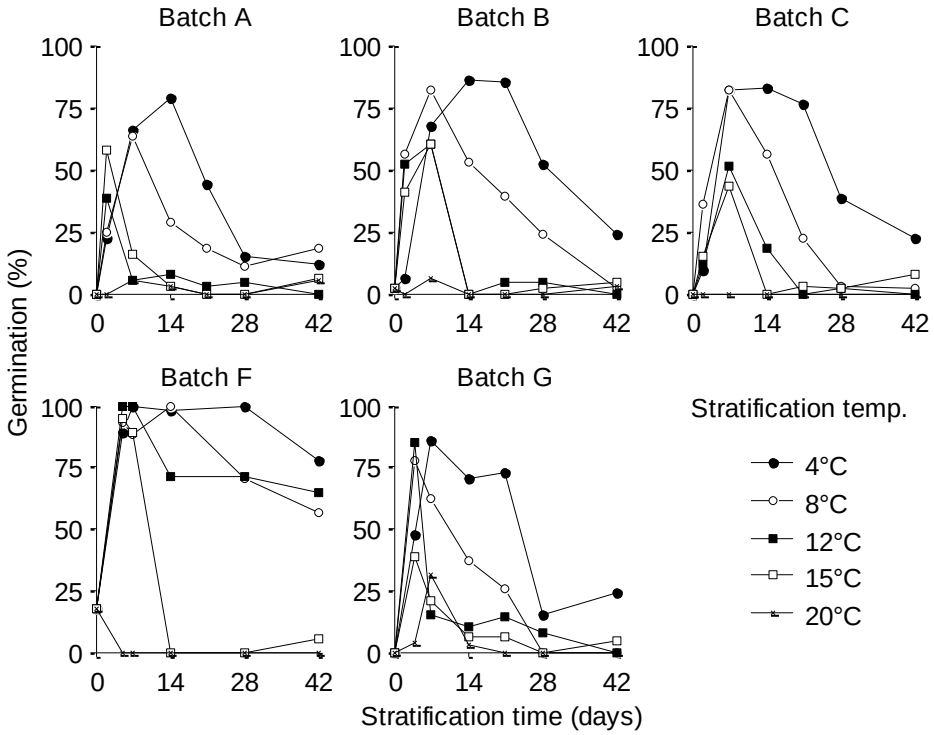

Figure 6.3 Germination responses of field grown seed batches to stratification at different temperatures

Seeds were sown onto agar and wrapped in foil. After a stratification period at the temperature indicted, dormancy was measured by transferring seeds into the light and counting germination after 7 days. Data represents the total % germination of seeds pooled from at least 6 individual plants in each batch.

The germination of field batches was tested after stratification at different temperatures to enable a comparison with seeds matured under constant temperatures in the laboratory (Figure 6.3). Batches A, B and C had winter annual life histories and very similar dormancy patterns, which resembled previous observations of Col-0 matured at 15°C (Figure 4.1). Seeds from these three batches were harvested on the same date in May 2012, although they had different histories having been transplanted to the field site on successive months from October to December 2011. Their similar responses to stratification however would suggest that temperatures closer to the harvest date, which were common to all three batches, were more important in determining their dormancy. Batch F was sown as a summer annual,

and also had the lowest dormancy of the five batches, resembling Col-0 matured at 17°C or 18°C (Figure 4.1). Batch G was also sown as a summer annual, however this batch had surprisingly high dormancy and the response to stratification was more similar to the winter annual batches.

By fitting the dormancy model to the germination data in Figure 6.3, it was possible to estimate maturation temperatures for each seed batch. The fitting procedure described previously was used with the model of germination in light, and values for T_m were varied to produce the closest fit between model predictions and the observed germination data. The resulting values provided an estimate of the maturation temperatures that would be required to elicit the same germination behaviour in seeds grown under constant laboratory conditions. It was hypothesised that a comparison between estimated T_m and actual temperatures during maturation in the field would reveal a period where they were equal, thereby revealing when temperature is most important for determining primary dormancy. In agreement with expectations, values for batches A-C were very similar (Table 6.1). Batch G, which was the most dormant, had the lowest estimated T_m, while the batch F was the least dormant and had the highest estimated T_m.

Table 6.1 Mean and estimated maturation temperatures for field grown seed batches

Mean maturation temperatures were calculated as the average mean daily temperature between bolting and harvest for each seed batch. Estimated maturation temperatures (T_m) were calculated for each seed batch by fitting the model of germination in light to the germination data from stratification experiments.

Batch	Mean maturation temperature (°C)	Estimated T_m (°C)
A	8.6	15.2
B	9.1	15.2
C	8.9	14.9
F	15.2	17.4
G	16.6	14.8

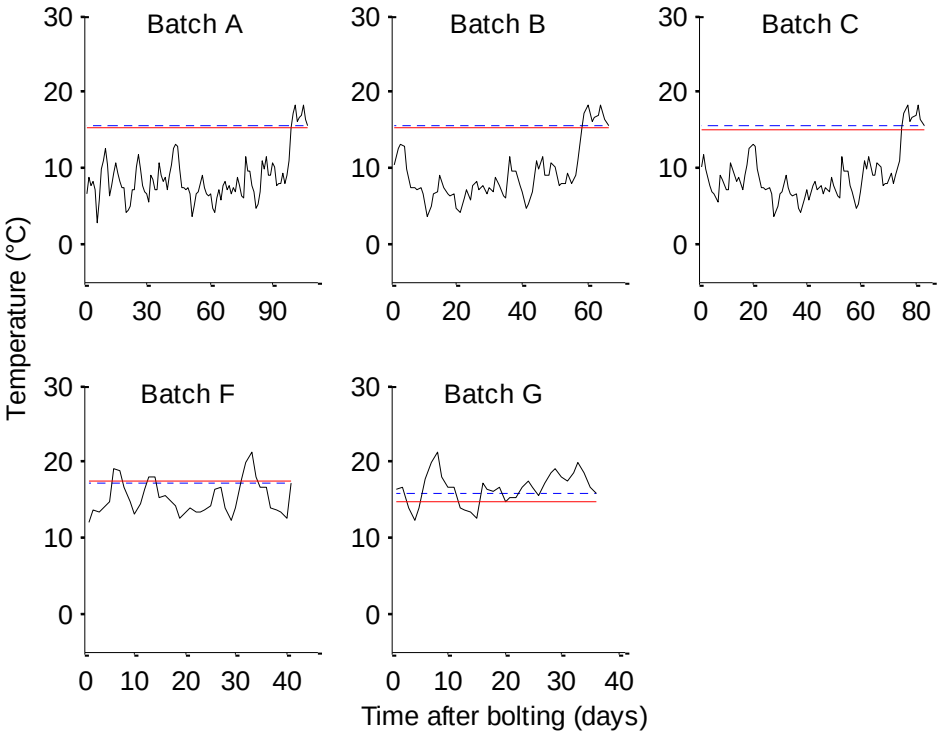

Figure 6.4 Mean daily temperatures from bolting to harvest for field grown seed batches

Mean daily temperature (solid black line) as measured by the nearby weather station. Also shown are estimated maturation temperatures predicted by the dormancy model (solid red line) and the mean daily temperature on the harvest date (dashed blue line).

Figure 6.4 shows a magnified view of the temperature during seed maturation (i.e. from bolting to harvest) for each seed batch, including estimated T_m values in red. Temperatures during maturation of batches A-C were generally much lower than the estimated T_m. However, there was a sustained temperature increase 9 days prior to the harvest date, which was much closer to the estimated T_m. This adds support to the hypothesis that temperatures closer to the harvest date are more important to the final dormancy than temperatures earlier in seed development. As further evidence of this, batches F and G both experienced a warm period where temperatures exceeded 20°C. For batch F, this occurred 9 days before harvesting, and therefore may have contributed to the reduced dormancy. However for batch G, this warm period occurred much earlier in development; only 8 days after bolting. Batch G was much more dormant than batch F, showing that the warm temperatures earlier in development had less of an effect on the final dormancy.

In order to pinpoint a specific time during seed development where the mean temperature closely matched the estimated T_m, an analysis comparing different temporal 'windows' was performed . Mean temperatures for all possible windows spanning lengths of up to 30 days and positioned at different points during seed development were calculated for each batch. For each window, the mean temperature was compared to estimated T_m values for each batch, and the squared differences were used to calculate the root-mean-square error (RMSE). The equation for this is given in [34]; where T_w is the mean window temperature, T_{m_i} is the estimated maturation temperature for the batch i, and n = 5 seed batches. Windows were then ranked in order of their RMSE, with lower scores providing the closest match (lowest error) across all batches.

[34] $$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(T_w - T_{m_i})^2}$$

In general, windows that were earlier in seed development had larger RMSE scores, and the lowest scoring 10 windows were all located within the last 11 days before harvest (Figure 6.5). Surprisingly, this analysis suggested that the best predictor of seed dormancy was the mean daily temperature on the day of harvest (RMSE = 0.58). This is shown in Figure 6.4 as a dashed blue line, which closely matches the predicted maturation temperature, shown in red. The second and third best scoring windows spanned 4-10 and 0-10 days before harvest respectively (RMSE = 1.36 and 1.4).

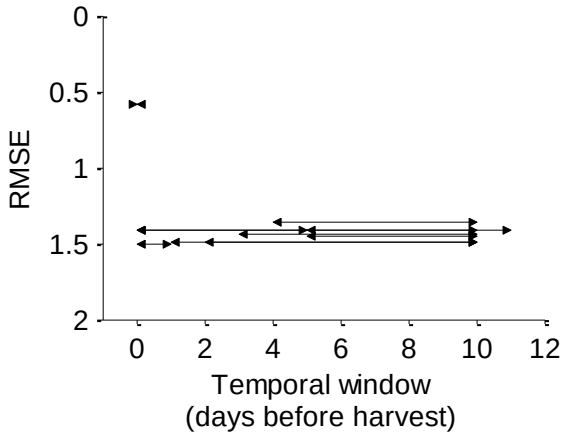

Figure 6.5 Temporal window analysis comparing mean window temperatures with predicted maturation temperatures for 5 field grown seed batches.

RMSE was calculated for 961 possible windows spanning up to 30 days, and at different times during seed development. Possible windows were ranked in order of smallest RMSE. Windows spanning anywhere between 0 and 10 days before harvest featured heavily in the lowest scoring ~50 windows. The top ten windows are represented here by arrows indicating their start and end position (in days before harvest), versus their RMSE. The best window was on day 0 (the harvest date).

Batch G proved anomalous in this analysis because the relatively high dormancy could not easily be explained by temperatures prior to harvest. This may have been due to uncontrolled factors such as the presence of unripe seeds, shortening photoperiod, or other environmental stress causing higher than expected dormancy. Consequently, RMSE scores for all windows were affected by larger errors for batch G in comparison to other batches. The same analysis was therefore repeated with batch G omitted. This confirmed that the top 10 ranked windows were still within the last 11 days before harvest. However the temperature on the day of harvest was ranked third (RMSE = 0.42), while first and second were windows spanning 3-10 and 2-10 days before harvest (RMSE = 0.24 and 0.40). This also demonstrates a larger sample size with more seed batches would be required to pinpoint a temporal window with greater confidence. However, it has clearly pointed towards temperatures the end of seed development, particularly in the final 11 days before harvest, as being most important for seed dormancy. The following experiments were therefore intended to test this prediction.

6.2.3 Effects of a change in maturation temperature prior to harvest

To test the prediction that the dormancy is determined in the final days of seed development, an experiment was set up in which plants were grown at constant 12°C from bolting onwards. Seeds matured at this temperature should have high dormancy and a weak response to stratification (e.g. Figure 4.1). Once seed shedding had begun all mature seeds were harvested. The plants were then moved to a constant 20°C environment, and further harvests from the same plants were performed at regular intervals. For comparison, plants were also grown at constant 20°C to produce seeds with very low dormancy.

The dormancy of harvested seeds was tested by imbibition in light at 22°C immediately after harvest, and also after a 3 day stratification treatment in darkness at 12°C. This treatment was intended to reveal any differences in dormancy that might not otherwise be visible in freshly harvested seeds. The overall aim was to test the prediction that dormancy is determined in the 10 or so days prior to harvest, and also to see what length of time at 20°C was required for the dormancy of seeds initially grown at 12°C to resemble that of seeds grown exclusively at 20°C.

As expected, seeds produced at constant 12°C were very dormant (Figure 6.6). Freshly harvested seeds did not germinate in light, and only 5% germinated after the stratification treatment. Moving plants to 20°C for 3 days did not have any effect, although after 5 days at 20°C there was a small increase in the germination of fresh and stratified seeds. No additional reduction in dormancy was observed after 7 days at 20°C, although after 10 days at 20°C germination of stratified seeds had increased to 42%. Overall, these results show a gradual reduction in seed dormancy with increasing time spent at 20°C prior to harvest. However, 10 days at 20°C was not sufficient to reduce the dormancy to the level of seeds matured at constant 20°C, which germinated to 87% when fresh and almost 100% after stratification.

