Adaptation of ash (*Fraxinus excelsior* L.) to climate change

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A thesis submitted for the degree of Doctor of Philosophy Bangor University April 2013

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Abstract

During the twentieth century, global surface air temperatures rose by 0.8 °C, largely as a result of anthropogenic driven climate change. During the twenty first century, the climate is projected to warm by 2 - 3 °C by 2050, and by as much as 5 °C towards the end of the century. Temperate forests are expected to be susceptible to the effects of warming as their long generations times limit the potential for rapid migration or adaptation.

Reciprocal transplant experiments were used to test populations of *Fraxinus excelsior* in local and non-local environments to investigate local adaptation across a 2000 km transect of the species natural range. Early results indicate that there is no home site advantage and local adaptation was not found in the populations studied. Phenological observations made in two provenance trials of *Fraxinus excelsior* revealed that provenances broke bud at different times at a single site, and also at different sites and in different years. Modelling of the phenological response found that mean spring temperature accounted for more variation than other environmental signals.

Germination testing of *Fraxinus excelsior* found that the seed from northern provenances required greater chilling to break dormancy than those provenances from further south. However, the chilling requirement was fully met in all provenances at present.

In summary, this study reveals that provenances of *Fraxinus excelsior* contain sufficient genetic variation to cope with medium term climate change through phenotypic plasticity.

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Chapter 1 Forest Robustness in a Changing Climate: Setting the Scene

1.1 Introduction

Britain's forests are of paramount importance for many environmental, social and economic reasons. In addition to biodiversity, habitat, landscape, amenity and recreation benefits, they provide economic returns from timber production and are one of few truly renewable, sustainable resources. Trees growing today and those in the future will face unprecedented rates of climate change, yet little is known about how tree species will respond to the predicted changes or how foresters and other land managers should prepare for them. It is vital to ensure that the species and genotypes that are being proposed for planting today will be able to cope with the changing climate. The question arises: will populations of native tree species be robust enough to survive and grow well until harvestable age, or should we be looking for different provenances of native trees?

1.1.1 The evidence for climate change

The fourth assessment report (AR4) by the Intergovernmental Panel on Climate Change provides the evidence for and the likely impacts of climate change (IPCC 2007). It is now widely accepted that climate change is largely due to increases in anthropogenic greenhouse gas emissions of carbon dioxide, methane and nitrous oxide since the middle of the 18th century. Of most concern is carbon dioxide (CO₂), which has risen considerably since pre-industrial levels of about 280 ppm to a current level of 395 ppm (NOAA 2012) and far exceeds the natural range over the last 650,000 years of 180 - 300 ppm of CO₂. This recent rise is largely due to the burning of fossil fuels, and also changes in land use.

Deforestation accounts for 18 - 20 % of CO_2 emissions (IPCC 2007). Carbon dioxide alone causes about 60 - 65 % of the human-induced greenhouse effect (Hulme 2002).

The global average surface temperature rise over the 100 year period from 1906 - 2005 was 0.74 °C (IPCC 2007). For the last 50 years of this period, the warming trend has been 0.13 °C (0.10 to 0.16 °C) per decade, almost twice that for the last 100 years. Diurnal temperature ranges have decreased because minimum temperatures are increasing twice as quickly as maximum temperatures. The AR4 ranks eleven of the twelve years from 1995 - 2006 among the twelve warmest years on record of global surface temperature (IPCC 2007). More recent data from the National Oceanic and Atmospheric Administration shows that each of the last 11 years (2001 - 2011) features as one of the 12 warmest on record (NOAA 2012) (Figure 1.1).



- The Met Office, in collaboration with the Climate Research Unit at the University of East Anglia (UK)
- The National Climatic Data Center (NCDC), part of the National Oceanic and Atmospheric Administration (NOAA) USA.
- The Goddard Institute for Space Studies (GISS), part of NASA (USA)
- Figure 1.1 Three global-average temperature records from 1850 2011. Source: The Met Office (2012).

The most recent climate change predictions for Europe suggest that surface temperatures will increase by 2 °C in Ireland and the UK, by up to 3 °C increase in central Europe and by 4 - 5 °C in northern boreal and some Mediterranean regions by the end of the 21st century (Christensen *et al.* 2007). The IPCC developed a set of six emission scenarios, based on different economic possibilities. For all scenarios, CO₂ concentrations are projected to rise dramatically, reaching levels of between 540 and 970 ppm by the year 2100 (Nakicenovic *et al.* 2000).

1.1.2 <u>Climate change predictions for the UK – UKCP09</u>

The 2009 UK climate projection model (UKCP09) is the most recent government funded assessment of future climate in the UK. Three IPCC emissions scenarios (B1, A1B and A1F1, Nakicenovic *et al.* 2000) are used to model the effects of low, medium and high carbon emissions. These represent, respectively, rapid economic growth with rapid changes towards a service and information economy (B1 – low emissions); rapid economic growth with new and efficient technologies, and a balanced emphasis on all energy sources (A1B – medium emissions), and rapid economic growth with new technologies as in A1B, but with an emphasis on fossil fuels (A1F1 – high emissions). These scenarios indicate that the UK climate will continue to warm through the 21st century and that there will be changes in rainfall and its seasonal distribution, which will vary regionally. Details of the UKCP09 medium emissions scenario for 2080 are given in Box 1 (Murphy *et al.* 2009). It should be noted that at the time of writing (2012), the A1F1 high emissions scenario is being realised globally, despite many governments' commitments to reduce emissions.

These projections of climate change take into account uncertainty due to natural variability and modelling errors by giving the probabilities of a range of outcomes. Climate projections for the UK indicate an increase in mean summer temperature of 3 - 4 °C by the 2080s under the medium emissions scenario, with the increase being greater in the south and east. Although there will be little change in total annual rainfall, summer rainfall with decrease while winter rainfall will increase. As a result, summer droughts may become more frequent and severe. A larger proportion of the rainfall is also projected to occur during extreme events, thereby extending the duration of winter waterlogging and increasing the

severity of summer flooding, as well as increasing the likelihood of soil erosion and frequency of landslips. Cloud cover, particularly in summer, will decline (Murphy *et al.* 2009).

Box 1.1 Climate change projections for the UK in 2080, under the medium emissions scenario (adapted from Murphy *et al.* 2009). Central estimates of change (at the 50 % probability level) are followed in brackets by changes which are very likely to be exceeded and very likely not to be exceeded (10 and 90 % probability levels respectively).

All areas in the UK warm, more so in summer than in winter. Changes in **summer mean temperatures** are greatest in southern England at 4.2 °C (2.2 to 6.8 °C) and smallest in the Scottish islands at 2.5 °C (1.2 to 4.1 °C).

Mean daily maximum temperatures increase everywhere, with summer average increases by 5.4 $^{\circ}$ C (2.2 to 9.5 $^{\circ}$ C) in parts of southern England and 2.8 $^{\circ}$ C (1 to 5 $^{\circ}$ C) in northern Britain. Increases in winter temperature are 1.5 $^{\circ}$ C (0.7 to 2.7 $^{\circ}$ C) to 2.5 $^{\circ}$ C (1.3 to 4.4 $^{\circ}$ C) across the UK.

Mean daily minimum temperature increases on average in summer by 2.7 °C (1.3 to 4.5 °C) to 4.1 °C (2.0 to 7.1 °C) with the biggest increase in southern England and the smallest in northern Scotland. In winter it increases by 2.1 °C (0.6 to 3.7 °C) to 3.5 °C (1.5 to 5.9 °C) depending on location.

The biggest changes in **precipitation in summer,** about 40 % reduction (65 to 6 %), are seen in the south of England with change close to zero (-8 to +10 %) over northern Scotland.

Precipitation in winter is projected to increase by up to 33 % (9 to 70 %) in the west with slight decreases over parts of the Scottish highlands.

Relative humidity will decrease by around 9 % (0 to 20 %) in summer in parts of southern England, but decrease less elsewhere. Winter changes are less significant.

Summer mean cloud will decrease by 18 % (2 to 33 %) in parts of southern England, but increase in parts of northern Scotland. Changes in **winter cloud cover** will be negligible.

Projected changes in **storms** are very different in various climate models, as are changes in anticyclonic weather, and so are difficult to predict accurately.

It is unlikely that an abrupt change to the Atlantic Ocean Circulation (**Gulf Stream**) will occur in the 21st century.

1.2 The Policy Framework

1.2.1 Legislation and government policy

The Climate Change Act 2008 sets a legally binding target for reducing total greenhouse gas emissions in the UK. Taking 1990 as the benchmark, it commits the UK to a reduction of at least 80 % by 2050. A framework of five-year targets has been set which includes a 34 % reduction by 2020 (DECC 2008). In Scotland, the **Climate Change (Scotland) Act 2009** also sets a limit of 80 % emissions reduction by 2050, with an interim target of a 42 % reduction by 2020.

To help with climate change mitigation, government places an emphasis on addressing the amount of carbon dioxide in the atmosphere. This can be achieved through the cutting of emissions by reducing reliance on fossil fuels, and also through the creation of new woodlands which lock up carbon for many years through sequestration. **The UK's Renewable Energy Strategy** sets a target for renewable sources to produce 15 % of the UK's energy requirement by 2020, a third of this through biomass (DECC 2009). The **Natural Environment White Paper** states that to achieve this, the aim is to increase woodland cover in England from 10 % to 13 % by 2060, an average of 8,000 hectares a year (Defra 2011). Similar amounts of woodland creation have been set as targets by other UK countries. However, the choice of planting stock deployed needs careful thought if it is to survive and thrive in today's climate and under future climate scenarios.

Forest Reproductive Material (FRM) regulations were first introduced in 1977 to control the quality and movement of material for forestry purposes. They introduced regions of provenance (RoP) which are based on broad geoclimatic and ecological factors, including major watersheds. In Britain, there are four RoP which are further subdivided into 24 native seed zones (Herbert *et al.* 1999) under the **Forest Reproductive Material (Great Britain) Regulations 2002** which implement EU directive 1999/105/EC. These regulations place basic material in to one of four categories listed below.

Source identified (SI) material comes from a specified location within a single region of provenance or seed zone and altitude band, and has no known superior quality.

Source selected (SE) material is collected from stands showing superior characteristics such as form, growth rates or health.

Qualified (QU) material is derived from the selection of superior phenotypes (such as plus trees) but has not undergone any form of testing.

Tested (TE) material is derived from selection of superior trees or stands that have undergone evaluation for genetic quality, or have been shown to be superior in comparison to accepted standards.

The UK Forestry Standard was launched in 1998. Along with good forestry practice guidelines, it gave recommendations on the use of planting stock for reforestation and aforestation to 'maintain genetic integrity of populations of native species'. It stipulated that for broadleaved planting in the vicinity of semi-natural woodlands, seed sources from local autochthonous stands were preferred, i.e. those of local origin, that arose in that location. In other areas, it recommended British origin material (Forestry Commission 1998). This practice was endorsed by the UK Woodland Assurance Standard, launched in 2000, and is still the case in the third edition (UKWAS 2012). This approach assumes that local stock is adapted to local conditions, and therefore more resilient. However, the nursery trade has been slow to adopt and promote local provenance material. In 2012, there are 14 registered seed stands for Fraxinus excelsior on the National Register of Approved Basic Material, but only four of these are of SE status, the others being SI, and many of which are not managed as seed stands. A survey of 24 forest nurseries and seed merchants reported that nearly all material supplied fell into the source identified category, and the demand for local provenance material was minimal. British material was regularly outperformed in the nursery by material from the European continent in terms of vigour and for some species, mildew susceptibility. This pushed production costs up for local provenance material, which required greater input of chemicals and another year in the nursery (Russell and Evans 2004). Also, British seed sources were often poor due to small,

fragmented populations and unmanaged seed stands making seed collection from a sufficient number of parent trees difficult and expensive. However, provenance trials have shown that some European continental material is poorly adapted to British growing conditions (Worrell 1992; Cundall *et al.* 2003) due to the prevalence of late spring frosts and its use often results in poor form due to forking.

The second edition of the UK Forestry Standard (Forestry Commission 2004) also recommended the use of local native material and referred the reader to Forestry Commission Practice Note 8 – (Using Local Stock for Planting Native Trees and Shrubs (Herbert *et al.* 1999)), and it was not until the third edition in 2011 that recommendations really changed to address possible impacts of climate change (Forestry Commission 2011a). Here, rather than the emphasis being on conservation or production, attention is given to adaptability. Recommendations include: the consideration of alternative species and increased species diversity; choice of trees that are well adapted to the site and of a wide genetic base to promote future adaptation; still encouraging natural regeneration to conserve distinctive genetic patterns, especially in semi natural woodlands (Forestry Commission 2011a).

1.2.2 <u>Climate change policy of non-governmental organisations</u>

The Woodland Trust believes that climate change is the biggest threat to the UK's native woodland and that it is already so apparent that change is inevitable and measures must be developed for adaptation (Woodland Trust 2006). Traditionally, the Woodland Trust has always strongly endorsed the use of local provenance in planting specifications to conserve locally adapted material. However, their *Position Statement on Tree Provenance* (Woodland Trust 2007) acknowledges that the exchange of genetic material between populations is now both more likely and desirable in a changing environment, as it will result in greater genetic diversity within populations, thereby making them more adaptable to change. They state that 'the balance between differently adapted populations in contrasting local environments and reproductive contact through long distance movement of pollen maintains genetic diversity and adaptability in tree species at a European level. The planting of native trees of non-local provenance should, therefore, pose little risk to existing tree

populations locally, particularly as it is likely only to form a small proportion of the total resource' (Woodland Trust 2007). This view has been further promoted in an updated *Position Statement on Tree Provenance* (Woodland Trust 2012) in light of the outbreak of ash dieback, *Chalara fraxinae*, stipulating the use of a wider range of non-local UK provenance material to help build resilience in to populations in the face of climate change and novel pests and diseases. They also advocate a precautionary approach to non UK material, but to trial small amounts of European material of UK native species.

While natural regeneration has been their preferred method for woodland creation, the Woodland Trust recognises that planting may now be necessary given rapid environmental change. They also state that non-native species should not be used as they may compound pressure on woodland ecosystems rather than enhance their resilience (Woodland Trust 2007).

Non-Governmental organisations Plantlife and Flora Locale both recognise that the best way of enhancing the prospect of long-term adaptation is through the maintenance of genetically diverse populations. They therefore promote use of British material from more southerly or lower elevation locations up to 250 km distance, and only promote the use of local origin material in ecologically sensitive areas (Plantlife 2005; Flora Locale 2007). Flora Locale also recommends that natural regeneration should be promoted where this is likely to work, e.g. for woodland expansion, rather than planting because natural selection processes are often strongest at the point of seed germination and seedling growth/survival. All organisations mentioned recognise that habitat fragmentation is one of the greatest threats to adaptation, and promote habitat connectivity through the creation and restoration of woodland.

Other organisations such as Wildlife Trusts, Woodland Heritage, and the Institute of Chartered Foresters make brief mention of climate change on their websites. However, they do not give specific position statements, nor do they make reference to use of provenance, novel species or adaptation, illustrating the lack of recognition of the importance of these issues and the role they may play in helping forests and woodlands adapt to climate change.

1.3 Forests and Climate Change

1.3.1 The effects of climate change on forests

The factors related to climate change that affect forest growth to the greatest extent are atmospheric carbon dioxide levels, temperature and precipitation. These factors impact physiological processes which in turn affect the distribution of species and ecosystems. Other factors are also expected to impact forests e.g. storms, wind, fire, humidity and snow cover, but these are more difficult to model and therefore predictions are less certain (Broadmeadow and Ray 2005). Any of these factors may cause trees to become stressed, making them more susceptible to pests and diseases, which are likely to exhibit increased activity and reduced winter mortality as a result of warmer summers and milder winters that are predicted for temperate regions (Evans *et al.* 2002). A summary of the possible effects that climatic variables may have on tree growth and function are given in Box 1.2.

Carbon dioxide

An increase in CO_2 concentration, as predicted in the IPCC special report on emission scenarios (Nakicenovic *et al.* 2000), will increase the rate of photosynthesis because rubisco, the enzyme responsible for fixing carbon, is not saturated at current ambient CO_2 levels (Ainsworth and Long 2005). This in turn affects net primary production. In young trees, growth could be increased by as much as 30 - 50 % (Broadmeadow and Randle 2002), although this is unlikely to be as great in mature trees. Stomatal conductance is also likely to decrease with elevated CO_2 resulting in better water use efficiency (Medlyn *et al.* 2001).

The magnitude of increased primary production varies among species and also with plant nitrogen status. Tree growth may not increase proportionally with increased photosynthesis where nutrients are limiting (Oren *et al.* 2001; Hungate *et al.* 2003). Norby *et al.* (2010) reported an initial fertilising effect under elevated CO₂ (550 ppm) in free air CO₂ enrichment (FACE) experiments of 24 % increase in net primary production compared to ambient levels of CO₂, but this dropped dramatically after six years as nitrogen became limited.

Variable	Positive effects	Negative effects
Increase in	An initial increase in growth rate due to	Increase in leaf area resulting in higher wind
atmospheric	a fertilizing effect through additional	resistance and water use (Quine and
CO ₂	carbon fixation (Drake <i>et al</i> . 1997)	Gardiner 2002)
	Increased water use efficiency due to	Possible changes in ground flora due to lower
	reduction in stomatal conductance and	light transmission (Broadmeadow and Randle
	transpiration (Medlyn <i>et al.</i> 2001.)	2002)
	Improved timber quality in broadleaves through faster growth rates (Savill	
	1991)	
Increase in	Longer growing season (Menzel and	Delayed hardening (Saxe et al. 2001)
temperature	photosynthesis (Drake <i>et al.</i> 1997)	Altered phenology resulting in species
	resulting in higher net primary	mistimings (Sparks and Gill 2002) and
	production	Increased risk of spring and autumn frost
	Lower risk of winter cold and snow	
	damage	Reduced winter mortality and increased
	The notential use of novel species	(Evans et al. 2002)
	which are currently not adapted to	
	British conditions (Cannell et al. 1989)	Potential for exotic pests to spread to the UK (Evans <i>et al.</i> 2002)
	Earlier budburst resulting in improved	Lack of natural regeneration where seed
	timber quality in oak (Savill and Mather	chilling requirements are not met (Gosling
	1990)	and Broadmeadow 2006)
Change in	Reduced intensity of some foliar	Summer drought more likely especially in
timing and	, pathogens	southern England, restricting productivity
amount of		(Broadmeadow and Ray 2005) and reducing
rainfall,		seedling recruitment (Silva et al. 2012).
increasing in		Waterlogging causing fine root death (Coutts
decreasing in		and Philipson 1978) which in turn makes
summer		trees more susceptible to summer drought
		and soil borne pathogens (Lonsdale and
		Drought stress, making trees more
		susceptible to pathogens (Huberty and Denno 2004)
Increase in wind		An increase in deep depressions, increasing
speed		the risk of wind damage (Quine and Gardiner
		2002)
Reduction in	Potential increase in productivity	Increased diurnal temperature range in
cioud cover	(Broadmeadow <i>et al.</i> 2003)	autumn due to reduced cloud cover,
		(Broadmeadow <i>et al.</i> 2003).

Box 1.2 Summary of possible effects on trees with changing climatic variables.

Temperature

Mean surface temperature is projected to increase by 2 °C above the 1961 - 1990 baseline for the UK by the end of the 21st century (IPCC 2007). Increases in winter temperatures are projected to be greater than increases in summer temperatures resulting in reduced incidences of frost, and the growing season is projected to increase by 5 - 10 days (IPCC 2007). These changes will have profound effects on tree physiology and other natural processes.