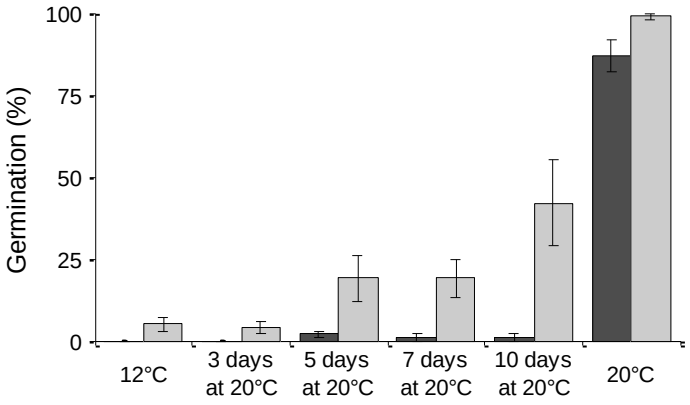

Figure 6.6 Germination of seeds grown at 12°C and moved to 20°C before harvest

Col-0 seeds were harvested at 12°C, and at intervals after transferring to 20°C. Seeds matured at constant 20°C are also shown for comparison. The germination in light was assayed in freshly harvested seeds (dark grey), and after stratification for 3 days at 12°C (light grey). Data represents the mean and SE of 5 replicate seed batches.

Notably, differences in dormancy were only visible after the stratification treatment, whereas the germination of freshly harvested seeds was relatively constant. This suggested that dormancy in freshly harvested seeds was generally too high to permit germination, even after 10 days at the warmer maturation temperature. The stratification treatment was therefore necessary to partially alleviate dormancy before any differences caused by changing the maturation temperature could be observed. Consequently this experiment was repeated, however a more detailed view of the dormancy was gathered by subjecting seeds to stratification at 3 different temperatures for up to 3 weeks. Plants were again grown at 12°C and seeds were harvested at intervals after transferring plants to 20°C. The reverse experiment was also performed, in which plants were first grown at constant 20°C before transferring to 12°C.

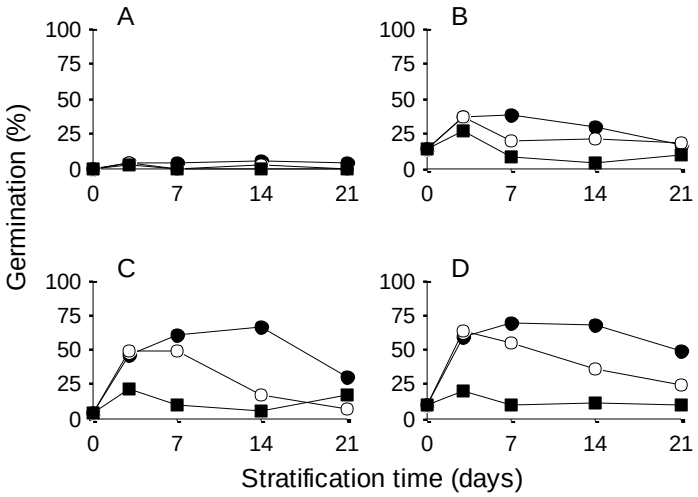

Figure 6.7 Germination of seeds matured at 12°C and moved to 20°C prior to harvest

(A) Seeds matured at constant 12°C until harvest; (B) Seeds matured at 12°C, and 20°C for 3 days; (C) Seeds matured at 12°C, and 20°C for 7 days; (D) Seeds matured at 12°C, and 20°C for 21 days. Germination in light was tested in freshly harvested seeds, and after stratification at 8°C (filled circles), 12°C (open circles) and 16°C (filled squares) for the time indicated. Data represents the total % germination of seeds pooled from 5 parent plants.

Figure 6.7 shows the germination of seeds from four successive harvests. The first harvest (Figure 6.7A) consisted of seeds grown only at 12°C. As expected, these were highly dormant and did not respond to stratification at any temperature. However, after spending 3 days at 20°C before harvest (Figure 6.7B), seeds were less dormant and their germination peaked at 38% following stratification for 7 days at 8°C. This shows that only a small amount of time was required for an increase in the maturation temperature to cause a reduction in the final dormancy. After 7 days maturation at 20°C (Figure 6.7C), the dormancy had been reduced further and the germination of seeds stratified at 8°C peaked slighlty higher at 66%. However, dormancy was not reduced any further by an additional 2 weeks maturation at 20°C (Figure 6.7D). This was surprising since seeds harvested 21 days after the transfer would have experienced the warmer temperature for most of their development.

Nevertheless, their dormancy did not resemble seeds matured only at 20°C (i.e. Figure 6.8A).

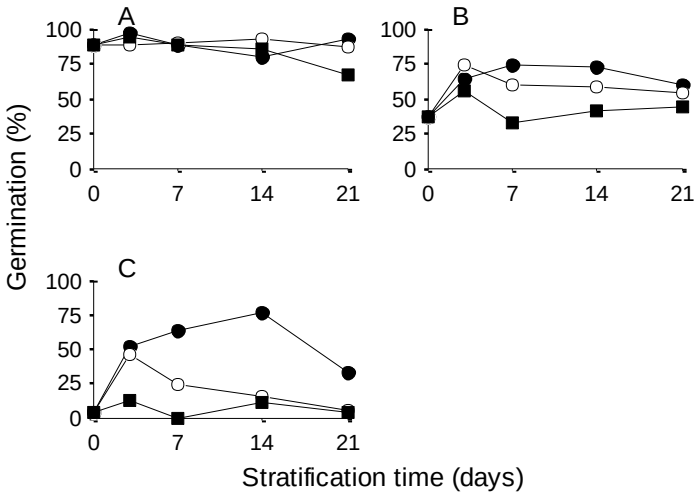

Figure 6.8 Germination of seeds matured at 20°C and moved to 12°C prior to harvest

(A) Seeds matured at constant 20°C until harvest; (B) Seeds matured at 20°C, and 12°C for 7 days; (C) Seeds matured at 20°C, and 20°C for 21 days. Germination in light was tested in freshly harvested seeds, and after stratification at 8°C (filled circles), 12°C (open circles) and 16°C (filled squares) for the time indicated. Data represents the total % germination of seeds pooled from 5 parent plants.

Figure 6.8 shows the reverse experiment, in which seeds were matured at 20°C and then moved to 12°C. Seeds matured only at 20°C germinated to around 90% after harvest, and were not affected by stratification (Figure 6.8A). After moving plants to the cooler maturation temperature of 12°C, rates of seed development were slowed down and an additional harvest after 3 days was not possible. However, after 7 days at 12°C the dormancy had increased, and germination without stratification was reduced to 36% (Figure 6.8B). After 21 days at 12°C, dormancy was increased further and germination was less than 5% in freshly harvested seeds. However the maximum germination in response to stratification at 8°C reached 76%, which was similar to seeds from the previous harvest (Figure 6.8C).

These results complement each other to show that dormancy can be altered by a change in temperature as little as 3 days before harvest. However, they also show that the response to temperature is complex and differences in dormancy are occasionally only visible after stratification. Compared to the prediction based on analysis of field matured seed batches, these experiments also showed that temperatures early in seed development do have a lasting effect on dormancy.

6.2.4 Alleviation of secondary dormancy

Most of the experiments described so far have focused on the dormancy of seeds as they are imbibed at constant temperatures. However, the temperature in natural environments constantly changes, and it has been suggested that alternating temperatures could play a role in dormancy alleviation (Ali-Rachedi et al., 2004). To investigate this further, seeds were stratified at a range of constant temperatures in darkness for 126 days in order to induce secondary dormancy. The long initial stratification period was required because secondary dormancy is induced more slowly at cold temperatures. Seeds incubating at 4°C or 8°C were then moved to a warmer (20°C) environment, and seeds incubating at 12°C, 15°C or 20°C were moved to a colder (4°C) environment. As a control, some seeds from each initial stratification temperature were also left at the same constant temperature. The dormancy was sampled by testing germination frequencies in light at intervals throughout the experiment.

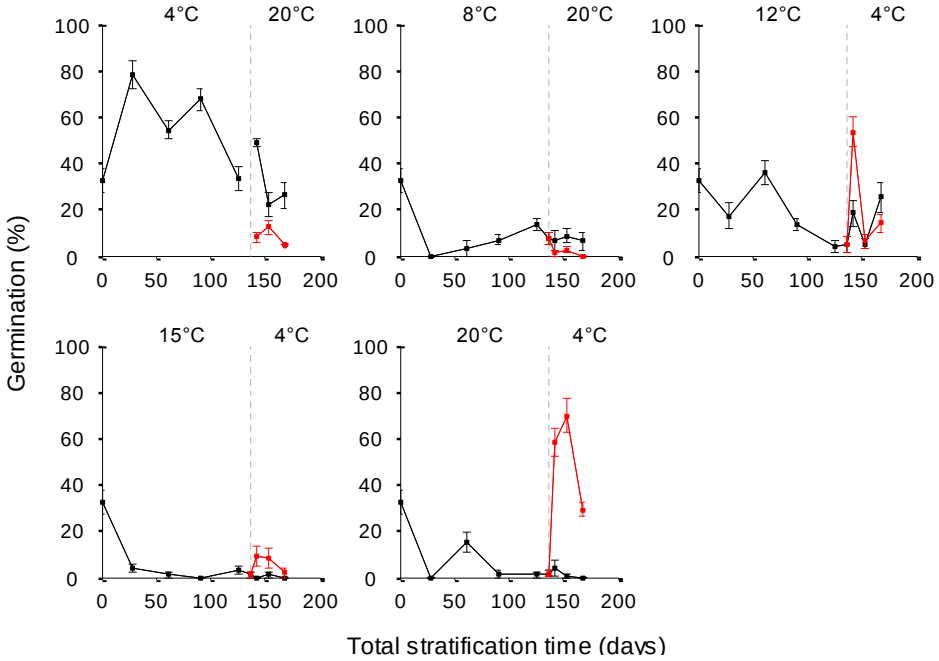

Figure 6.9 Germination of Col-0 seeds after a change in stratification temperature

Col-0 seeds were initially stratified in darkness at the indicated temperature to induce secondary dormancy. After 126 days (dotted line) half the seeds were moved to a different temperature (red), while the other half were left at the same constant temperature (black). Dormancy was sampled at intervals by measuring the germination in light. Data represents the mean and SE of 5 replicate seed batches.

In Col-0, stratification at 4°C resulted in an initial increase in the germination, followed by an overall decrease which was incomplete after 126 days (Figure 6.9). This is consistent with an initial loss of primary dormancy followed by very gradual induction of secondary dormancy. At all other temperatures the germination decreased after the first month of stratification, showing that secondary dormancy was induced more quickly. In L*er*, seeds were generally more dormant and very few seeds germinated after stratification at constant temperature; except at 20°C, where the germination transiently increased after 2 months (Figure 6.10).

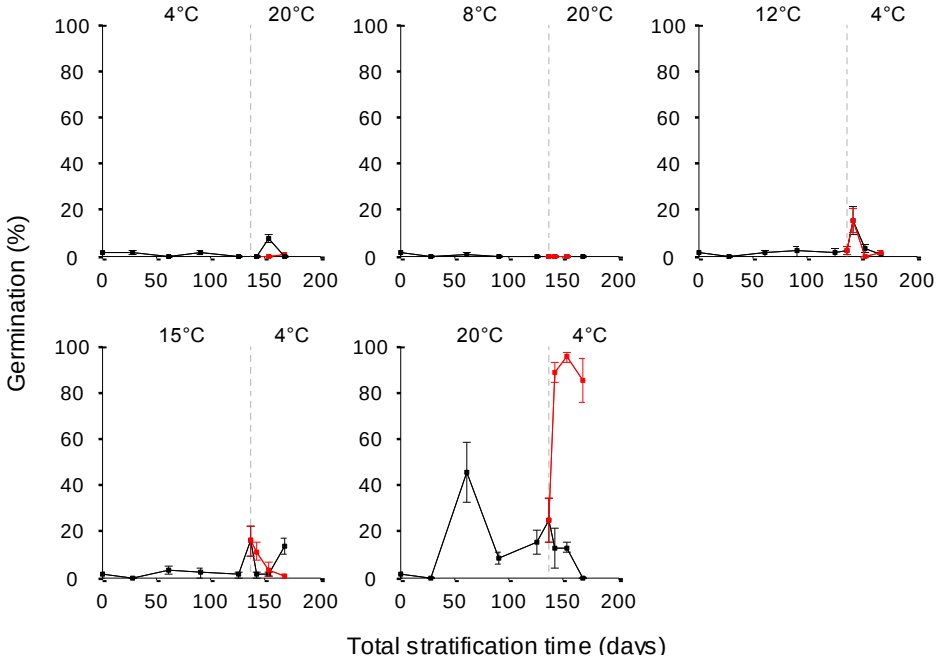

Figure 6.10 Germination of L*er* seeds after a change in stratification temperature

L*er* seeds were initially stratified in darkness at the indicated temperature to induce secondary dormancy. After 126 days (dotted line) half the seeds were moved to a different temperature (red), while the other half were left at the same constant temperature (black). Dormancy was sampled at intervals by measuring the germination in light. Mean Data represents the mean and SE of 5 replicate seed batches.

In both ecotypes, switching from a cold to a warm temperature reinforced dormancy and reduced germination. However, switching from a warm to a cold temperature caused a reduction in secondary dormancy and increased germination. This effect was greatest when seeds were moved from 20°C to 4°C, however it was unclear from this experiment whether the seeds were responding to these specific temperatures, or whether the size of the temperature differential was responsible for the larger response.

Interestingly there was also evidence of oscillations in the dormancy of seeds incubating at constant temperatures, shown by the germination increasing and decreasing at alternate sampling points. This was particularly pronounced in L*er* incubating at 20°C, where there was a sudden large increase in the germination after

2 months which was not seen at any other temperature. Similar effects were also visible in Col-0, although the oscillations were not as large. Since time of sampling was not controlled, this could potentially be caused by circadian oscillations, which is investigated further in section 6.2.6.

6.2.5 Seedling emergence in the field

A experiment was performed to investigate field conditions which prompt germination of two seeds batches; one from plants grown in the lab at 18°C which were expected to have relatively low primary dormancy, and one from plants that were grown at the field site in York. Pots were filled with sterile soil and covered with a fine nylon mesh to prevent seeds from becoming buried too deeply. Approximately 500 seeds were sown onto the mesh and then covered with an inch of sterile soil. Pots were then buried to soil level at the field site. Periodically, the soil inside the pots was disturbed in order to expose seeds to light, and seedling emergence was monitored weekly. A data logger was also used to monitor the soil level temperature. At the end of the experiment, the percentage emergence was calculated as the number of seedlings that had emerged during each time period, expressed as a percentage of the cumulative total (Figure 6.11).

The results revealed two main germination flushes; one in autumn, which peaked in September and October and was followed by a period of low germination over winter; and another the following spring which began in April and continued through to July. As expected, seeds which had been grown in the lab at 18°C were less dormant initially, and a higher percentage of these germinated during the first flush in autumn. Consequently, fewer remained to germinate during the subsequent spring

germination flush. In comparison, more of the field grown seeds delayed their germination until October during the first year. This batch also germinated more synchronously compared to lab grown seeds, whose emergence was more spread out during the first 4 months. In addition, the germination of field grown seeds was spread more evenly between autumn and spring flushes.

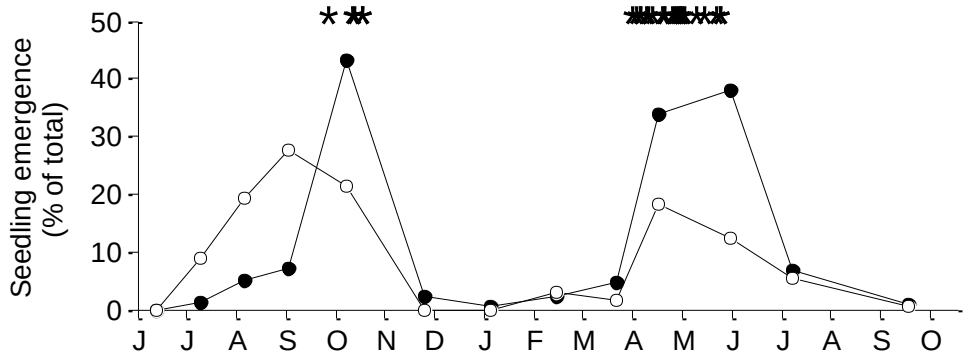

Figure 6.11 Seedling emergence in York, UK

Laboratory grown Col-0 seeds matured at 18°C (open circles), and field grown Col-0 seeds harvested on 30th May 2012 (closed circles) were covered in sterile soil in pots buried up to soil level at the field site. The surface soil was periodically disturbed to expose buried seeds to light, and seedling emergence was checked at weekly intervals. Data points represent the number of emerged seedlings between each soil disturbance event, expressed as a percentage of the cumulative total. Days where the maximum temperature exceeded 20°C and the minimum fell below 4°C are marked with an asterisk.

Daily temperature ranges were greatest during the summer of 2013, when seedling emergence had begun to decrease (Figure 6.12). This suggests that the magnitude of the temperature range does not in itself promote dormancy loss. Instead it is likely that the elevated temperatures caused secondary dormancy induction. However, high levels of seedling emergence were found to be closely correlated with dates on which the maximum temperature was 20°C or more, and the minimum temperature was 4°C or less (asterisks in Figure 6.11). This supports the notion that a change in

temperature from 20°C to 4°C, which caused a loss of secondary dormancy in lab experiments (Figure 6.9), could also be promoting germination under field conditions. However, effects of additional environmental factors such as water availability or day length cannot be ruled out.

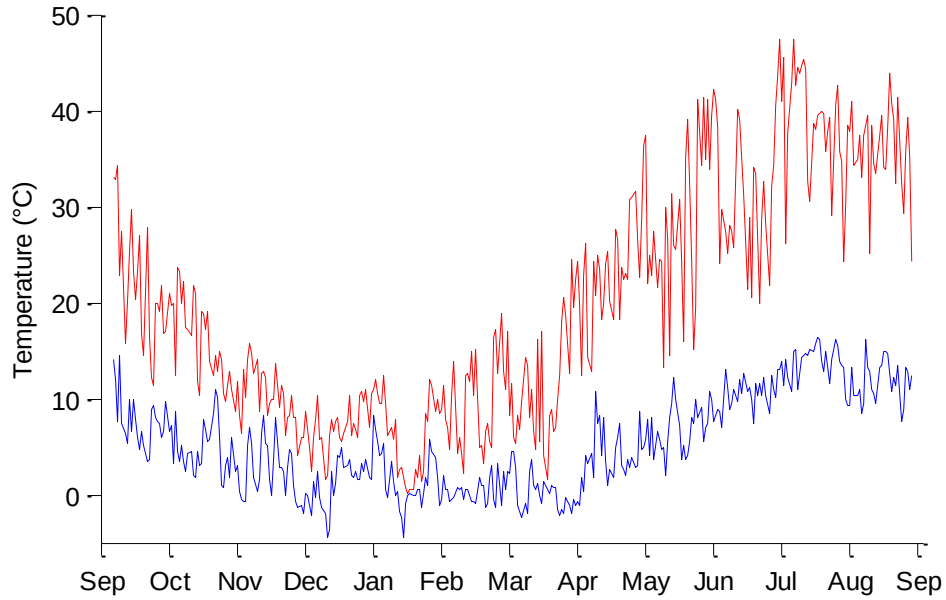

Figure 6.12 Ground level temperatures recorded at the field site during 2012 and 2013

A temperature logger was set to take readings every 10 minutes at ground level. Daily maximum (red) and minimum (blue) temperatures are shown.

6.2.6 Investigation into the circadian control of dormancy

To investigate a possible link between dormancy and the circadian clock, an experiment was set up with the aim of comparing dormancy in wild type Col-0 and a circadian clock mutant. *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) is a key component of the central circadian clock oscillator (Locke et al., 2005), and constitutive over expression of this gene abolishes the rhythmic expression of many other genes in continuous conditions (Wang and Tobin, 1998). Col-0 and *CCA1*-

overexpressing seeds (*CCA1*-ox) were matured at 16°C, and imbibed darkness at constant 8°C, 12°C or 16°C. The maturation temperature of 16°C was intended to confer a moderate level of primary dormancy in freshly harvested seeds, which would be lost within the timeframe of the experiment. The stratification temperatures were also chosen to provide a range of secondary dormancy induction rates, with the expectation that complete dormancy induction would require 4-6 weeks at 8°C, and 2-5 days at 16°C. During stratification in constant conditions, dormancy was sampled every 3 hours to determine if any circadian rhythm could be detected, and if so whether it would be abolished by overexpression of *CCA1*.

There was no difference between seeds stratified at 8°C and 12°C. Figure 6.13 therefore only shows a comparison between seeds stratified at 8°C and 16°C. At 8°C there was a gradual loss of primary dormancy and increase in the germination. As expected, there was minimal induction of secondary dormancy at this temperature, and the germination eventually reached 97% in Col-0 and 90% in *CCA1*-ox after 5 days. There was also no evidence of any circadian rhythm at this temperature. However, seeds incubating at 16°C displayed a clear rhythmic germination response, with daily oscillations. The height of the peaks increased for the first 3 days, and then began to decrease. This was interpreted as an initial loss of underlying primary dormancy, with a simultaneous rhythmic, and progressively increasing induction of secondary dormancy. Overexpression of *CCA1* resulted in slightly higher dormancy overall, however did not affect the oscillations at 16°C which occurred with the same period as wild type.

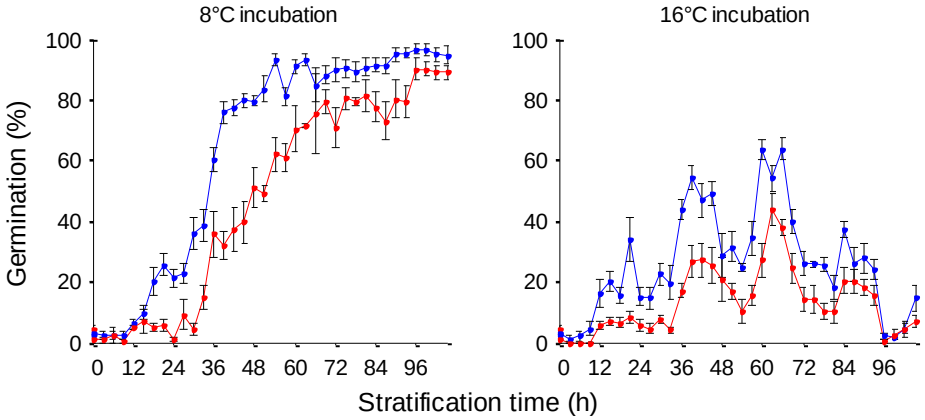

Figure 6.13 Germination of Col-0 and CCA1-ox stratified at constant temperatures

Seeds were matured at 16°C, imbibed on water agar, and stratified in darkness for the time indicated. Germination percentages of Col-0 (blue) and *CCA1*-ox (red) were calculated by counting the total number of germinated seeds after moving plates into 22°C, 16h light per day for 7 days. Data represents the mean and SE of 5 replicate seed batches.

Secondary dormancy induction was only expected during stratification at 16°C, and oscillations were only observed at this temperature. This therefore suggests a link between secondary dormancy and the circadian clock, which occurs independently of constitutive *CCA1* expression. The fact that primary dormancy loss at 8°C and 12°C was not rhythmic also suggests that primary dormancy is not controlled by the same mechanism.

6.3 Discussion

6.3.1 Primary dormancy is determined by a combination of long and short term temperature signals

A comparison between the dormancy of seeds matured in naturally fluctuating temperature environments and seeds matured at constant temperatures suggested that temperatures during the last 10 days of seed maturation are the most important for primary dormancy establishment in *Arabidopsis* (Figure 6.4). This is consistent with

results in barley, where susceptibility to pre-harvest sprouting was shown to be correlated to the temperature during the final stages of seed development (Rodríguez et al., 2001; Gualano and Benech-Arnold, 2009). Furthermore, a temporal window analysis suggested that the mean temperature on the day of harvest was the best predictor of dormancy in five field grown seed batches (Figure 6.5).

An ability to adjust seed dormancy according to environmental temperatures allows germination responses to be highly plastic, and responses to temperature on such a short term basis would allow plants to have the greatest flexibility in responding to their current environment. However, this could also leave them vulnerable to short term temperature fluctuations caused by unseasonal weather. Since climate change is anticipated to increase the frequency of unusual weather patterns (Karl and Trenberth, 2003; Hansen et al., 2012), this could increasingly affect the seasonal regulation of dormancy and germination in seeds.