An increase in temperature is expected to be mostly beneficial for tree growth since it should result in an extended growing period and reduced risk of frost and other winter related damage. Of perhaps the greatest concern is the effect of temperature on phenology and the possible mistimings that may result (Sparks and Gill 2002; Visser and Both 2005). Budburst has been reported to be occurring earlier over the latter half of the 20th century (Menzel and Fabian 1999; Menzel 2000; Parmesan and Yohe 2003; Menzel *et al.* 2006), with oak in southern England advancing by approximately three weeks, or six days for every 1 °C increase in spring temperature (Figure 1.2).



Figure 1.2 Observed first budburst date for oak in Surrey between 1950 and 2007, showing approximately three weeks advancement. Reproduced from the UK Phenology Network (2012).

In Britain, spring frosts are still likely to occur, and these may negate any benefits from earlier budburst (Bennie *et al.* 2010). In a study of 30 years of phenological observations in Europe, Menzel and Fabian (1999) reported spring budburst occurring six days earlier and a delay in autumn events such as leaf colouring of 4.8 days, extending the growing season by 10.8 days since the early 1960s. However, as photoperiod is the trigger for trees to harden in preparation for dormancy, trees may fail to take full advantage of an extended growing season (Saxe *et al.* 2001). In addition, milder winters may result in the chilling requirements for flowering and budburst or seed germination not being met.

Precipitation

Rainfall is projected to be less in summer, leading to periods of drought. Increased winter rainfall on the other hand may lead to waterlogging, resulting in reduced tree stability (Ray and Nicholl 1998) and possible death of fine roots (Coutts and Philipson 1978). Death of fine roots will compound stress induced by summer drought.

1.3.2 <u>Tree responses to a changing climate</u>

The distribution of plant species is strongly influenced by climate and water regime (Woodward 1987; Huntley 1991). Plants have evolved in such a way that they are finely tuned to the seasonality of their environment. They exhibit both phenotypic plasticity and genetic adaptation to different growth environments (Sultan 1995). Phenotypic plasticity is the ability of a single genotype to produce more than one form of morphology, physiological state and/or behaviour in response to environmental conditions (West-Eberhard 1989), thereby allowing an individual to endure a broad array of environmental conditions (Eriksson *et al.* 1993), as would be experienced during a life cycle by long-lived perennial species such as trees.

There is a good correlation between January isotherms and the distribution of northern hemisphere tree species, with low winter temperatures being the limiting factor for frost-sensitive species (Saxe *et al.* 2001). However, with climate warming, these 'climate envelopes' of species are expected to shift towards the poles or higher altitudes as land

availability allows. There are therefore several options for tree populations in a warming climate: they can adjust to novel conditions at the individual level through phenotypic plasticity; they can adapt at the population level through natural selection; they can migrate to follow conditions to which they are already adapted, or they face extirpation (local extinction) (Davis *et al.* 2005; Aitken *et al.* 2008; Nicotra *et al.* 2010).

Both the degree and the rate of climate change are of concern for several reasons: the effects of climate change may be so great that species may not have adequate genetic variation to adapt (Hamrick 2004); the rate of change may be so rapid that trees may not adapt fast enough because of their long generation times (Davis and Shaw 2001; Jump *et al.* 2006); or trees may lack the ability to disperse quickly enough to match the rate of change (Clark 1998).

Phenotypic plasticity and genetic adaptation

Whether a species colonises a new area or stays within its traditional range, individuals and populations have to respond to environmental change. This can be through either phenotypic plasticity to buffer environmental change at the individual level or by changes in the genetic composition of populations as a result of selection pressure (genetic adaptation). Genetic diversity provides the raw material upon which natural selection works thereby allowing for adaptation to the novel environment, and thus has an important role in maintaining the resilience of forest ecosystems to threats associated with climate change.

Natural selection, genetic drift and mutations tend to increase genetic differentiation among populations, while phenotypic plasticity and gene flow tend to reduce it. However, plasticity is under some genetic control and can therefore be acted on by selection to fit the demands of different environments and thus contributes to genetic adaptation (Bradshaw 2006; Lindner *et al.* 2010).

The high levels of genetic diversity in tree species (Hamrick *et al.* 1992) increase the probability that a proportion of genotypes will survive in to the future by increasing the

adaptability of that population (Hamrick 2004). Genotypes with a high degree of phenotypic plasticity also have a greater probability of surviving temperature extremes if they can activate genes that produce phenotypes with better resistance to a particular stress (Saxe *et al.* 2001). Linking and expanding native woods using natural regeneration or planting with well adapted stock will increase gene flow and strengthen the capacity of tree populations to adapt. This can then allow the time for natural selection and evolutionary adaptation to take place.

There is ample evidence of phenotypic plasticity as a response mechanism to environmental change (Barnett and Farmer 1980 (*Prunus serotina*); Kramer 1995 (seven tree species); Grantani *et al.* 2003 (*Quercus ilex*) Rehfeldt *et al.* 1999 (*Pinus contorta*)). In a warming climate, budburst, which is largely governed by temperature, is expected to occur earlier in the season (e.g. Cannell and Smith 1983; Murray *et al.* 1989; Hänninen 1991). Kramer (1995) investigated the budburst and leaf fall of seven tree species in relation to climate warming by analysing data from clones transplanted over a large latitudinal range in Europe, and compared the results with those from other trees of the same species in the same parts of that range, which were assumed to have adapted to their local climates. The clone response to temperature was found to be of the same order of magnitude as that of the other trees, indicating that the former had considerable phenotypic plasticity.

It is often expected that evolutionary change occurs over long time scales and that the tolerance range of a species remains the same as it shifts its geographical range (Pearson and Dawson 2003). However, studies have shown that climate-induced range shifts can involve not only migration into newly-suitable areas, but also selection against phenotypes that are poor dispersers or are poorly adapted to local conditions (Davis and Shaw 2001). Rapid evolutionary change can and does occur as demonstrated by some plant species tolerance to heavy metals. For example, at a site adjacent to a copper refinery in Lancashire, UK, evolution of copper tolerance was found in populations of *Agrostis stolonifera* (creeping bent grass) exposed to copper in the soil after four years (Wu *et al.* 1975). Individual heterozygosity has been correlated with adaptation in beech (*Fagus sylvatica*) populations to heavily-polluted soils (Muller-Starck 1985), on which tolerant populations have higher levels of heterozygosity than sensitive ones. Thus, there is genetic

variability in tolerance to environmental stresses in *Fagus sylvatica* populations on which selection can act on rapidly.

Gene flow is extensive in forest trees (Savolainen *et al.* 2007). Forest tree populations may be locally adapted over large parts of their range, but at range edges local adaptation may break down. Current migration in trees is slow relative to the rate of climate change, and gene flow from the interior of a species' range may negate response to selection at the edges, even when sufficient genetic variation exists (Parmesan 2006). Even if populations have extensive genetic variation, limited dispersal and suitable habitat for establishment of new populations may hamper an evolutionary response to climate change.

Migration

Species distributions are thought to be in equilibrium with climate (Huntley *et al.* 1989; Sykes *et al.* 1996; Chuine and Beaubien 2001; Aitken *et al.* 2008) although some authors argue that some species are still restricted to refugia determined by the last glacial maximum when the ice was at its greatest extent (Bergeron *et al.* 2004; Svenning and Skov 2007a; 2007b). At the end of the last glacial period, mean global temperatures rose by about 5 °C (Schneider 1989). Fossil pollen records indicate that tree migration rates across Europe ranged from 200 - 250 m/yr (Huntley and Birks 1983). However, more recent cpDNA analysis of low-density refugial populations also show that refugia were more widespread and occurred at more northerly latitudes than previously thought (McLachlan and Clark 2004; McLachlan *et al.* 2005; Anderson *et al.* 2006; McLachlan *et al.* 2007), and therefore maximum migration rates were more likely to have been in the region of 100 m/yr (Aitken *et al.* 2008).

Seed dispersal in a forest situation is very limited (in the region of 10 - 20 m/yr) for most tree species and these rates are incompatible with migration rates at the end of the last glacial period (Clark 1998). Long-distance seed dispersal events (e.g. of acorns by mammals and birds) that increased the effective migration rate relative to average seed dispersal are also believed to have occurred (Le Corre *et al.* 1997; Clark *et al.* 2003; Petit *et al.* 2004) resulting in satellite populations that grew and merged with the expanding main population

(Clark *et al.* 1998), but these are thought to be rare and are hard to quantify. Moreover, range expansion through long-distance dispersal assumes that there is land available that is suitable for colonization, and also good seed viability (Higgins and Richardson 1999). When long-distance dispersal is included in tree migration models, migration rates were found to typically be in the region of 160 m/yr, and, for one species, maritime pine, (*Pinus pinaster*), a maximum of 750 m/yr (Higgins and Richardson 1999); such rates can account for the rapid expansion of species realised after the last glacial period.

Previous rates of migration are of considerable interest in order to quantify possible migration rates resulting from climate change in the 21^{st} century. For tree species, range changes occur though the slow process of population extinctions and colonisations (Walther *et al.* 2002). In a study of 14 combinations of global circulation models and species distribution models it was found that all models predicted that migration rates of 1000 m/yr or more would be necessary for species to track their climatic envelope under a doubling of atmospheric CO₂ concentrations (Malcolm et *al.* 2002). Model predictions for five tree species in the United States indicated a very low probability that migration will be greater than 10 - 20 km (an average of 100 - 200 m/yr) by the end of the 21^{st} century (Iverson *et al.* 2004).

A 3 °C increase in mean annual temperature corresponds to a shift in isotherms of approximately 300 - 400 km north in temperate zones, or 500 m in elevation (Davis 1989; Hughes 2000). The southern limits of boreal forests are predicted to move 500 -1000 km north within the 21st century. In temperate forests of central Europe, *Picea abies* is expected to decline and *Fagus sylvatica* to become less dominant, while other broadleaves such as *Fraxinus excelsior*, *Populus tremula*, *Alnus glutinosa*, *Acer spp.*, *Quercus spp.*, *Carpinus betula*, *Tilia spp.* and some conifers are projected to thrive (reviewed in Saxe *et al.* 2001).