However, laboratory experiments showed that primary dormancy was not solely explained by temperatures immediately before harvest. A change in temperature was capable of causing a change in dormancy in as little as 3 days, and a larger change after 7 days (Figure 6.7 and Figure 6.8). However, the change in maturation temperature was never sufficient to completely overcome the effects of temperatures experienced earlier in development. Even providing constant temperatures for 3 weeks prior to harvest had no additional effect beyond the initial 7 days at the altered temperature. Similar results have also been reported in *Arabidopsis*, where the temperature experienced by mother plants even before flowering has a lasting effect on seed dormancy (Kendall and Penfield, 2012). This strategy seems to provide the best of both worlds by allowing immediate response to temperatures at the time of

dispersal, whilst also preventing vulnerability to changes in weather by retaining a longer term memory of past temperature.

6.3.2 Evidence for a link between seed dormancy and the circadian clock

Throughout this chapter, several experiments have hinted at some involvement of the circadian clock in regulating seed dormancy. Figure 6.1 shows subtle differences depending on whether warm or cold temperature treatments were given during the day or at night. These differences were amplified in L*er* which lacked the *FT* gene, a transcription factor well known for its role in floral induction (Mathieu et al., 2007), and whose expression is known to be linked to several circadian clock regulated pathways (Suarez-Lopez et al., 2001; Helliwell et al., 2006; Salathia et al., 2006). Further evidence of a shared pathway between flowering and germination has also been found, which involves *FLC, FT, SOC1* and *AP1* (Chiang et al., 2009). Together, these findings suggest that *FT* is pleiotropic, and in addition to its central role in flowering is also required to establish normal dormancy levels in response to temperatures during seed development. A role for *FT* in dormancy establishment may also help to explain why it is highly expressed in developing siliques (Kobayashi et al., 1999).

A clear oscillation in the germination response to light was also found in seeds incubating in constant darkness at 16°C (Figure 6.13). Similar results have previously been reported by Oliverio et al., 2007, who demonstrated that a rhythmic germination response to a FR pulse in seeds incubating at 22°C is mediated by PHYA, and is gated by the circadian clock. Their study also showed that the rhythmicity of the response was maintained in *gi* mutants. Both *CCA1* and *GI* are

considered to be core clock components (Locke et al., 2005; Locke et al., 2006; Pokhilko et al., 2010; Pokhilko et al., 2012). Constitutive expression of *CCA1* abolishes rhythmicity in many clock associated genes in constant conditions (Wang and Tobin, 1998), and mutations at the *GI* locus also disrupt normal clock function (Fowler et al., 1999a; Mizoguchi et al., 2005; Locke et al., 2006). The finding of rhythmicity in germination responses of *CCA1*-ox seeds during stratification in constant darkness is therefore surprising. This result, and those published by Oliverio et al., 2007 seem to suggest that seeds may possess an oscillatory mechanism which is independent from the central shoot oscillator. Further support for this possibility comes from the finding that clock architecture can indeed be tissue specific, as expression of only a subset of shoot circadian clock genes were found to oscillate in root tissue (James et al., 2008).

Interestingly, results in Figure 6.13 also support the notion that primary and secondary dormancy operate through distinct mechanisms. Seeds stratified at 8°C and 12°C gradually lost primary dormancy and did not enter secondary dormancy within the timeframe of the experiment. Additionally, there was no evidence of any rhythmic response to light. These results therefore suggest that warm temperature induced secondary dormancy is under circadian control, while loss of primary dormancy is not. Secondary dormancy is often said to control seasonal dormancy cycling (Hilhorst, 1998; Baskin and Baskin, 2004). Therefore the existence of a circadian controlled pathway regulating secondary dormancy in relation to seasonal cues, would not be surprising.

*6.3.3 Secondary dormancy in Col-0 and L*er *ecotypes is broken by a switch from warm to cold temperatures*

For seeds within the soil seed bank, the timing of germination is largely governed by the level of secondary dormancy. To predict seasonal emergence, it is therefore necessary to understand what conditions result in secondary dormancy alleviation. Responses to changes in stratification temperatures were investigated, and in agreement with warm temperature induction of secondary dormancy, a switch from a cold to warm environment reinforced dormancy and reduced germination in both Col-0 and L*er* seeds (Figure 6.9 and Figure 6.10). However, a shift from a warm to a cold environment caused a reduction in dormancy, and an increase in germination. Furthermore a switch from 20°C to 4°C caused the greatest increase in germination, whereas a smaller temperature difference caused little or no response.

This observation suggested that germination would occur in autumn after a transition to cooler temperatures at the end of summer, a suggestion which has also been made by previous authors (Donohue et al., 2007). This was confirmed by observations of seedling emergence in the field (Figure 6.11), however large numbers also emerged in spring which could not be explained in the same way. However, analysis of ground level temperature measurements showed that emergence patterns were correlated with days on which the maximum temperature was ≥20°C, and the minimum temperature was ≤4°C. This correlation supports a hypothesis that diurnal oscillations of this magnitude or greater may be sufficient to break secondary dormancy and trigger seasonal germination flushes. Emergence experiments also suggested that the specific temperature range was important, since an increased diurnal amplitude at warmer temperatures, which occurred during summer, was not

correlated with high levels of seedling emergence; although effects of other germination inhibiting factors such as water availability could not be ruled out.

The effect of alternating temperatures on dormant Cvi seeds has been examined previously, and the highest germination was reported when seeds were at constant 13°C (Ali-Rachedi et al., 2004). However, it was unclear whether these seeds were in a state of primary or secondary dormancy. In primary dormant seeds for example, high germination would be expected in cool temperatures that inhibit induction of secondary dormancy. High levels of germination were also maintained if temperatures during the day were increased, provided that temperatures at night remained low (13°C), whereas germination levels were reduced if the day and night temperatures were reversed (Ali-Rachedi et al., 2004).

In light of results presented in this chapter, it is possible that the sensitivity to alternating temperatures are linked to a circadian clock in seeds, and perceptions of 'warm' or 'cold' are modified according to the time of day. A moderately warm temperature during the day for example would not necessarily be a good indicator of the season, and could just as easily occur on a sunny day in winter or on an overcast day in summer. The same temperature at night however would be a more reliable indicator of summer, and is likely to have a stronger dormancy inducing effect in winter annuals such as Cvi.

Near constant low temperatures in winter however are likely to have a gradual secondary dormancy inducing effect, whereas relatively warm days combined with cold nights are indicative of spring and autumn. This combination of diurnal temperatures is therefore a reliable indicator of a favourable season for germination, and provides a cue which alleviates secondary dormancy. It has also recently been

shown that FT binds to diurnally oscillating phospholipids to accelerate flowering (Nakamura et al., 2014). FT was also found to modulate primary dormancy in response to temperatures at particular times of day (Figure 6.1). Adaptations in the sensitivity and specificity of genes such as *FT* may therefore provide a way of adapting both flowering and germination timing to particular climates and annual habits.

6.3.4 Conclusions

Results in this chapter have demonstrated links between seed dormancy and the circadian clock. *FT* was found to be involved in the establishment of primary dormancy in response to temperature at night, and oscillations of dormancy were observed in seeds stratified in constant darkness at 16°C. A lack of oscillations at 8°C or 12°C suggested this interaction with the clock was specific to warm temperature induced secondary dormancy, and did not apply to primary dormancy loss at lower temperatures. Oscillations were also present in *CCA1*-ox seeds, despite previously published findings that *CCA1*-ox plants are arrhythmic in all aspects of circadian control so far examined (Wang and Tobin, 1998; Green et al., 2002). This suggests the existence of an alternative circadian mechanism which is specific to seeds, and oscillates independently of normal rhythmic *CCA1* expression. Lab experiments also revealed that secondary dormancy in Col-0 and L*er* ecotypes is broken by a transition from 20°C to 4°C. Daily minimum and maximum temperatures of ≤4°C and ≥20°C were also correlated with high levels of seedling emergence in the field, indicating that temperature fluctuations within this range could serve as a cue to break secondary dormancy.

Chapter 7: Simulating Life History Phenology and Seed Dormancy

7.1 Introduction

Flowering and germination are the two major developmental transitions which combine to determine generation time, and overall life history in many plant species, including *A. thaliana* (Evans and Ratcliffe, 1972; Nordborg and Bergelson, 1999; Griffith et al., 2004). There is also significant interaction between these two traits. The seasonal timing of germination determines the conditions experienced during vegetative growth, thereby affecting flowering time. Reproductive timing also influences germination through a combination of maternal effects on seed dormancy, and more directly by determining the conditions experienced immediately after seed dispersal.

Interactions between these two transitions can also be significant and long lasting, with potentially dramatic effects between generations. For example, manipulation of flowering time in *Campanulastrum americanum* affected the frequencies of annual and biennial life histories in the offspring generation (Galloway and Burgess, 2009). However studies such as this are relatively rare, and the adaptive significance of this phenotypic plasticity is not well understood. This chapter therefore aimed to bring together predictive models of flowering and seed dispersal timing with models of seed dormancy in order to examine interactions between life history transitions and seed dormancy and germination behaviour in *Arabidopsis*.

7.2 Results

7.2.1 Simulations in a range of locations

A model of flowering time (Wilczek et al., 2009) was combined with models of seed dispersal timing and seed dormancy described in Chapters 3, 4 and 5. The aim of combining these models was to create an integrated life cycle model capable of simulating a complete generation, from seed to seed. This integrated model was then used to investigate how the seasonal timing of developmental transitions can affect growth dynamics and influence later life stages, and to investigate the role and significance of reproductive timing and seed dormancy in spring and winter annuals. Simulations of the combined flowering and seed set models were initiated on different germination dates as described previously in Chapter 3. Predicted bolting and seed dispersal dates were plotted for each germination date, and the resulting graphs were then overlaid onto colour maps representing germination probabilities, calculated using the dormancy models (Figure 7.1).

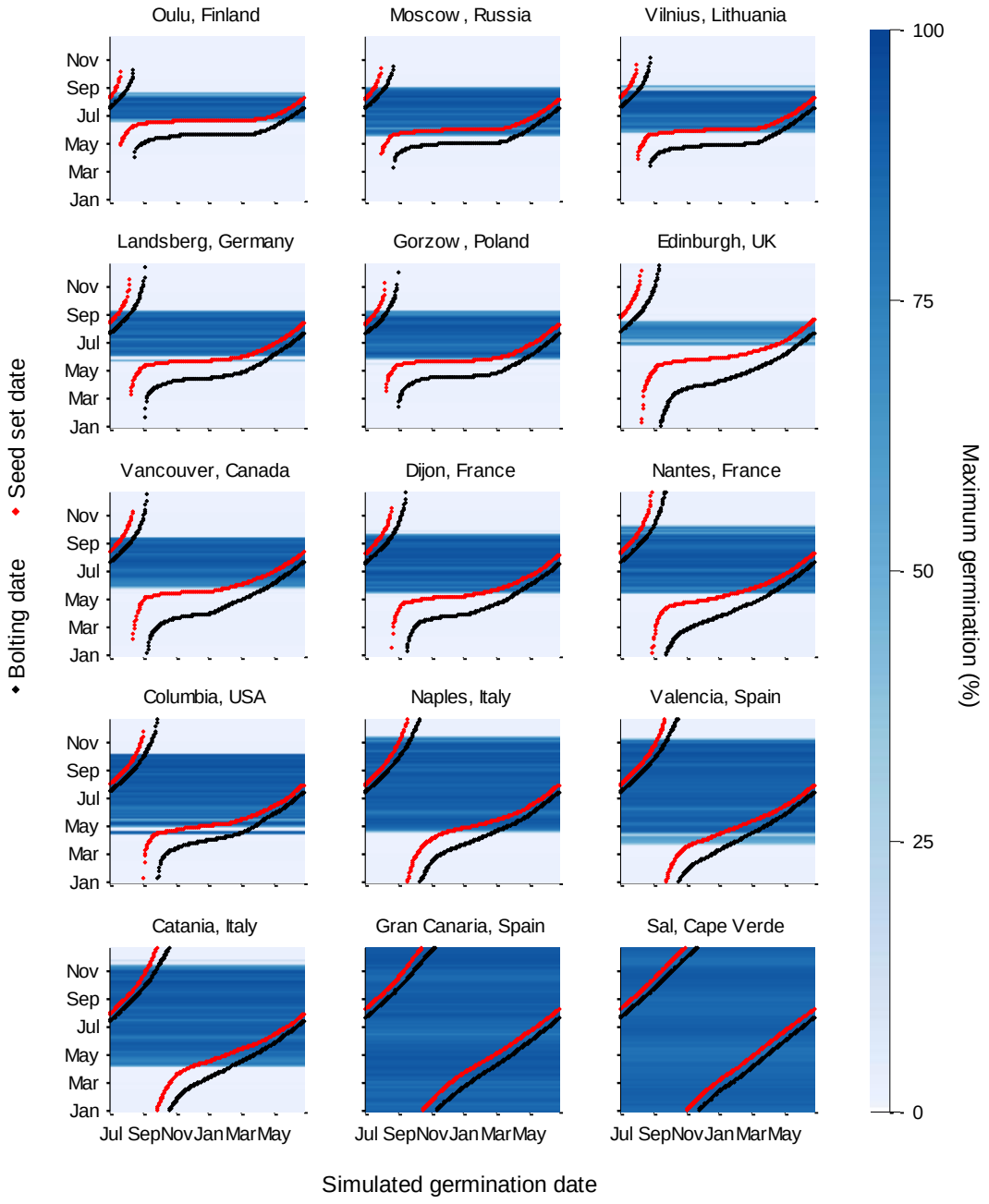

Figure 7.1 Simulations of bolting, seed set, and seed dormancy in a range of locations

Predictions for bolting (black) and seed set dates (red) were generated for all possible germination dates in each location. Dormancy model simulations used the mean temperature during the week prior to seed set as the estimated maturation temperature. The maximum probability of germination for seeds set on each date along the vertical axes is indicated by horizontal shading.

The colour maps were produced by simulating seed dispersal on each possible date, and using mean temperatures during the week prior to seed dispersal to predict primary dormancy. Results in Chapter 6 suggested that this was a reasonable assumption, as the dormancy model could predict the behaviour of field grown seed batches if temperatures closer to the end of seed development were used. Germination probabilities were then calculated for each day after dispersal, according to the predicted frequencies of primary and secondary dormancy within the seed population. In general, germination probabilities reached a maximum within 3 weeks of dispersal, beyond which secondary dormancy induction caused an overall reduction. Probabilities were therefore only calculated for the first 100 days after seed dispersal, since after this time secondary dormancy induction would be complete and there would be no further change in the predicted germination. The maximum germination probability reached within the first 100 days was then used to determine the shading of the colour map according to the colour scale shown. Furthermore, these simulations combined output of both light and dark germination models. The horizontal shading of the colour maps therefore represents the maximum probability of germination for seeds dispersed on a given date, assuming that seeds are exposed to light, and including seeds which lack a light requirement.

In general, summer dispersal dates resulted in darker horizontal bands, since warmer maturation temperatures resulted in reduced primary dormancy. Given adequate light and moisture, seeds dispersed within the dark blue regions (red dots in Figure 7.1) are therefore likely to germinate to high levels shortly after dispersal. In contrast, seeds dispersed within pale blue regions would have a low probability of germination, and are likely to enter secondary dormancy within the soil seed bank.

In most cases, the transition from high to low germination probabilities on different dispersal dates was very sudden. As discussed previously in Chapters 4 and 5, this was caused by the linear relationship between the maturation temperature and the rate of primary dormancy loss. This linear relationship causes an abrupt transition from negligible, to positive rates of dormancy loss when temperatures begin to exceed 14°C. However, it is interesting to note that predictions of seed dispersal timing often closely coincided with this transition. Furthermore, this apparent behaviour was most prevalent in winter and spring annuals, and was consistent in many locations, particularly in Northern Europe. In these locations, autumn or spring germination resulted in fulfilment of photothermal requirements for flowering and seed maturation just before mean weekly temperatures reached 14°C. The full duration of the seed dispersal period would therefore most likely transition the 14°C temperature threshold, resulting in the production of offspring with a full range of dormancies. The consistency of this outcome suggests that developmental timing of flowering and seed maturation, combined with the specific temperature sensitivity of primary dormancy, may be configured deliberately in this way.

Simulations using climate data from Edinburgh proved to be an exception to this rule due to its unusually small annual temperature range in comparison to locations with similar mean annual temperatures. Due to its maritime climate, winter temperatures in Edinburgh are relatively mild, and spring temperatures increase slowly. This caused photothermal requirements to be fulfilled before mean weekly temperatures reached 14°C. Winter temperatures, and the rate of temperature increase during the transition from winter to spring therefore seem to be important factors. This shows that adaptation to local climates may be required in some cases to produce offspring with the same dormancy characteristics.

Winter temperatures also affected the degree of synchronicity in flowering and seed set for winter and spring annuals. In locations where winter temperatures remained below 3°C for longer, plants germinating on a wider range of dates were predicted to flower and set seed at the same time. For example, the coldest climate used for simulations was Oulu in Finland, where the temperature remained below 3°C from October through to April. The minimum growth temperature for Col is 3°C (Granier et al., 2002), therefore photothermal unit accumulation within this date range was halted. Consequently, there were no differences in terms of photothermal unit accumulation between plants germinating on different dates within this period, leading to synchronous flowering. Germination after temperatures increased above 3°C however resulted in later flowering, and caused seed set to begin after temperatures exceeded the 14ºC dormancy threshold. Similarly, in locations with milder winters spring synchronicity was reduced, and progressively earlier germination dates resulted in seed dispersal during the window of reduced primary dormancy. These models therefore predict an increase in rapid cycling behaviour in warmer climates, as increased proportions of seeds would germinate during the same season as dispersal.

7.2.2 Simulations with climate change

The previous simulations suggested that plastic responses to environmental cues would allow a single ecotype to adjust its phenology, and maintain specific conditions at seed dispersal in a wide range of locations and climates without requiring any specific adaptations. To test the robustness of this finding, various degrees of climate change were imposed on model simulations, with the aim of finding the limits which cause the system to break down. Temperature data from

Gorzow, Poland was altered in 1°C increments from -7°C to +7°C, while the photoperiod information was unchanged. Simulations were then performed in each modified climate using all parameters and procedures as previously described. Gorzow was chosen as the basis for this because the Col-0 ecotype is said to have originated there (Röbbelen, 1965).

The results showed that cooling would lead to a delay in the onset, and also a reduction in the duration of the low primary dormancy window in summer (Figure 7.2). A reduction of 7°C meant that summer temperatures never reached the 14°C threshold required for the low dormancy window, which was completely abolished by this level of cooling. Extended winters, with an increased duration of temperatures below 3°C also led to increased synchronicity in reproductive timing. Consequently, maturation temperatures were also more consistent across a greater range of parental germination dates (Figure 7.3). Flowering was also delayed relative to current climates, however the shift in flowering time served to maintain seed maturation temperatures at 14°C, provided the cooling was not greater than 3°C. A reduction of 4°C or more would therefore result in higher proportions of dormant offspring than predicted in current climates.

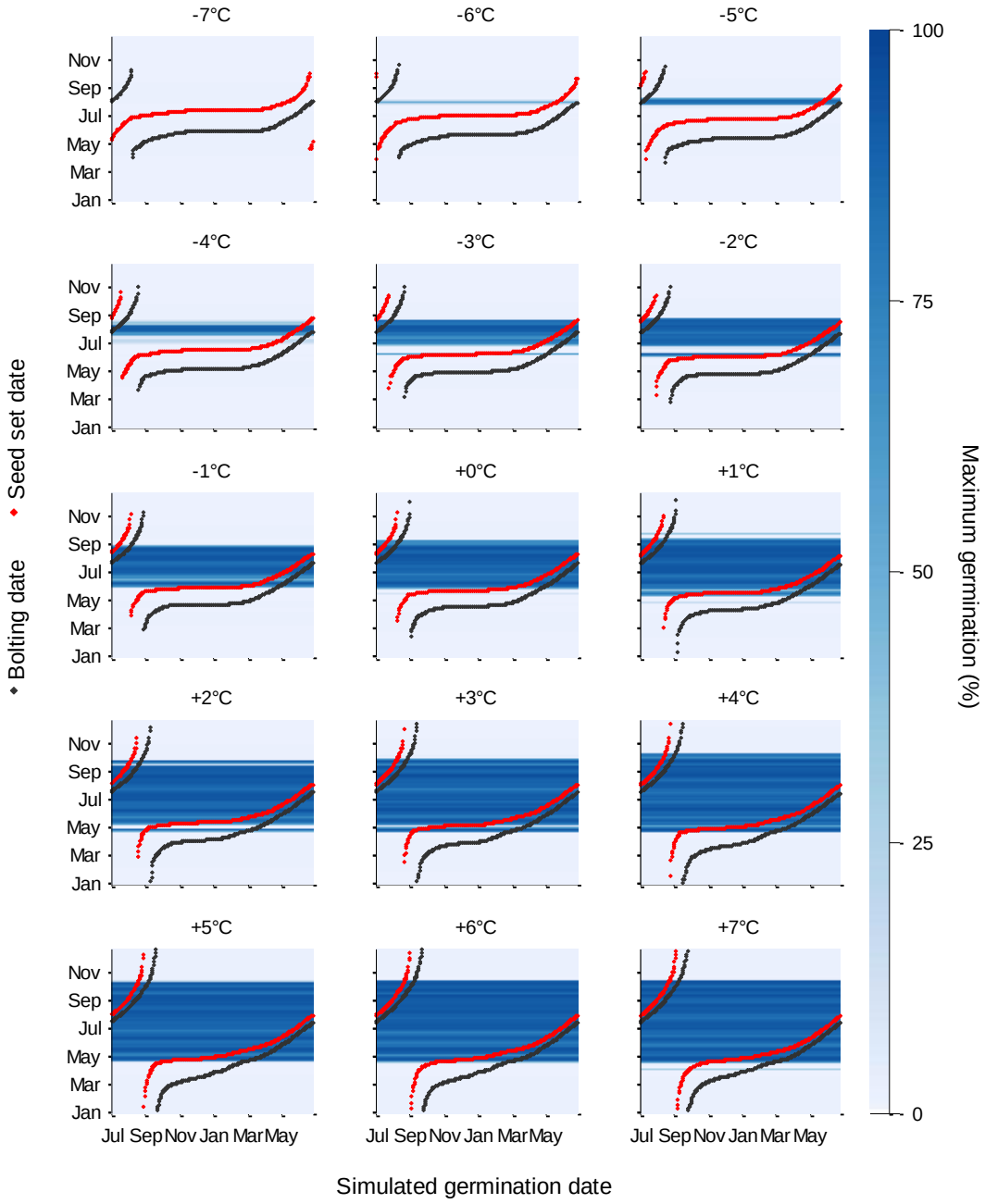

Figure 7.2 Effects of altered climates on bolting, seed set, and seed dormancy

Simulations were performed using current average annual temperatures in Gorzow, Poland. Climate change was then imposed by adding or subtracting up to 7°C as indicated. Predictions for bolting dates (black), seed set dates (red) and seed dormancy (blue shading) were generated in each simulated climate, as described previously.