Thus, although tree species have efficient dispersal mechanisms, have broad ecological tolerances and are often prolific seed producers, there is concern that many tree species may not be able to disperse quickly enough to track climate. Migration is likely to be slower than that realised after the last ice age due to disjunct populations and habitat

fragmentation. Even with long distance dispersal events taken into account, the migration rate of 1000 m/yr that may be required to track projected climate change is unlikely to be realised. Assisted migration of populations from more southerly latitudes that may be pre-adapted to the local climate may therefore be necessary (McLachlan *et al.* 2007; Atkins and Travis 2010; Seddon 2010; Grey *et al.* 2011).

Extinction

Some authors believe that climate change will be a significant driver of extinction (McCarty 2001; Root *et al.* 2003; Thomas *et al.* 2004). Some population extinctions have already occurred, more commonly at the southern limits of species ranges. Habitat loss and fragmentation will make it difficult for species to migrate to new climatically-suitable areas and so species ranges will contract. Also, the ability of species to persist in existing climate niches may be affected if they are outcompeted by new invasive species. Both processes will put some species at risk of extinction. A study modelling the likely extinction of all species globally under low, mid and high climate change scenarios, assuming maximal possible dispersal and no dispersal, indicated that 9 to 52 % of species studied were likely to go extinct, with 15 % (with dispersal) to 37 % (no dispersal) of species likely to go extinct under mid-range scenarios (Thomas *et al.* 2004).

Extinction of plant species are rarely described in the literature, suggesting that at the species level plants have been able to respond to climate change over the past glacial and interglacial periods since. *Picea critchfeldii* is one example of a tree species extinction; it is thought to have died out due to an inability to disperse and colonise newly available habitat at the end of the last ice age (Jackson and Weng 1999).

As much of the genetic variation within tree species is found within rather than among their populations, the extinction of a large proportion of a tree species' populations would result in relatively little overall loss of genetic diversity (Hamrick 2004). Also, because trees tend to be long lived, have high levels of genetic diversity and the potential for high rates of pollen flow, they should prove resistant to extinction during changing environmental conditions.

1.4 Adapting Forestry to Climate Change

To ensure healthy productive forests for the end of the 21st century 'business as usual' is no longer an option. The UK government commissioned the "Read Report", *Combating Climate Change* (Read *et al.* 2009), which presented the role that UK forests can play in climate change mitigation and adaptation. Its recommendations are followed through in the UK Forestry Standard (Forestry Commission 2011a).

1.4.1 Mitigation measures

Forests play an important role in the carbon cycle, accounting for almost three quarters of the annual exchange of carbon between the land and atmosphere. Land use change over the 20th century accounted for approximately a quarter of the increase in atmospheric CO₂ levels (Forestry Commission 2011b).

The IPCC AR4 (2007) defines climate change mitigation as 'a human intervention to reduce the sources or enhance the sinks of greenhouse gases' (IPCC 2007). For forestry, this means establishing new woodlands, managing them and existing woodlands and wood products sustainably to enhance their potential as sinks for greenhouse gases, and making more use of wood to replace fossil fuels and carbon intensive materials. For Europe, the AR4 suggests that the amount of carbon sequestered could be in the region of 1,980 Mt of CO₂ per year by 2030 through a combination of aforestation (660 Mt), reduced deforestation (95 Mt) and forest management (1225 Mt).

Mitigation measures include the following:

- 1. Carbon sequestration creation of new woodland and management of neglected woodland
- 2. Emissions reduction carbon storage through conservation and maintenance of soils
- 3. Substitution wood products used in place of concrete, especially in construction

 Bioenergy – replacement of fossil fuels with renewable energy (wind, solar, wave) including bioenergy through forestry. The UK target is for 30 % of renewable energy to come from bioenergy by 2020 (DECC 2009).

In 2009, annual UK greenhouse gas emissions were 566 Mt of CO₂ equivalents per year of which about 474 Mt were CO₂ (Forestry Commission 2012a). Established mixed oak-ash forest in southern England removes CO₂ from the atmosphere at around 15 t CO₂ ha/yr (Read *et al.* 2009). With planned woodland creation projected to be 8,000 ha/yr, woodland creation plays a minor role in meeting carbon reduction targets.

1.4.2 Adaptation measures

The IPCC AR4 defines adaptation as 'initiatives and measures to reduce the vulnerability of natural and human systems against actual or expected climate change effects'. When forest management strategies are incorporated into models of forest responses to climate change, results indicate that production losses may be at least partially lessened through planned adaptive management (Saxe *et al.* 2001). Creating well-structured and species-diverse forests should allow greater resilience to a changing climate than continuing to manage forests with a simpler structure. This could involve the introduction of novel species and provenances, and the use of silvicultural techniques such as continuous cover systems and assisted migration. Continuous cover forestry may promote structural and species diversity and evolutionary adaptation through the promotion of natural regeneration and in some circumstances can make forests more resilient to wind damage (Kerr 2008).

The Forestry Commission outline a number of adaptive measures to promote resilient woodlands (Ray *et al.* 2010). They suggest a mixed provenance approach to sourcing planting material. This should comprise material from 2° latitude south of planting site, and a small amount of material from as much as 5° south of planting site while also incorporating some local provenance material. Provenances selected should be adapted to a maritime climate, and species should be considered which may require less cold to harden. Changes in silvicultural systems could be employed such as shorter rotation times and increased thinning. Although species migrated during past climate change, it is
generally accepted that migration rates necessary to track climate envelopes are unlikely to be realised during the 21st century due to such rapid climate change (Malcolm *et al.* 2002; Iverson *et al.* 2004). As a consequence, building robustness into populations through wider provenance and species use seems the best alternative to enable species to adapt to a changing environment.

Adaptation benefits of forest expansion may also include the regulation of water flow thereby alleviating floods, reducing soil erosion and providing habitat linkages for biodiversity and genetic diversity through gene flow (Forestry Commission 2011a).

1.5 Thesis Outline – aims and objectives

The aim of this work is to investigate the implications of climate change for common ash, *Fraxinus excelsior* L. The null hypothesis put forward is that '*predicted climate change will have no adverse effect on the survival and growth of native populations of Fraxinus excelsior.*'

To investigate this, populations of *Fraxinus excelsior* are selected from locations that match future predicted climate for a site in southern England and mirror predictions for climate change over a range of latitudes. DNA fingerprinting, using microsatellite markers, is carried out to test for autochthonicity (Chapter 2). A common garden experiment is established to assess local and non-local populations in various climates (Chapter 3). Phenological (budburst) observations are undertaken in field and controlled conditions with the aim of predicting the effects of climate change on budburst (Chapter 4). Chilling requirements for germination of seed from the selected populations are investigated, with their implications for natural regeneration (Chapter 5). Finally, overall conclusions are discussed and recommendations are made for the future of *Fraxinus excelsior* in Britain as a productive timber species (Chapter 6).

The specific research questions asked are:

- 1. What does climate matching suggest would be suitable provenances for planting in future predicted climatic conditions at a range of UK locations?
- 2. Do reciprocal transplant experiments show that provenances are adapted to local climatic conditions?
- 3. Can observations of budburst under natural and controlled conditions suggest which provenances of *Fraxinus excelsior* will be suited to predicted future climatic conditions in the UK?
- 4. With milder winters projected, will seed of *Fraxinus excelsior* receive enough chilling for natural regeneration to occur across the UK?

Table 1.1 details field and lab work that was carried out within this study.

Table 1.1 Timeline of work throughout the study.

Task	When	Where
Site selection	Spring 2006	
Leaf collection	June 2006	8 UK and 6 French populations
DNA analysis	September 2006	University of Oxford
Seed collection	October - November 2006	6 UK sites, 4 French sites
Seed stratification (1)	December 2006	Forest Research, Alice Holt
Soil collection (1)	May 2007	3 UK and 2 French sites
Seedling production (1)	June 2007	Maelor Forest Nurseries
Soil analysis	June 2007	Bangor University
Phenology assessments under	October 2007 – May 2008	Forest Research, Alice Holt
controlled conditions		
Seed stratification (2)	August 2007	Forest Research, Alice Holt
Soil collection (2)	May 2008	3 UK and 2 French sites
Seedling production (2)	May 2008	Maelor Forest Nurseries
Germination testing (1)	February 2007 - August 2007	Forest Research, Alice Holt
Germination testing (2)	October 2007 - March 2008	Forest Research, Alice Holt
Trial establishment	November 2008 - February 2009	3 UK and 2 French sites
Phenology field assessments	Spring 2007 - 2010	2 UK sites
RTE assessments	Winter 2008 - 2011	3 UK sites, 2 French sites

Chapter 2 Selection of Sites and Populations

2.1 Introduction

Genetic variation within a species can be either neutral or adaptive (i.e. variation that is influenced by natural selection). Information on adaptive variation is required to decide on provenances that may be suitable for growing in the UK within predicted future climates. While neutral variation can be relatively easily assessed by genetic markers and give information on population differences, field trials are required to assess adaptive variation. Reciprocal transplant experiments (RTEs), where populations from various locations are planted at their own and every other location, can provide information on adaptive variation on adaptive variation.

The overall objective in this study, to investigate the adaptive potential of *Fraxinus excelsior*, is tested through an RTE. This chapter describes the underlying criteria for population and site selection, and DNA analysis of the populations to ensure autochthenicity.

2.2 Study Species – Fraxinus excelsior L.

Fraxinus excelsior is one of the most common and important hardwood species in Britain. It is the foundation of many valued ecosystems, an important element in the British landscape, and produces valuable timber on relatively short rotations (Kerr and Evans 1993). Several reviews of *Fraxinus excelsior* have been published which cover various aspects of its ecology (Wardle 1961; Gordon 1964), silviculture (Kerr 1995; Kerr and Cahalan 2004; Dobrowolska *et al.* 2011) and genetics and phylogeography (Pliûra and Heuertz 2003;

Heuertz *et al.* 2004a, 2004b; FRAXIGEN 2005). However, other characteristics are less well understood. There is little in the literature on leaf phenology of *Fraxinus excelsior*, and information on gender expression and seed biology is of variable quality and often conflicting. As a study species, *Fraxinus excelsior* is interesting in that its distribution is broad throughout Europe, and although quite demanding in terms of site conditions for producing high quality timber, it grows on many different site types.

2.2.1 Distribution

Fraxinus excelsior has a broad distribution throughout Europe and occurs in widely different climates of both maritime and continental character (Figure 2.1). It covers 2.6 % of the land surface and 2.7 % of the total volume of timber removed annually from European forests (Spiecker *et al.* 2009). The expansion of *Fraxinus excelsior* in Europe is a recent phenomenon which Margio *et al.* (2000) attribute to a decline in the rural human population. It occupies large areas, owing to its presence in both the early and the mature stages of woodland succession (Peterken 1993; Tapper 1996; Pliûra and Heuertz 2003).



Figure 2.1 Distribution of *Fraxinus excelsior* throughout Europe. Reproduced from Pliûra and Heuertz (2003).

Fraxinus excelsior reaches its most northern limits in Norway just south of the Arctic Circle at 63 °40' N (Wardle 1961), with the southern limit reaching 37 °N in Iran (Pliûra and Heuertz 2003). These northern and southern limits coincide with January isotherms of 0 °C and 5 °C respectively. However, at the eastern limit in central Russia it can withstand temperatures as low as -15 °C. It is native throughout the UK except in the extreme north-west of Scotland. Wardle (1961) reports that the whole of lowland Britain falls within the range of temperature and rainfall tolerances for *Fraxinus excelsior*, with a combination of exposure and unsuitable soil probably being responsible for its altitudinal limits.

Of the 43 species of *Fraxinus*, only three are native to Europe: common ash (*Fraxinus excelsior*); narrow leaved ash (*Fraxinus angustifolia* Vahl) and manna ash (*Fraxinus ornus* L.) of which only *Fraxinus excelsior* is native to the UK (Wallander 2008). Hybridisation may occur between *Fraxinus excelsior* and *Fraxinus angustifolia* where their ranges overlap (Heuertz *et al.* 2006; Fernandez-Manjarres *et al.* 2006). This is undesirable from a forestry point of view as the timber of *Fraxinus excelsior* is more valuable than that of *Fraxinus angustifolia*.

2.2.2 Site factors

Fraxinus excelsior is an ecologically flexible species, found naturally on many different site types. However, it is considered to be demanding in terms of nutrition, growing best on deep, fertile well-drained soils where it can reach a yield class of 10 to 12 (Evans 1984). Wardle (1961) reports *Fraxinus excelsior* growing in soils with a pH greater than 4.2, Evans (1984) quotes 5.5, and Savill (1991) reports *Fraxinus excelsior* growing best on calcareous loams with a pH of 7 - 8. There is little information in the literature regarding the specific nutrient requirements of *Fraxinus excelsior*, although all reviews note that to grow high quality *Fraxinus excelsior* for timber requires sites rich in nitrogen.

Fraxinus excelsior is shade tolerant when young but becomes a strong light demander at around seven to eight years of age (Savill 1991). It rarely forms stands of any great extent, occurring more commonly as a component of mixed broadleaved woodland (Joyce 1998). Under the National Vegetation Classification (NVC), *Fraxinus excelsior* is the dominant

species of woodland types W8 (*Fraxinus excelsior – Acer campestre – Mercurialis perennis*) and W9 (*Fraxinus excelsior – Sorbus aucuparia – Mercurialis perennis*), is locally common in W7 (*Alnus glutinosa – Fraxinus excelsior – Lysimachia nemorum*) and W12 (*Fagus sylvatica – Mercurialis perennis*) and forms a component of several other woodland communities (Rodwell 1991). Its drought tolerance and frost sensitivity make *Fraxinus excelsior* a species likely to be favoured in the short term by climate change (Scherrer *et al.* 2011).

2.2.3 <u>Reproductive biology</u>

Fraxinus excelsior is a wind-pollinated and wind-dispersed species. Trees are polygamous and show a continuum of gender from pure male to pure female with several stages of hermaphrodite in between. In young trees male flowers tend to be apparent before female flowers are evident which is a possible explanation for the observation in the literature that *Fraxinus excelsior* sometimes changes it sex, exhibiting maleness one year and femaleness the next (Bignelli and Power 1991). Hermaphrodite flowers are most common, but are usually functionally female or functionally male, with either abortive anthers or ovary depending on the degree of sex expression (Picard 1982; Morand-Prieur *et al.* 2003).

Good seed crops are produced from around age 25 - 30 (Savill 1991; Joyce 1998), although isolated trees can produce seed in small quantities from age ten. Masting occurs every 2 - 3 years. The seed is a samara which ripens on the tree in bunches referred to as keys. They are shed from October up to the following spring, but the embryos are immature, requiring warm temperatures to complete elongation. A period of cold is then required to break dormancy, and seedlings do not appear until the second spring after seed formation (Wardle 1961).

2.2.4 Forest cover in the UK

Forest cover in the UK is estimated at around 3.1 million hectares, constituting approximately 13 % of the total land surface area (Forestry Commission 2012b). In England, there are almost three times as many broadleaved trees as conifers, whereas in the UK as a whole conifers account for a little over half of all trees. *Fraxinus excelsior* is the third most

common broadleaved species in the UK after oak (*Quercus robur* and *Quercus petraea*) and birch (*Betula pendula* and *Betula pubescens*), and the second most widely planted, after oak. However, *Fraxinus excelsior* has seen a 64 % increase in planting from 1980 to 1998, from 72,503 ha to 119,232 ha – more than twice the percentage increase of any other broadleaved species (Forestry Commission 2003).

With climate change rising up the political agenda, increased tree planting is seen as highly desirable and this is reflected in the recent increase in forest cover (Table 2.1). Due to this increase, which is projected to continue, questions are being asked about the adaptability of our trees to projected climate change.

		England			UK		
	conifers	broadleaves	total	conifers	broadleaves	total	Forest cover (%)
2001	372	728	1100	1660	1130	2790	11.5
2007	367	757	1124	1640	1197	2837	12.0
2009	365	764	1129	1628	1213	2841	12.0
2010	364	766	1130	1625	1220	2845	12.0
2011	411	886	1297	1724	1355	3097	13.0
2012	334	961	1295	1610	1487	3097	13.0

Table 2.1 Area of woodland in England and the UK in thousands of hectares. Source: Forestry Commission (2012b).

2.3 Modelling and Site Selection

The Forestry Commission has developed an ecological site classification (ESC) system and associated decision-making tool to assist foresters in Britain in matching tree species and native woodland communities to site types. The classification uses four climatic variables (accumulated temperature, moisture deficit, windiness and continentality) and two edaphic factors (soil moisture regime and soil nutrient regime) to classify sites, and species suitability for a given site is then estimated (Pyatt *et al.* 2001). The underlying climatic data are based

on the 1961 - 1990 average climate at a spatial resolution of 10 km. By incorporating the UKCIP02 climate change scenarios (Hulme *et al.* 2002), predictions of the effects of climate change on tree species suitability for timber production have been modelled up to 2050 as climate change predictions beyond 2050 are less certain (Broadmeadow *et al.* 2005).



Figure 2.2 The effect of climate change on the potential suitability of *Fraxinus excelsior* as predicted by Ecological Site Classification for the UKCIPO2 2050s Low and High emission scenarios. Yield data in cubic metres per hectare per year. Reproduced from Broadmeadow *et al.* 2005.

The ESC based predictions indicate that there are likely to be significant changes in productivity and probably survival of hardwood timber species, making much of southern England unsuitable for commercial timber production, although genetic variability in native populations may enable a degree of adaptation. By 2050, under low or high emission scenarios, *Fraxinus excelsior* is expected to decrease in productivity in the west of England but increase throughout south-east and north-east England and much of Scotland. In

Wales, productivity is expected to be largely unaffected. In east England, productivity is predicted to be reduced due to increased drought (Figure 2.2; Broadmeadow *et al.* 2005).

2.3.1 Climate matching

It is widely suggested that native populations may not be able to adapt to a changing climate, particularly when the predicted fast rate of change is considered (Menzel and Fabian 1999; Saxe *et al.* 2001; Broadmeadow *et al.* 2005). Sourcing planting material from regions with a current climate similar to that of predicted future climates could help populations adapt. However, in Britain it will be important to match suitable provenances carefully, to protect against late spring frosts, the frequency of which is not expected to change (Hulme *et al.* 2002). Existing provenance trials of *Fraxinus excelsior* in Britain indicate that the best performing provenances are those from regions with a climate similar to that of the trial site (Cundall *et al.* 2003). This suggests that a provenance for climate change adaptation should be selected from a region with a current climate well matched to the planting site's future predicted climate.

Climate matching analysis takes the predicted future climate at a given location and matches it to the current climate at another location, so that material from forest stands at the latter can be used as a source of planting material presumably adapted to future climate at the former (EUFORGEN 2013). Climate matching analysis for Little Wittenham, Oxfordshire, UK, was carried out (Broadmeadow 2004) using a global gridded dataset at a resolution of 10 minutes developed by the Climatic Research Unit (CRU) of the University of East Anglia (New *et al.* 2002) and UK Climate Impacts Programme 2002 (UKCIP02; Hulme *et al.* 2002) with the low and high emissions scenarios for 2050 and 2080 (Figure 2.3). For full details of the methodology, see Broadmeadow *et al.* (2005).



Figure 2.3 Climate matching analysis for Little Wittenham, Oxfordshire, UK. The red and yellow areas represent respectively the 0.2 % and 1 % of grid-squares in Europe best matched to each scenario. Climate matching was performed on the basis of monthly mean temperature, diurnal temperature range and precipitation, weighted according to the annual range for each variable. Baseline data are provided by the Climate Research Unit, University of East Anglia, and the future scenarios are based on the UKCIP02 50 km gridded dataset. Reproduced from Broadmeadow (2004).

Under a low emissions scenario, the climate at Little Wittenham in 2050 would be like that of the Loire Valley in France today; under a high emissions scenario this would include regions of the Mediterranean at high elevations. By 2080 the climate of Little Wittenham is matched to the Bordeaux area under the low emissions scenario (not shown) and to regions of the Adriatic coast at high elevations under the high emissions scenario (see Figure 2.3).