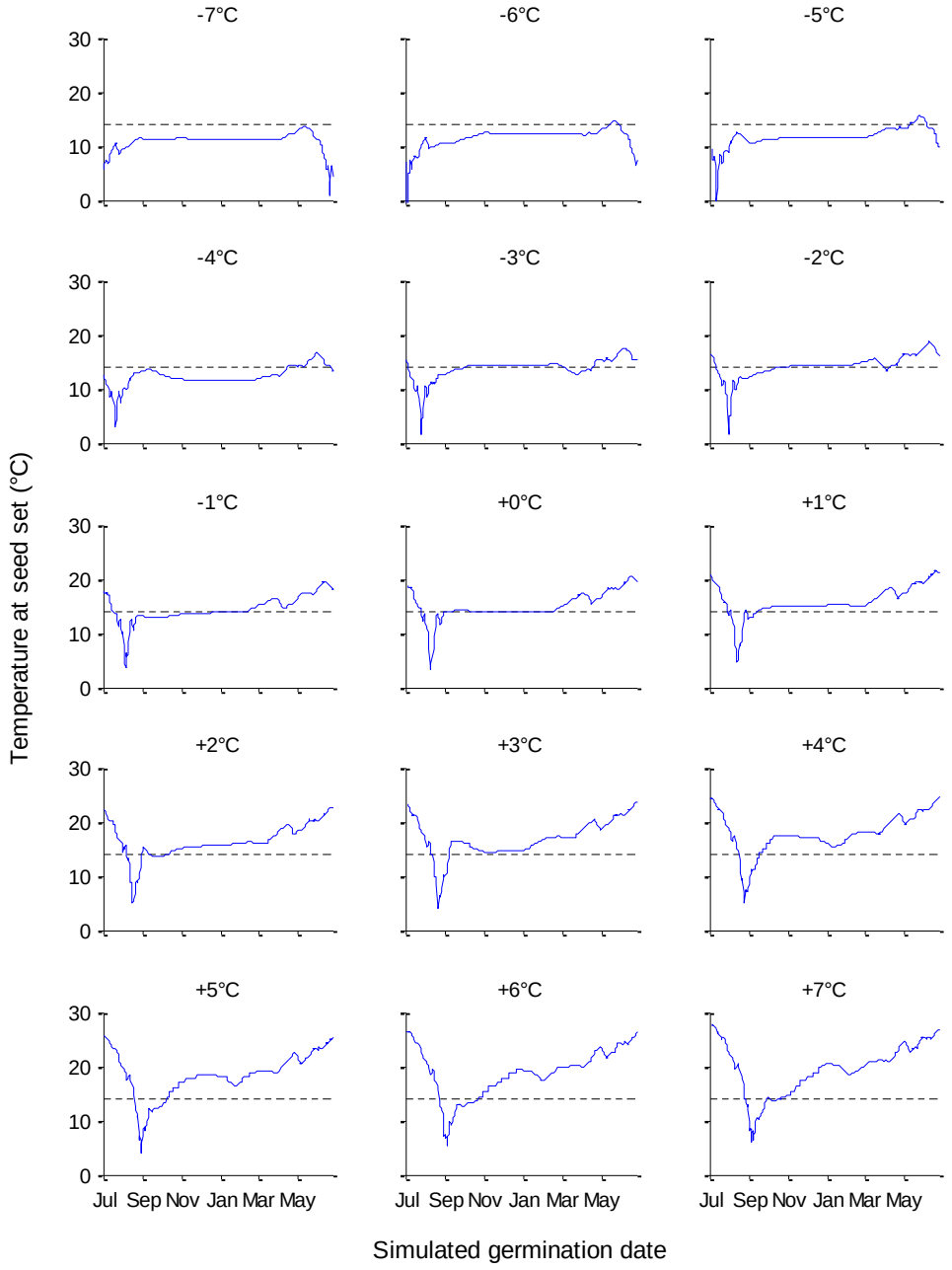

Figure 7.3 Effects of altered climates on temperatures at seed set

Bolting and seed set dates were predicted for each germination date in various simulated climates as indicated (see Figure 7.2). Blue lines represent the mean weekly temperature at seed set, plotted against the parental germination date. The dotted black line indicates the 14°C dormancy threshold.

Conversely, increasing the temperature caused a reduction in flowering synchronicity, and a shift to earlier flowering and seed set dates. The window of reduced primary dormancy also occurred earlier in spring and persisted later into autumn. The earlier flowering of winter annuals resulted in seed dispersal temperatures of approximately 14°C, provided that warming was not more than 3°C from the current average. However, seed set in spring annuals was increasingly pushed into warmer conditions, resulting in reduced dormancy. The models therefore predict that spring germination would increasingly result in rapid cycling in warmer climates. However, the winter annual habit could potentially be maintained in climates much warmer than the current average, as emergence in autumn would combine with earlier flowering to produce seeds capable of remaining dormant until the following autumn.

7.2.3 Effects of altered flowering time

Mutations at many different loci have been shown to result in altered flowering time under laboratory conditions, in comparison to wild type plants. Loss of growth repressors such as the DELLA proteins for example, result in early flowering phenotypes (Cheng et al., 2004), whereas loss of flowering promoters such as *FT* result in late flowering phenotypes (Michaels et al., 2005). The presence of dominant *FRI* and *FLC* alleles also cause delayed flowering in many ecotypes (Koornneef et al., 1994), although functional *FRI* has also been associated with early flowering under winter conditions (Stinchcombe et al., 2004). Simulations of altered flowering time were therefore used to shed light on the roles of these genes in the wider context of whole life cycle phenology and seed dormancy. Combined flowering, seed set, and dormancy model simulations were run as described previously, using the

unmodified climate data from Gorzow. However, predictions from the flowering time model were adjusted before being used as input for the seed set model (Figure 7.4).

Early flowering in winter and spring annuals had very little effect on the timing of seed set, and maturation temperatures were maintained at approximately 14°C even with perturbations of up to 60 days from the normal flowering date. This was because if flowering occurred while average temperatures were still below the minimum required for photothermal unit accumulation, seed development was simply delayed and the final date of seed set was largely unaffected. The seed set model therefore acted as a buffer against earlier flowering.

In contrast, late flowering resulted in later seed set dates, which were increasingly pushed into warmer spring temperatures. This was because late flowering meant there was not enough time to fulfil photothermal requirements for seed maturation before spring temperatures increased beyond the reduced dormancy threshold. Late flowering is therefore more likely to result in reduced dormancy and an increase in rapid cycling. Together these results suggest that early flowering may not have much of an effect on overall life history in comparison to late flowering. Early flowering phenotypes may therefore be favoured if maintaining specific conditions at seed dispersal is important for offspring survival.

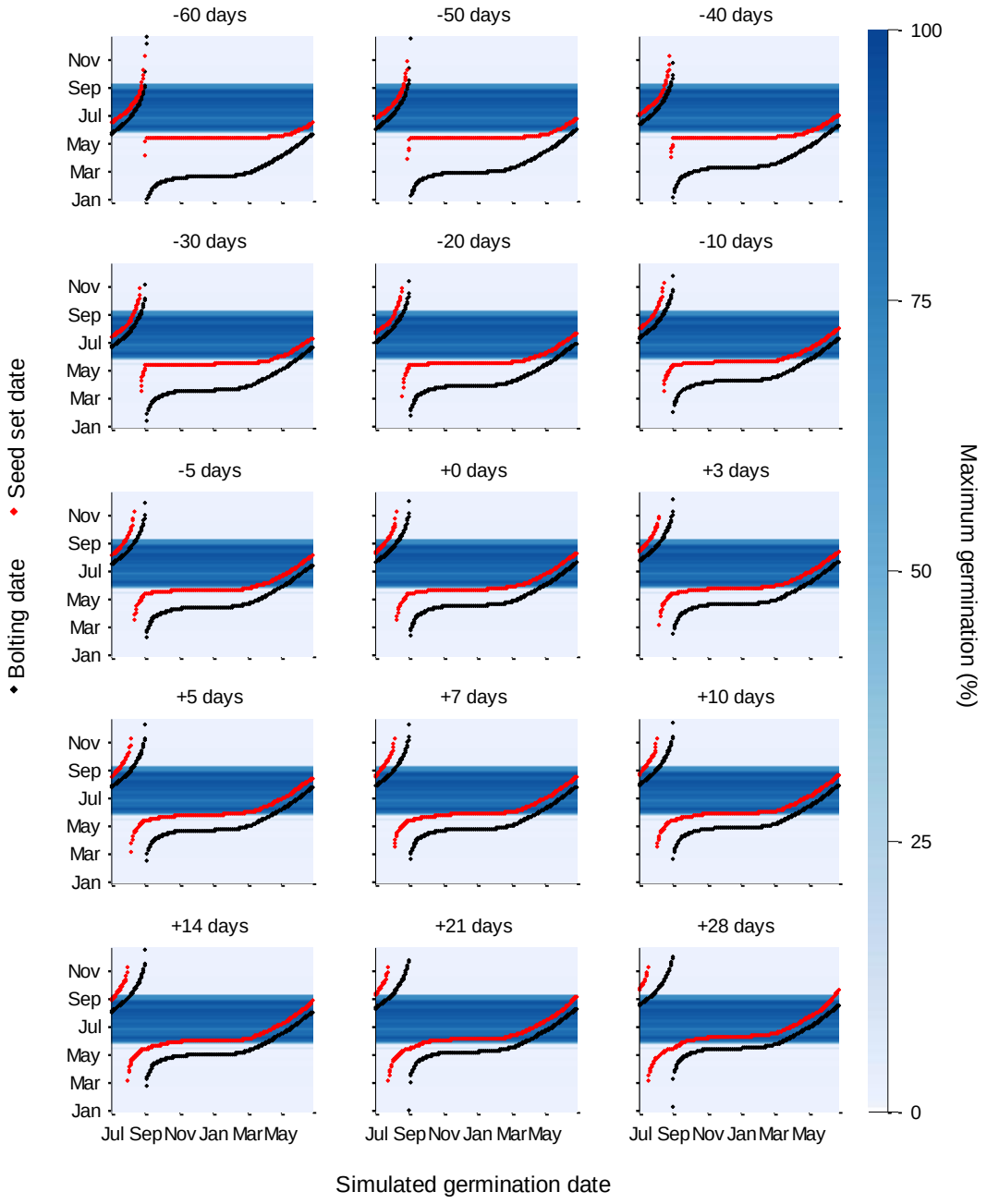

Figure 7.4 Effects of altered flowering time on seed set timing, and seed dormancy

Bolting dates were predicted for each germination date as previously described, using the current average temperatures in Gorzow, Poland. Bolting predictions (black) were then adjusted by the number of days indicated, and used as input for seed set models. Adjusted bolting dates are shown in black, and the resulting seed set dates are shown in red. Predicted seed dormancy (blue shading) was also generated as described previously.

7.3 Discussion

7.3.1 Temperatures at seed dispersal are maintained for spring and winter annuals

Models of flowering time, seed set and seed dormancy were combined to examine the effects of location, climate, and perturbations in flowering time on *Arabidopsis* phenology. A persistent outcome was that germination in spring and autumn resulted in seed dispersal when average daily temperatures were approximately 14°C. This was significant because dormancy model simulations predicted an abrupt change in the germination behaviour around this temperature. At temperatures below 14°C, primary dormancy was sufficient to prevent germination, most likely causing seeds to enter secondary dormancy and remain in the soil seed bank. However, at temperatures above 14°C, primary dormancy was reduced and high levels of germination were predicted. This abrupt transition in seed behaviour was also evident in laboratory experiments (Figure 4.1).

The extent to which temperatures at seed dispersal were maintained at 14°C across a wide range of parental germination dates was surprising, however this behaviour was also consistent when simulations were repeated with climate data from different locations. A latitudinal cline was observed in which this behaviour was most pronounced in Northern European climates, however began to break down in equatorial climates (Figure 7.1). This behaviour also persisted if mean annual temperatures were reduced. Simulations in altered climates showed that predicted flowering and seed set dates were delayed to compensate for the later onset of 14°C conditions (Figure 7.2). However if the climate was artificially warmed, models predicted that only overwintering plants would be able to adjust their reproduction enough to compensate for the advancing warm temperatures. In contrast, plants germinating in spring would be forced to produce seeds under warmer conditions,

resulting in lower dormancy (Figure 7.3). Altered flowering time also had relatively little effect on seed set dates and maturation temperatures (Figure 7.4). Early flowering had virtually no effect on seed set in winter and spring annuals, however late flowering of 2 weeks or more forced seed dispersal to occur later, and in warmer temperatures.

7.3.2 Evidence for a robust bet-hedging strategy

These results suggested that the growth of the Col ecotype in typical Northern European climates is configured to maintain specific seed maturation and dispersal conditions, and is resistant to perturbations in flowering time and climate. The fact that these particular conditions correspond to a change in seed dormancy behaviour is a surprising coincidence, which suggests it could be a deliberate strategy. A seed dispersal period which transitions the predicted 14°C dormancy threshold would enable a single parent plant to producing offspring with a full range of dormancies. This would constitute a diversified bet-hedging strategy (Slatkin, 1974; Philippi and Seger, 1989), in which the risk of germinating at a particular time is spread out amongst individuals of the same genotype. This strategy is classically associated with seed dormancy in desert annuals, and can provide an evolutionary advantage in unpredictable environments (Cohen, 1966).

7.3.3 Conclusions

Simulations combining flowering, seed set and dormancy models suggested that growth of spring and winter annuals of the Col ecotype is configured so that seeds with a range of dormancies are produced from a single parent. This is evidence of a

diversified bet-hedging strategy that allows plants to spread the risk of germination timing amongst their offspring to maximise fitness in an unpredictable environment. The strategy was also found to be common across typical Northern European locations, and was resistant to simulated perturbations in climate and flowering time.

Chapter 8: General Discussion

The broad aim of this research was to further an understanding of how plants regulate phenology in relation to seasonal cues. The genetic pathways that regulate flowering phenology in response to cues such as photoperiod and temperature, are now relatively well understood (Andres and Coupland, 2012; Khan et al., 2013). However, a growing number of studies are reporting changes in flowering times, that are correlated with rising global temperatures (Fitter and Fitter, 2002; Parmesan, 2006). It is unclear whether this change is a result of phenotypic plasticity or genetic adaptation to the changing climate, however it has highlighted a need to improve our understanding of the potential effects on other aspects of plant growth. For example, early flowering can also lead to early fruit maturation (Sherry et al., 2007; Post et al., 2008). This trend coupled with a warming climate could result in altered seed dormancy, which as well as having a genetic component, is determined by the temperature during seed maturation. The environment that seeds are dispersed into can also affect whether dormancy is broken or induced. Some species require long periods of chilling before they will germinate, and milder winters may delay their germination in spring.