2.3.2 Selection of study sites

On the basis of the climate matching data (Figure 2.3), sites were selected in France and the UK from which to sample populations of *Fraxinus excelsior* to test for adaptability to projected future climates for the UK. Populations of *Fraxinus excelsior* were sought from the Loire Valley and the Bordeaux region of France. These are approximately 3° and 6° latitude south of Little Wittenham and correspond to the 2050 and 2080 climate matching analyses of Broadmeadow (2004). As the aim was to investigate adaptation of *Fraxinus excelsior* to climate change in Britain, population were also sought from 3° and 6° latitude north of Little Wittenham and this roughly corresponds to north Yorkshire and Invernessshire. The pattern of temperature and rainfall at Little Wittenham was matched to proposed sites using data provided by the CRU of the University of East Anglia (Figure 2.4).

At each location, two populations were selected (Table 2.2). The aim was to try to separate the effect of climatic from edaphic factors on tree growth, although finding two contrasting sites with sufficient pure *Fraxinus excelsior* that fitted the climate matching analysis and for which weather data were available ultimately proved extremely difficult. Therefore two sites were selected that had a) similar rainfall and precipitation patterns (Figure 2.4), b) ease of access and c) were thought to be pure *Fraxinus excelsior*. Where possible, these were from within a forest stand. The location of these sites is given in Figure 2.5.

Little Wittenham is a semi-ancient (400 years old) semi-natural woodland where *Fraxinus excelsior* forms a major component of the canopy along with *Acer pseudoplatanus*. The other site selected from this latitudinal level is Ranmore Common in Surrey, a native woodland, again largely of *Fraxinus excelsior* and *Acer pseudoplatanus*. In north Yorkshire, *Fraxinus excelsior* grows well due to favourable soils and underlying geology. The two sites selected here were Settrington and Parlington and on both estates the woodlands are managed for production forestry. Three degrees further north is Inverness-shire where *Fraxinus excelsior* only exists as a widely scattered marginal species. Trees selected were from roadside verges and hedgerows across Ballindalloch and Cawdor estates.



b)



Figure 2.4 Mean monthly a) temperature (°C) and b) precipitation (mm) at site of populations selected for inclusion in trials to test the adaptability of *Fraxinus excelsior* to climate change. Baseline data (1961-1990) are from a global dataset of 10 minute resolution provided by the Climatic Research Unit of the University of East Anglia.



Figure 2.5 Geographic distribution of populations selected for inclusion in the reciprocal transplant experiment. Outline map from <u>http://maps.hist-geo.com/</u>

Table 2.2 Details of populations used in the reciprocal transplant experiments (chapter 3) and seed germination study (chapter 5). Populations in parentheses are those where leaf samples were collected for DNA analysis, but were not used in these trials.

	Latituda	Longitudo	A N 4 T		Elevation	Stand Tuna	Soil Turpo	
	Latitude	Longitude	AIVH	AIVIP	(m.a.s.l.)	Stand Type	5011 iype	
Scotland								
Cawdor	57.52 °N	3.61 °W	6.6	884	150	scattered roadside trees	sandy loam	
Ballindalloch	57.40 °N	3.36 °W	6.5	902	220	scattered roadside trees	podzol	
(Altyre)	57.56 °N	3.63 °W	-	-	50	scattered single trees	-	
Yorkshire								
Settrington	54.13 °N	0.70 °W	8.4	737	110	ash sycamore woodland	brown earth	
Parlington	53.81 °N	1.36 °W	9.1	666	65	ash sycamore woodland	brown earth	
Oxfordshire								
L. Wittenham	51.63 °N	1.17 °W	9.5	669	50	ash sycamore woodland	clay loam	
R. Common	51.14 °N	0.38 °W	9.8	687	190	mixed woodland	brown earth	
(Alice Holt)	51.15 °N	0.98 °W	-	-	100	mixed woodland	-	
lle de France								
Marly	48.89 °N	2.05 °E	10.3	644	115	registered seed stand	brown earth	
Dourdan	48.51 °N	1.96 °E	10.5	628	125	mixed woodland	heavy clay loam	
Pyrenees								
Monein	43.33 °N	0.54 °W	13.2	991	138	registered seed stand	silty clay loam	
Le Tech	42.33 °N	2.66 °E	12.2	919	800	scattered roadside trees	silty clay	
(Saint Paul)	45.13 °N	2.52 °E	-	-	1000	scattered riparian copse	-	
(Lestiac)	44.70 °N	0.39 °W	-	-	10	scattered riparian copse	-	

AMT = annual mean temperature (°C). AMP = annual mean precipitation (mm) from baseline data of 1961-1990, CRU University of East Anglia.

Sites in France (Table 2.2) were selected with assistance from the Institut National de la Recherche Agronomique (INRA) and the Office National des Forêts (ONF). In the Ile de France, (matched to the climate of Little Wittenham in 2050, Broadmeadow *et al.* 2005) *Fraxinus excelsior* is a major component of many of the forests, and the Forêts du Marly and Dourdan were selected as sites well known to the ONF and with easy access. Lestiac and Saint Paul, near Bordeaux, were originally selected to match the predicted climate of Little Wittenham by 2080, but germination of seed from these stands showed that they were not pure *Fraxinus excelsior* (suspected hybridisation with *Fraxinus angustifolia*; Dufour, pers comms) and therefore sites further south were selected. Monein, at the foothills of the

Pyrenees, is another forest managed by the ONF and was selected to match the climate of Little Wittenham by 2080. However, it was necessary to go further east, and to a higher elevation than ideal to find a second population (Le Tech). This population is from 800 m altitude, and is the only one considered to be from high elevation (over 300 m). The rainfall pattern for this site, and also to a lesser extent for Monein, is different from the rainfall pattern at the other sites, with more rain falling in late spring, and less in midsummer (Figure 2.4b).

The populations selected are putatively autochthonous, with the exception of the population at Settrington. Here, estate records indicated that some planting of non-local material, perhaps of French origin, had occurred in some stands managed for timber production (Taylor, pers comms). Efforts were made to collect seed, soil and leaf samples from the indigenous parts of this woodland, rather than the managed timber production stands.

2.4 Phylogeography of *Fraxinus excelsior* and Microsatellite Analysis

Phylogenetic analysis is concerned with the geographic distribution of genealogical lineages (Avise *et al.* 1987) and can be used to map postglacial recolonization routes. Several studies have been carried out using DNA profiling techniques to assess neutral genetic variation within and among *Fraxinus excelsior* populations in Europe. In a nuclear microsatellite study, Heuertz *et al.* (2004a) found that western and central Europe were recolonised by *Fraxinus excelsior* after the last glaciation from several refugia located in the eastern Balkan Peninsula and north east Europe. They also reported that *Fraxinus excelsior* populations showed almost no differentiation from the British Isles to Lithuania, and attributed this to strong gene flow, as did Morand *et al.* (2002), looking at *Fraxinus excelsior* populations in France, Hebel *et al.* (2006) in Germany and Ferrazini *et al.* (2007) in Italy.

Chloroplast DNA differs from nuclear DNA in that it is generally inherited maternally (Reboud and Zeyl 1994) in angiosperms and is slow to evolve, and so haplotypes remain essentially the same from generation to generation. This makes chloroplast DNA analysis a useful method of interpreting recolonization routes. Heuertz *et al.* (2004b) showed relatively high genetic differentiation among populations of *Fraxinus excelsior* using chloroplast DNA analysis, suggesting a separate recolonization history from different refugia of the Iberian Peninsula, the Apennines, the Eastern Alps and the Balkans (Figure 2.5). They reported that *Fraxinus excelsior* in Britain comprised a single haplotype (H4) originating from the Iberian Peninsula and not found in France. *Fraxinus excelsior* in western France comprises the H3 haplotype from a probable refugium in the Apennines, while the H2 haplotype is found throughout Germany and eastern France. It is thought that the H2 haplotype originated from the eastern Alps.

In Britain, FRAXIGEN (2005) and Sutherland *et al.* (2010) also used chloroplastic microsatellites to investigate genetic diversity in *Fraxinus excelsior*. FRAXIGEN (2005) reported the H2 haplotype in a population from north-east England (Settrington). Sutherland *et al.* (2010) also reported the H2 haplotype in north-east England, but also the H9 haplotype from Slovakia reported by Heuertz *et al.* (2004b), in five locations in Scotland. They also found three new haplotypes in: southern Scotland and northern England (H13), western Scotland (H14) and eastern Scotland (H15).

Chloroplast DNA microsatellite analysis was carried out on leaf samples from the populations selected for this study (Table 2.2), to determine if populations are autochthonous.



Figure 2.6 Geographical distribution and frequency of chloroplast microsatellite haplotypes in *Fraxinus excelsior*. Reproduced from Heuertz *et al.* 2004b.

2.4.1 Plant material

Leaf samples were collected for DNA analysis during the early summer of 2006. Five trees from 14 populations were selected at random (Table 2.2), except at Settrington, where samples from seven trees were collected. Estate records from Settrington indicated that some stands were planted, and previous DNA analysis from this site had identified a different haplotype from the H4 haplotype common to the British Isles (FRAXIGEN 2005; Sutherland *et al.* 2010). In total, 72 trees were sampled. Trees selected were widely spaced (a minimum of 100 m apart) to avoid sampling close relatives. Leaflets of one to two

compound leaves were removed from the petiole and placed in a sealed plastic bag with silica gel. The dried leaf samples were kept at room temperature until DNA extraction.

2.4.2 DNA extraction and microsatellite analysis

Total DNA was extracted from 60 - 90 mg of dried leaves using a DNeasy 96 Plant Kit (Qiagen) at the Department of Plant Sciences, University of Oxford. Chloroplast fingerprinting of individuals was performed with six of the ten universal dicotyledonous angiosperm primers developed by Weising and Gardener (1999) that were known from previous studies to be polymorphic in *Fraxinus excelsior*: ccmp2, ccmp3, ccmp4, ccmp6, ccmp7 and ccmp10.