The importance of germination timing has already been highlighted in many previous studies (Donohue, 2002; Donohue et al., 2005c; Korves et al., 2007; Huang et al., 2010; Chiang et al., 2013). It is a trait that can define which particular life history is expressed by a particular genotype (Boyd et al., 2007; Galloway and Burgess, 2009; Wilczek et al., 2009) . In the case *A. thaliana* for example, germination timing can determine whether plants behave as summer annuals and flower in the same season as germination, or as winter annuals where flowering is

delayed until the following spring. This plasticity allows plants to alter their growth according to their environment, but could also potentially disrupt the normal life history strategies of plants. This was illustrated in a recent study which showed an increase in life history variation in *Arabidopsis* under artificially warmed climates (Li et al., 2013).

Primary dormancy in *Arabidopsis* seeds is reduced if seeds are matured in warm temperatures. This can also cause them to lose their requirement for light, and such an extreme lack of dormancy would mean germination timing would become completely unregulated. Seedling survival could be affected, since germination in summer can lead to high rates of mortality (Donohue et al., 2005c). It has therefore been suggested that climate change could threaten the survival of many European plant species (Thuiller et al., 2005). A holistic understanding of plant responses to seasonal environments is therefore crucial to anticipate the potential effects of climate change on plant life cycles as a whole. This knowledge will also facilitate the breeding of improved crop varieties, for example by revealing phenological traits which confer optimal growth in particular locations and climates.

To meet these aims, growth and seed germination responses in the model plant species *A. thaliana* were measured in a range of controlled environments, and used to inform predictive models of seed development, and seed dormancy. These models were combined with a previously described model of flowering time (Wilczek et al., 2009), and simulations were used to investigate the effects of germination date, flowering date, location, and climate on the timing of seed dispersal, seed dormancy, and anticipated germination behaviour.

8.1.1 Dynamics of primary and secondary dormancy in relation to temperature can explain germination behaviour

The results presented here have shed light on seed dormancy and its regulation by temperature. It was discovered that germination percentages could be predicted using a small number of simple assumptions: dormancy levels of individual seeds are normally distributed, such that any change in the mean dormancy of a population results in a cumulative increase or decrease in the frequency of dormant seeds; primary and secondary dormancy are distinct processes, and vary independently within a seed population; and rates of primary dormancy loss and secondary dormancy induction are governed by seed maturation temperature and imbibition temperature respectively. This basic framework was capable of accurately describing germination in light after varying periods of stratification (Figure 4.6), and also predicting germination during stratification in darkness (Figure 5.9). This suggests the same fundamental processes of primary and secondary dormancy regulate germination in relation to temperature in both light and dark environments.

The level of primary dormancy is already known to be affected by the temperature during seed development (Schmuths et al., 2006; Kendall et al., 2011; Penfield and Springthorpe, 2012; Huang et al., 2014). Unexpectedly however, the effect of imbibition temperature on the rate of primary dormancy loss was found to be relatively minor (Figure 4.5), and warm temperatures were almost as effective at relieving primary dormancy as cold temperatures. This has been reported previously (Derkx and Karssen, 1993b; Toorop et al., 2005), however is contrary to the established view that primary dormancy in *Arabidopsis* is removed by chilling.

Results presented within this thesis instead point towards two alternative explanations for the promotion of germination by low temperatures. Firstly,

secondary dormancy induction is inhibited by low temperatures. Seeds in a state of primary dormancy are therefore more likely to germinate after chilling because secondary dormancy induction is repressed, and primary dormancy loss occurs irrespective of the imbibition temperature.

Secondly, if primary dormancy is lost relatively quickly upon imbibition at any temperature, and secondary dormancy is quickly induced by the warm conditions typically used to promote germination; seeds which would normally be classified as being in a state of primary dormancy, may in fact be in a state of secondary dormancy. Secondary dormancy can be broken by a switch from a warm to a cold temperature (Figure 6.9 and Figure 6.10). Therefore the transition from a warm to a cold environment during the chilling treatment may in fact be breaking secondary dormancy, not primary dormancy.

The response of secondary dormancy to temperature also provides an explanation for thermoinhibition, which can be thought of as a rapid induction of secondary dormancy promoted by high germination temperatures. This altered perspective of thermoinhibition could also facilitate future research into secondary dormancy, which is still poorly understood although undoubtedly plays an important role in determining germination timing in the wild.

The idea that dormancy loss and induction are acting independently and simultaneously to regulate seed germination was first proposed by Totterdell and Roberts (1979), and has been used previously to model germination in *Polygonum aviculare* (Batlla et al., 2009). The model presented here based on *Arabidopsis* can also be used to explain a variety of previously puzzling germination behaviours. For instance, a commonly cited phenomenon is that dormancy status determines the

range of environmental conditions which permit germination (e.g. Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006). In general, non-dormant seeds are capable of germinating in the widest range of environmental conditions, such as temperature and light, while increasing dormancy leads to a narrowing of this range. Seeds are said to be fully dormant when they are no longer capable of germinating in any conditions.

Figure 8.1 illustrates this behaviour in terms of primary and secondary dormancy. In a population with low primary dormancy (Figure 8.1A-B), a high proportion of seeds would germinate at either 4°C or 16°C. However, high levels of germination would only be expected at 4°C in a population with higher dormancy (Figure 8.1C - D). A comparison between panels A and C also illustrates why the time required for a particular fraction of the population to germinate may be greater in populations with higher dormancy (e.g. Chantre et al., 2009). Figures such as these showing cumulative germination patterns after different dormancy breaking or inducing treatments are common in the literature, and are reported for a wide range of species. Some examples include germination in wild rice (Figure 8 in Probert and Longley, 1989), wheat (Figure 1 in Walker-Simmons, 1988), sugar beet (Gummerson, 1986), onion (Figure 7 in Ellis and Butcher, 1988), *Jatropha* (Figure 1 in Windauer et al., 2012), and poplar (Figure 1 in Wang et al., 2013). This suggests the basic mechanism may be common to many species, and models such as this may be useful in explaining their germination behaviour.

It is also common to see final germination percentages plotted at different temperatures to illustrate how various treatments, such as pulses of light (Figure 1 in Saini et al., 1989) or after-ripening (Figure 11.1 in Probert, 2000) can alter dormancy. Figure 8.2 illustrates how the dormancy model predicts this type of

behaviour. Effects of two dormancy breaking treatments were simulated by altering the maturation temperature, thereby simulating different levels of primary dormancy. A similar method could also potentially be used to incorporate effects of additional factors such as light, water potential, nitrogen and hormone concentrations.

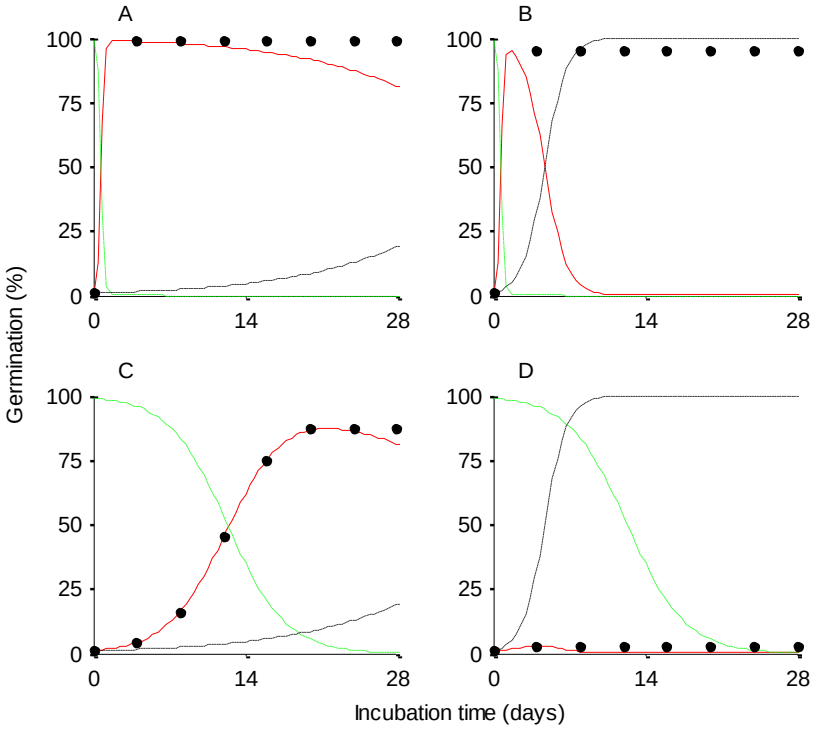

Figure 8.1 Predicted germination of two seed populations at two different temperatures

(A) and (B); A population with low primary dormancy (high maturation temperature) incubated at 4°C and 16°C respectively. (C) and (D); A population with high primary dormancy (low maturation temperature) also incubated at 4°C and 16°C respectively. The dormancy model was used to predict frequencies of primary dormancy (green), secondary dormancy (black) and germination (red) during stratification over 4 weeks. Black circles indicate the cumulative germination.

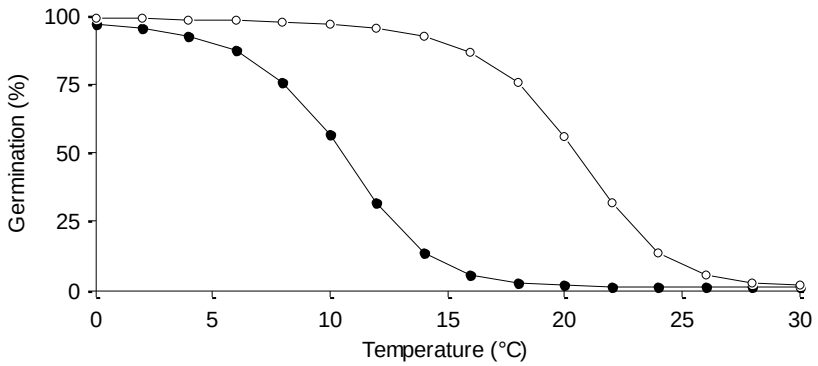

Figure 8.2 Predicted effects of dormancy levels on germination at different temperatures

The maximum germination percentages at a range of temperatures were predicted for two seed populations. Filled circles indicate a population with high initial primary dormancy, and open circles indicate a population with low initial primary dormancy.

8.1.2 A role for the circadian clock in seed dormancy

Several pieces of evidence point towards involvement of the circadian clock in the control of seed dormancy. Small differences were observed depending on whether warm or cold phases occurred during light or dark cycles during seed maturation. These differences were also amplified in *ft* mutant seeds (Figure 6.1), suggesting that FT is involved in modulating sensitivity to temperature at certain times of day during seed maturation. Because FT is also known to be involved in the circadian control of flowering in relation to photoperiod (Suarez-Lopez et al., 2001), this result potentially suggests a related role for FT in the circadian control of primary dormancy establishment. However, a recently published study found evidence that FT binds to phospholipids that fluctuate diurnally due to the influence of light on fatty acid synthesis (Browse et al., 1981; Nakamura et al., 2014). Furthermore, constitutively increasing the proportion of unsaturated fatty acids, by overexpression of *FATTY ACID DESATURASE 3* (*FAD3*) caused a significant delay in flowering (Nakamura et al., 2014). Interestingly *FAD3* expression is also upregulated in seeds matured at low temperatures (Kendall et al., 2011). The effects of FT on primary

dormancy establishment could therefore be linked to the regulation of fatty acid synthesis by light and temperature, rather than being linked to the circadian clock.

A more reliable indication that a process is under circadian control is rhythmic behaviour which persists in constant conditions. Accordingly, a rhythmic oscillation in response to light was observed in imbibed seeds incubating in constant darkness at 16°C (Figure 6.13). Primary dormancy loss at lower temperatures did not exhibit any circadian oscillations, which were therefore presumed to be exclusive to warm temperature induced secondary dormancy. This can also be interpreted as further evidence that primary and secondary dormancy operate with distinct mechanisms (Bouwmeester and Karssen, 1993; Derkx and Karssen, 1993a; Barua et al., 2012).

Oscillations were also found to be unaffected by constitutive *CCA1* expression; a core component of the shoot clock (Locke et al., 2005; Locke et al., 2006; Pokhilko et al., 2010; Pokhilko et al., 2012). This was surprising since constitutive expression of *CCA1* has been shown to abolish rhythmic expression of many other genes in constant conditions (Wang and Tobin, 1998). However, previous findings have also shown that oscillations in responses to germination in light were independent of *GI*; another clock component (Oliverio et al., 2007). These results hint at the existence of a seed specific clock, which oscillates independently of *CCA1* or *GI*, and may therefore have a different architecture to the shoot clock. It is possible for example that a seed specific clock may share some similarities to the root clock which was previously reported to be a simplified version of the shoot clock (James et al., 2008).