DNA was diluted 1:10 with sterile water prior to amplification by polymerase chain reaction (PCR), performed on a Geneamp PCR system 2700 (Applied Biosystems) thermocycler using fluorescent labelled primers. The PCR reaction mix (20 μ l) contained: 1.0 μ l DNA, 15.1 μ l distilled water, 2.0 μ l of PCR buffer (15 mM MgCl₂), 1.0 μ l of dNTPs (25mM), 0.8 μ l primer mix (5 μ M, Qaigen) and 0.1 μ l of Hotstart *Taq* polymerase (5 U/ μ l, Qiagen). The PCR reaction proceeded as follows: 15 minutes denaturing at 95 °C, followed by 25 cycles of one minute at 94 °C, one minute at 50 °C, one minute at 72 °C.

The PCR products were multiplexed by size into groups A (ccmp2, ccmp3, and ccmp10) and B (ccmp4, ccmp6, and ccmp7) (Table 2.3) and loaded on to polyacrylamide gels with a GS-LIZ 500 size ladder (Applied Biosystems). Electrophoresis and detection of PCR products were carried out using a 3730 DNA analyzer (Applied Biosystems) and the resulting electrophoregrams analysed with Genemapper 4.0 software (Applied Biosystems) (Table 2.4). Allele binning (allele specification) was based on graphics with consecutive fragment lengths per locus and checked by an independent researcher.

Table 2.3Primer sequence and PCR details for six chloroplast microsatellite loci in *Fraxinus*
excelsior after Weising and Gardener (1999).

Primer	Sequence	Colour	Multiplex
ccmp2-for	GATCCCGGACGTAATCCTG	Red	В
ccmp2-rev	ATCGTACCGAGGGTTCGAAT		В
ccmp3-for	CAGACCAAAAGCTGACATAG	Black	В
ccmp3-rev	GTTTCATTCGGCTCCTTTAT		В
ccmp4-for	AATGCTGAATCGAYGACCTA	Black	А
ccmp4-rev	CCAAAATATTBGGAGGACTCT		А
ccmp6-for	CGATGCATATGTAGAAAGCC	Red	А
ccmp6-rev	CATTACGTGCGACTATCTCC		А
ccmp7-for	CAACATATACCACTGTCAAG	Blue	А
ccmp7-rev	ACATCATTATTGTATACTCTTTC		А
ccmp10-for	TTTTTTTTAGTGAACGTGTCA	Blue	В
ccmp10-rev	TTCGTCGDCGTAGTAAATAG		В

2.4.3 <u>Results and discussion</u>

The characteristics of the haplotypes found in the fourteen populations are summarised in Table 2.4. Full details of all allele sizes of amplification products and corresponding haplotypes are given in Appendix 1. Previous studies of British populations of *Fraxinus excelsior* found ccmp2 and ccmp4 to be monomorphic, but in this study, two alleles were found at both these loci, although ccmp3 was monomorphic, as found by Hebel *et al.* (2006). Populations from Altyre, Cawdor, Parlington, Little Wittenham, Alice Holt and Ranmore Common all comprised a single haplotype, H4, consistent with the findings of Heuertz *et al.* (2004b) and FRAXIGEN (2005). However, trees sampled from Ballindalloch (north-east Scotland) were mixed, with a new haplotype with allele 190 at the ccmp2 locus: this allele has not been reported in British populations before. Of the seven trees sampled at Settrington, six had the H4 haplotype and the seventh had allele 98 at ccmp6. This

corresponds to haplotype H14 reported by Sutherland *et al.* (2010) from western Scotland and contrasts to the previous studies which found haplotype H2 at Settrington.

Table 2.4 Characteristics of the haplotypes detected with six universal chloroplast microsatellite primers in *Fraxinus excelsior*. Haplotypes in the final column are as reported by Heuertz *et al.* (2004b: H2, H3, H4, H10) and Sutherland *et al.* (2010: H14). Alleles in bold are new.

Haplotype	n	ccmp2	ccmp3	ccmp4	ccmp6	ccmp7	ccmp10	Haplotype
А	13	191	93	136	98	113	101	H2
В	10	191	93	136	98	113	100	H3
С	44	191	93	136	97	114	101	H4
D	1	191	93	136	97	113	101	H10
E	1	191	93	136	98	114	101	H14
F	1	190	93	136	97	114	101	New
G	1	191	93	135	98	113	101	New
Total		(2)	(1)	(2)	(2)	(2)	(2)	

Both the H2 and H3 haplotypes were found in populations from France, as reported by others (Heuertz *et al.* 2004b, FRAXIGEN 2005). As expected, populations from the IIe de France (Dourdan and Marly) comprised the H2 haplotype and the populations from Lestiac and Le Tech comprised the H3 haplotype. The population from Saint Paul mostly comprised the H2 haplotype, but a new allele (135) was found at the ccmp4 gene giving a previously unreported haplotype. It is possible that the new allele found in the Saint Paul population is due to hybridisation with *Fraxinus angustifolia*, as the species is known to occur in this region and to hybridise with *Fraxinus excelsior*.

Monein is at the foothills of the Pyrenees. Contrary to the findings of other authors, the H4 haplotype was most common here. Heuertz *et al.* (2004b) suggest that in post-glacial

recolonization, H4 haplotype populations of *Fraxinus excelsior* skirted the Pyrenees, making their way north up the Atlantic coast. It would appear from the study reported here that the H4 haplotype did in fact make its way over the Pyrenees. One individual from this population also clearly had a different allele from the others at the ccmp7 locus, making its haplotype H10. This was unexpected as H10 has previously only been found in the Czech Republic (Heuertz *et al.* 2004b).

Based on these findings, populations from Saint Paul and Lestiac were excluded from reciprocal transplant experiments looking at adaptive variation (Chapter 3) due to possible hybridisation with Fraxinus angustifolia. Due to the isolation of Ballindalloch and that ash is a minor species this far north, nor a species used for forestry purposes, it is highly unlikely that this haplotype is not of native origin. Similarly, the additional haplotype found in one sample from Settrington (with all samples being taken from non-productive forestry stands) was also found by Sutherland *et al* (2010) from western Scotland and is thus likely to be of native origin. It was therefore judged that the populations tested, except Lestiac and Saint Paul, were pure *Fraxinus excelsior*, and fulfilled the criteria of autochthonicity and hence were suitable to be used in this study.

Chapter 3 Investigation of the Adaptive Potential of Ash (*Fraxinus excelsior* L.) through a Reciprocal Transplant Experiment

3.1 Introduction

Species are composed of populations that are genetically adapted to different environments. This was demonstrated in early trials by Turesson (1922) and Clausen *et al.* (1948) who showed that plant populations will exhibit adaptations to the environments from which they originated, even when cultivated in common garden trials. Provenance trials have historically been used to investigate transfer responses of different populations to find the most productive (best growing) seed sources of a species for particular areas. For example, for *Pseudotsuga menziesii* (Douglas fir) growing in the UK, provenances from coastal Washington are best suited to western parts of the UK, while provenances from southern Washington are better suited to the drier, eastern parts of the UK (Forestry Commission 2013). Foresters can also use provenance trials to look at levels of adaptation in trees, as each trial provides information on quantitative genetic variation in certain traits and the extent of genotype x environment interaction for those traits (Morgenstern 1996).

In most trees, most genetic variation occurs within populations rather than between populations (Hamrick 2004). Studies in the US on conifers have shown that 25 - 45 % of the genetic variation within populations is accounted for by climatic or geographic variables that reflect a range of environmental factors specific to each location (Rehfeldt *et al.* 1999). In the UK, this information is mainly lacking, although Worrell (1992) showed that British material of some native British species (*Pinus sylvestris* (Scots pine), *Betula pendula* (silver

birch), *Quercus petraea* (sessile oak) *and Alnus glutinosa* (common alder)) is adapted to British conditions whereas material from continental Europe can suffer from frost damage.

Local adaptation occurs when natural selection acts on the resident population which then shows, on average, a higher fitness than genotypes originating from other habitats (Williams 1966). The extent of local adaptation is governed by the amount of genetic variation within populations, as well as gene flow between populations. Kawecki and Ebert (2004) define local adaptation as 'patterns and processes observed across local populations of the same species connected, at least potentially, by dispersal and gene flow', thereby acknowledging the differentiating effects of natural selection and the homogenising effect of gene flow. However, low genetic variation resulting from genetic drift can limit adaptation (Travisano *et al.* 1995). This is particularly important in small populations which may not be well adapted to their environment because drift can make it difficult for advantageous alleles to reach high frequency (Whitlock 2003) or because the chance fixation of deleterious alleles can lead to lower fitness (Whitlock *et al.* 2000). Gene flow can also hinder local adaptation by homogenizing allele frequencies and limiting the response to selection within an environment (Garcia-Ramos and Kirkpatrick 1997; Hendry and Taylor 2004).

The fitness that present day populations may express in future climates in the absence of evolutionary change can be inferred through reciprocal transplant experiments (RTEs) that include environments similar to those predicted under climate change scenarios. RTEs test the performance (fitness) of individuals in both their native habitat and simultaneously in a foreign habitat. Local adaptation is thought to have occurred if native individuals show greater fitness than foreign transplants in a given environment (Lowry 2012; Figure 3.1). However, there are trade-offs involved, as local adaptation to one environment comes at a cost of lower fitness in foreign habitats (Hereford 2009; Lowry 2012). Thus, new climates may reduce an individual's growth as well as its fecundity (fitness). The reduction in fitness and other variables can be large e.g. 30 % reduction in seed production in some herbaceous species (Etterson 2004a) and 20 % reduction in biomass in *Pinus contorta* (lodgepole pine) in Canadian populations (Rehfeldt *et al.* 1999).



Figure 3.1 Hypothetical patterns of genotype x environment interaction for fitness. Circles: the average of populations originating from habitat 1; diamonds: the average of populations originating from habitat 2. In a) each population has highest absolute and relative fitness at its home site showing local adaptation. In b) both populations have highest absolute fitness at habitat 1, but each has highest relative fitness at its local site, again showing local adaptation. Adapted from Kawecki and Ebert (2004).