A role for flowering genes in the control of dormancy in *Arabidopsis* has already been described in the case of FLC (Chiang et al., 2009), and similarities between flowering pathways and control of bud dormancy in perennial plants have also been

described (Horvath, 2009). Pleiotropy in genes controlling seasonal events such as flowering and germination, could therefore be common. Further similarities, particularly those conferring links between the circadian clock and primary dormancy establishment during seed maturation, and also with regulation of secondary dormancy are therefore anticipated to be an important aspect of future dormancy research.

8.1.3 The significance of flowering timing for germination strategies

The development of a seed dormancy model incorporating both maternal, and post-dispersal temperature effects allowed the link between reproductive timing and seed dormancy to be investigated. Simulating seed dispersal on different dates revealed a distinct transition in germination behaviour when mean daily temperature exceeded 14°C (Figure 4.11 and Figure 4.12). The model predicted seeds dispersed at temperatures below this threshold would possess long term dormancy. This was because the level of primary dormancy would prevent germination until secondary dormancy was induced. Seeds would therefore remain in the soil seed bank until the return of conditions that alleviate secondary dormancy. However, if seeds were dispersed at temperatures above 14°C, the model predicted high levels of germination shortly after dispersal, since the rate of primary dormancy loss would surpass rates of secondary dormancy induction. The timing of dispersal in relation to the annual temperature cycle is therefore crucial in determining germination behaviour.

To integrate these findings with reproductive phenology, a photothermal model of seed set timing was also developed. Three models were initially suggested, which

differed in the way photoperiod was used to modify rates of seed maturation (Figure 3.3). However in practice, model choice made little difference owing to the relatively minor effect of photoperiod in comparison to temperature (Figure 3.6). Simulations showed that the time required to transition from flowering to seed dispersal was largely dependent on the flowering date, which ultimately determined the temperature experienced. However, when combined with a model of flowering time (Wilczek et al., 2009) it was apparent that not all flowering dates were equally likely. Autumn and early spring germination, which are the most common emergence times for *Arabidopsis*, often resulted in synchronous spring flowering (Figure 3.8A). This also meant seeds would be produced and dispersed in the same environment, despite parent plants having a potentially large range of germination dates (Figure 3.8B). This behaviour was also resistant to modest changes in flowering date. For example, if flowering of a typical winter annual was shifted earlier or later than originally predicted, the time required for seed development would simply increase or decrease, meaning that roughly the same dispersal date was maintained (Figure 3.11 and Figure 7.4). These results illustrated that plasticity of growth rates in each growth phase (vegetative to flowering; and flowering to seed dispersal) served to buffer against perturbations in germination and flowering date, and ensured the environment at seed dispersal was maintained.

Combining these results with the simulations of the dormancy model suggested this particular seed dispersal environment was highly relevant, since it was typically very close to the 14°C dormancy threshold (Figure 7.3). It was therefore suggested that seeds produced early during dispersal would be highly dormant owing to the relatively low seed maturation temperature. However, during the course of the dispersal period, newly matured seeds would be increasingly less dormant as

temperatures rise above 14°C. This would mean offspring germination would be spread across multiple seasons, therefore creating a bet-hedging strategy.

Bet-hedging was first described in desert annuals, which stagger their emergence over multiple years (Cohen, 1966). This was seen as a risk spreading strategy which evolved to avoid catastrophic reproductive failure in an unpredictable environment. Results of field experiments presented in this thesis also demonstrated how the fitness associated with germination in any particular season is not always predictable (Table 3.2). Sowing in autumn in 2011 led to a high proportion of plants reaching reproductive age, whereas sowing in spring led to high mortality due to early drought. In contrast, sowing in autumn the following year led to high mortality due to late snowfall in spring when plants had already begun flowering, but sowing in spring led to good survival. A bet-hedging strategy would ensure a fraction of the seed population germinates in the best season, whilst preventing synchronous germination in a bad season, and may therefore be advantageous in this location.

Diversifying bet-hedging is defined as a strategy in which individuals of a single genotype express multiple phenotypes (Philippi and Seger, 1989). Applied to seed dormancy, this means a single parent should produce offspring which emerge at different times without any heritable differences between them. Maternal effects are thought to play an important role in producing this variation (Fenner, 1991). Model simulations suggested that spring and winter annuals are likely to produce both dormant and non-dormant seeds owing to the continuously increasing mean daily temperature in spring. However, small differences in the microenvironment caused by the positioning of individual seeds on the parent plant can also create variation in seed dormancy (Philippi, 1993; Gutterman, 2000). A field emergence experiment

demonstrated that even a seed batch produced under constant 18°C can spread emergence timing over at least two seasons (Figure 6.11).

8.1.4 Predicted effects of climate change on Arabidopsis phenology

Spreading emergence timing has been documented in a diverse range of species, including some animals (Evans and Dennehy, 2005). In plants however, this strategy relies on the maintenance of a viable seed bank (Fenner and Thompson, 2005). Current climate models forecast potential mean surface temperature increases of up to 4.8°C by the end of the century (Stocker et al., 2013). The frequency and intensity of heat waves is very likely to increase, and in mid-latitude and subtropical regions drought and flooding events will become more frequent, causing greater disparity between wet and dry regions. Bet-hedging could therefore be an increasingly important strategy as the environment becomes more unpredictable. However, increases in mean global temperatures could lead to reduced seed dormancy and viability, and changes in precipitation could lead to increased seedling mortality. Climate change could therefore threaten seed bank persistence; an issue which few studies have so far addressed (Ooi, 2012). Model simulations also showed that for seeds dispersed at temperatures above 18°C, at least 50% would germinate without a requirement for light. Summer dispersal in many locations was therefore predicted to result in high levels of germination (Figure 5.14), which was not repressed until temperatures reached in excess of 31°C. Under these circumstances very few seeds would be capable of long term dormancy, and the soil seed bank would not be replenished.

However, results presented in this thesis also suggest that phenotypic plasticity would play a significant role in mediating the effects of changes in climate. When simulations were run using photoperiod and temperature data from different locations, plasticity in flowering time meant that spring and winter annuals maintained similar seed dispersal conditions (Figure 7.1), meaning a seed bank and bet-hedging strategy would most likely be maintained. This was generally consistent in climates similar to the Col ecotype's native environment in Northern Europe, and suggests a single ecotype could be capable of a wide geographic distribution without requiring genetic adaptation, or significant alterations to annual habits.

The same was also true if the climate in a particular location was modified. Mean annual temperatures in Gorzow, Poland were altered to simulate climate change and examine the effects on seeds at dispersal. With small increases in temperature, flowering time in winter annuals became earlier (Figure 7.2) and this did not have a large effect on the predicted seed maturation temperature (Figure 7.3). Early spring flowering has already been observed in many plant species as a result of anthropogenic climate change (Fitter and Fitter, 2002; Parmesan, 2006; Cleland et al., 2007), and was also recently demonstrated experimentally in *Arabidopsis* with artificial warming experiments (Li et al., 2013). However, simulations showed that raising the temperature by more than 3°C increasingly pushed seed set into a dormancy inhibiting environment, as increases in growth rates could not keep up with advancing warm temperatures in spring. This was partly due to the vernalisation requirement built into the flowering model (Wilczek et al., 2009), which caused a reduction in the rate of photothermal unit accumulation if the vernalisation requirement was not fully met. An increase in sensitivity to vernalising temperatures

may therefore be required to allow plants to flower earlier if winters become milder (Stinchcombe et al., 2005b).

The effects of altered flowering time were also investigated, and while seed set timing was resistant to late flowering of up to two weeks, earlier flowering had virtually no effect (Figure 7.4). This suggests late flowering phenotypes may be selected against, due to the potential effect on seed dispersal. Genetic variation at the *FRI* locus has been interpreted as selection for early flowering (Le Corre et al., 2002), and analysis of over 100 plant species also showed that early flowering was generally favoured due to environmental factors (Munguia-Rosas et al., 2011). However these types of studies generally measure fitness by seed yield, and offspring fitness is rarely considered. The direction of selection may also be dependent on germination timing, since flowering early in autumn rather than delaying flowering until spring can be detrimental (Donohue et al., 2005c).

The results of these simulations suggest the winter annual habit would convey the greatest resilience to climate warming, by enabling a persistent seed bank and bet-hedging strategy to be maintained. In current climates across Northern Europe, the models predict the same seed dispersal conditions are maintained for both spring and winter annuals. This is because flowering time is relatively insensitive to germination dates from autumn to early spring. However as the climate warms, the models predict that flowering time will become more sensitive to germination date, and the range of germination dates which result in these seed dispersal conditions will be reduced. The timing of germination would therefore become increasingly important. Seasonal emergence timing is still poorly understood, and is generally assumed to be a result of secondary dormancy cycling. It was demonstrated that secondary dormancy was broken in Col and L*er* ecotypes by a shift from a 20°C to

4°C (Figure 6.9 and Figure 6.10), and diurnal temperature fluctuations in this range correlated with seedling emergence in the field (Figure 6.11). Given the importance of germination timing, more research is needed to understand these processes. In particular, the regulation of secondary dormancy which remains largely mysterious, should be the focus of future dormancy and germination phenology research.

To summarise, the results in this thesis have shown that it is possible to reproduce the effects of pre- and post- dispersal temperature on seed dormancy by modelling simultaneous primary dormancy loss and secondary dormancy induction. Combining models of flowering and seed set timing revealed a conserved seed dispersal environment for spring and winter annuals. This environment coincided with a predicted shift in dormancy behaviour that occurred when mean daily temperatures exceeded 14°C. This would most likely result in the production of seeds with a range of dormancies, therefore spreading emergence timing across multiple seasons. It was suggested that this could be a deliberate bet-hedging strategy to reduce the chance of reproductive failure in unpredictable environments.

Simulations also suggested that plasticity in flowering and seed dispersal timing would buffer against changes in germination and flowering date, and small changes in climate. This could allow a single ecotype to exist in a wide geographic range, and withstand small changes in mean annual temperature without the requirement for genetic adaptations. Models also predicted that winter annuals would be most resilient to climate change, however beyond temperature increases of 3°C adaptations such as increased vernalisation sensitivity may be required. This study also highlighted the importance of seasonal germination timing, and pointed towards links between dormancy and the circadian clock.

Abbreviations

ABA	Abscisic Acid
Col	Columbia ecotype of *A. thaliana*
Cvi	Cape Verdi Islands ecotype of *A. thaliana*
FR	Far-red light
GA	Giberrelic acid
L*er*	Landsberg *erecta* ecotype of *A. thaliana*
LFR	Low Fluence Response
PD	Physiological dormancy
Pfr	Inactive, far-red light absorbing phytochrome
Pr	Activated, red light absorbing phytochrome
R^2	Coefficient of determination; defined in equation [7]
RMSE	Root Mean Squared Error; defined in equation [34]
SE	Standard Error
VLFR	Very Low Fluence Response

Glossary

After-ripening	A period of dry storage to alleviate dormancy
Bolting	First visible sign of flowering, appearance of the flowering stem, or inflorescence
Circadian rhythm	An endogenous oscillation which persists in constant conditions, and has a period of approximately 24 hours
Dehiscence	The splitting of seed containing siliques at maturity
Dormancy	Failure of a viable seed to germinate in favourable conditions
Homoscedasticity	In statistics, the property of having equal variance
Imbibibition	The process by which the seed absorbs water
Life history	The history of an organism's life cycle
Maternal effect	The phenotype of an individual is affected not only by its genotype and the environment, but also by the maternal genotype or phenotype
Maturation	The phase of seed development after embryogenesis, in which primary dormancy is established
Phenology	The study of periodic natural events
Phenotype	An organism's observable characteristics or traits
Phenotypic plasticity	Ability of an organism to change its phenotype in response to changes in its environment
Phytochromes	A family of light sensitive photoreceptors in plants
Primary dormancy	Dormancy which is established during seed development
Quiescence	A non-dormant seed which does not germinate due to a lack of suitable environmental conditions (e.g. light, moisture,O_2)
Scarification	Scratching or cracking the seed coat to encourage germination
Secondary dormancy	Dormancy which develops after seed dispersal
Seed set	Gone to seed (i.e. seed shedding or dispersal)

Stratification	A pre-germination treatment given to seeds to break dormancy, usually consisting of a period of moist incubation at low temperatures (between 1 and 5°C); however warmer temperatures may be used instead
Thermal time	Cumulative temperature or heat-sum, usually has units of degree-days (°C days)
Thermoinhibition	Inhibition of germination at high temperatures
Vernalisation	The acquisition of flowering competence in spring by exposure to winter chilling
Vivipary	Giving birth to live young in animals, or germination while still attached to the parent (pre-harvest sprouting)

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