RTEs commonly show that herbaceous plants species comprise a series of populations, each of which is relatively well adapted to its local environment (Lacey 1988; Nagy and Rice 1997; Montalvo and Ellstrand 2000; Etterson 2004a, 2004b; Waser and Price 1985). In the model plant *Arabidopsis thaliana* (mouse-ear cress), RTEs carried out over five years at the full extent of the species natural range (Sweden to southern Italy), showed that the fitness of the local population was significantly higher than that of the non-local population: 3.1 - 22.2 times higher at the southern site, and 1.7 - 3.6 times higher at the northern site (Ågren and Schemske 2012).

Leimu and Fischer (2008) carried out a meta-analysis of local adaptation in plants (but no tree species) using RTEs and reported local adaptation in 71 % of populations studied. However, local plants performed better than foreign plants at *both* sites of a pair-wise comparison (the strict definition of local adaptation sensu Kawecki and Ebert (2004)) in only 45.3 % of 1032 compared population pairs, suggesting that local adaptation is less common in plants than often presumed. They also found local adaptation to be more common in large populations with local adaption being rare in populations with fewer than 1000 flowering individuals, indicating a clear influence of population size on the likelihood that

adaptation will occur. However, RTEs have also shown lower fitness in the local population compared with foreign transplants (Rice and Mack 1991; Galloway and Fenster 2000; Hereford and Winn 2008), and also that many populations are not optimally adapted to their local environment (Gould 1998; Rehfeldt *et al.* 2001; Hansen *et al.* 2006). Hereford (2009) carried out a quantitative survey of local adaptation and fitness trade-offs and found that, while not ubiquitous, local adaptation was common (found in 71 % of plants and animals studied) and that on average, the local population had 45 % greater fitness than foreign populations. However, of the 74 experiments examined, 21 % showed maladaption to the local climate, suggesting that foreign population advantage is not rare.

There are several key differences between relatively short lived herbaceous plant species and perennial tree species, which have long life cycles, wide distributions and extensive gene flow, which might lead one to expect more extensive scales and patterns of adaptation than in herbaceous plants. There is evidence of adaptive variation occurring over short distances in some tree species; Rehfeldt (1983) demonstrated intraspecific adaptation across a 600 m altitudinal gradient in *Pinus contorta* that paralleled the frost free period in northern Idaho. When transplanting acorns of *Quercus rubra* (red oak) within a 4 ha plot, Sork *et al.* (1993) found that seedlings showed least damage from insect herbivory when planted at the site of the mother plant, indicating the occurrence of local adaptation. This was unexpected in an outcrossing tree on the small geographic scale studied.

Carter (1996) examined data from provenance trials of ten north temperate tree species in the USA, and found that growth tended to be highest at test sites with temperatures similar to the source location with growth tending to be less and mortality higher at both warmer and colder locations. Moving provenances of *Fraxinus americana* (white ash) to both warmer and colder sites from the seed source site resulted in reduced mean height growth (Carter 1996). In European provenance trials of *Fraxinus excelsior*, Kleinschmit *et al.* (1996) reported significant differences among provenances for form and vigour and strong provenance x site interactions. Cundall *et al.* (2003) also reported significant provenance x site interactions in UK provenance trials of *Fraxinus excelsior*, although this only accounted for 6.5 % of the total variation, compared to 60 % of total variation accounted for by site and 24 % by provenance. The FRAXIGEN project (Boshier and Stewart 2005) used RTEs to

estimate local adaptation in populations of *Fraxinus excelsior* from England, varying in distances from seven to 283 km apart. They reported no evidence of local adaptation in three year old plants, with the best provenance growing well on most sites.

As forest managers seek to minimize the costs of establishing new forests, compromises are made in matching planting material with ecological site conditions, and in ensuring high genetic and physiological quality of the material used. Climate change will alter the environmental conditions to which European forests are adapted and may even create novel climatic conditions (EUFORGEN 2013). The question then arises as to how native UK populations of *Fraxinus excelsior* will cope with this predicted rise in temperature. Are UK populations locally adapted and are there possibilities for phenotypic plasticity that will enable them to survive and grow in a novel climate?

In this study an RTE is used to investigate the adaptive potential of *Fraxinus excelsior* in the face of a warming climate. Ten populations are tested over five sites (two populations selected from each site location and thus the RTE is not fully reciprocal) to examine home site advantage, and whether populations are adapted to local conditions, and how they may perform in other environments.

3.2 Methods

3.2.1 Seed collection and treatment

Approximately 200 g of fresh seed was collected from each of 20 trees from ten populations of *Fraxinus excelsior* selected across a 15° latitude range (Table 2.2) in autumn 2006 using pole pruners and a shotgun and the seed was bulked at source. Seed were collected from trees at least 100 m apart to minimise the chances of sampling close relatives. Seed were dried to 10.5 % (\pm 0.4 %) moisture content and held in a cold store at 4 °C until pretreatment.

Approximately half the collected seed entered warm pretreatment at 15 °C on 7th December 2006 using standard methods (Suszka *et al.* 1996). Twenty five seed from each seedlot were assessed every two weeks to determine embryo occupancy. After ten weeks, average embryo occupancy was 72.3 % (range 68 – 80 %) across all seedlots. Seed entered chilling pretreatment at 4 °C on 15th February 2007 for 16 weeks until 7th June 2007, when they were delivered to Maelor Forest Nurseries¹, Shropshire. Moisture content was determined after pretreatment and averaged 45.1 % across all seedlots (range 37 - 58 %).

3.2.2 Soil analysis

Approximately 500 kg of soil were collected from each of the five trial sites (Cawdor, Parlington, Little Wittenham, Dourdan and Monein) in May 2007 for raising seedlings. Soil analyses were carried out on soil samples from all ten seed collection sites, for comparison purposes. Nine soil samples were collected randomly from across each trial site and five samples from each non trial site (Ballindalloch, Settrington, Ranmore Common, Marly and Le Tech) for mechanical and chemical analysis. Samples were kept in a fridge until analysis, which was undertaken for: soil moisture content, organic matter content, pH, nitrogen content (%), sodium, potassium, calcium, phosphorus, nitrate and ammonia concentration (mg/l) using standard laboratory techniques (ISO 11464 2006).

3.2.3 <u>Seedling production</u>

Seedlings were raised in 175 cm³ Sherwood Root Trainers in soil from the site that they would be planted into (e.g. for those seedling to be planted at the Cawdor trial site, all seedlings were raised in soil from Cawdor). This aimed to reduce the extent and length of nursery effects, so that adaptive differences would be visible at a much earlier age (Cundall *et al.* 2003, Boshier and Stewart 2005). Two stratified seed were sown per cell for all seedlots in mid June 2007, except for Le Tech, where four seed per cell were sown due to seed degradation. Overhead watering and shade were provided. Germination was recorded at regular intervals throughout the growing season. However, germination was

¹ Maelor Forest Nurseries, Fields Farm, Bronington, Whitchurch, SY13 3HZ

highly variable between provenances and soil types, and insufficient seedlings were produced for trial establishment over the winter of 2007 - 2008.

Seedling production was repeated in 2008, with seed entering pretreatment much earlier in the year than in 2007. All seedlots received 12 weeks warm pretreatment at 15 °C commencing 6th August 2007 and were washed after six weeks to aid removal of germination inhibitors. Chilling pretreatment commenced 29th October 2007 for 27 - 28 weeks. Moisture content was checked regularly throughout the pretreatment period and adjusted to keep seedlots as close as possible to 45 % moisture content. Seedlings were again produced at Maelor Forest Nurseries in root trainers, with seed being sown between 6th and 15th May 2008. Soils from Dourdan and Little Wittenham had previously been noted as particularly heavy clays, unsuitable for seedling production, and so were mixed with one part inert grit to two parts soil to aid water penetration. Numbers of seed sown per cell varied based on germination percentages from the 2007 sowing: two seed per cell were sown for Ranmore Common, Dourdan, Marly and Monein, three per cell for Little Wittenham, Settrington, Parlington and Ballindalloch, four per cell for Cawdor and five per cell for Le Tech. Overhead shade and watering were again supplied. Figure 3.2 shows seedling production at Maelor Forest Nurseries. Different germination rates of different provenances are evident with some trays having almost no seedlings present.



Figure 3.2 Seedling production at Maelor Forest Nursery, Shropshire. Different coloured labels represent soil from each trial site. Each tray contains a single provenance.

3.2.4 Trial design

The reciprocal transplant experiment comprised five trials, established at sites approximately 3° latitude apart, from Inverness in Scotland to the foothills of the Pyrenees, spanning a north - south distance of approximately 2000 km. Each trial comprised a complete randomised block design of the ten provenances collected as seed (Table 2.2) and aimed to also include two commercial bare root provenances as nursery standards. These were planned to be a Dutch provenance of selected material, and a Romanian provenance, as this material was widely planted in Britain during the 1980s and 1990s, but has since been observed to be unsuitable for the British climate due to its early flushing and subsequent frost damage (Cundall *et al.* 2003). It was impossible to source either, and a German provenance, Hügelland, was substituted for Dutch material, supplied by Nicholson

Nurseries², Oxfordshire, as 1-year-old undercut 40 - 60 cm bare root trees. The Romanian provenance was substituted by left over seedlings and the trees not assessed.

Trial sites were approximately 0.25 ha, and experiments comprised three blocks of 12 provenances, planted as 6 x 6 plots at 1 x 1 m spacing. Each trial was surrounded by a double guard row, planted with the same material as the adjacent plot. The exception was at Monein where due to space constraints, the two nursery standards were omitted, plots were of 25 trees (rather than 36), and the trial was surrounded by a single guard row (Appendix 2). In total, 5,934 test trees excluding guards were planted (12 provenances x 3 blocks x 36 trees x 4 sites, and 10 provenances x 3 blocks x 25 trees x 1 site).

3.2.5 <u>Trial establishment and assessments</u>

Trials were planted between November 2008 and February 2009 at the five sites (Table 3.1). As multiple seed germinated in each cell of some provenances, seedlings were carefully teased apart to minimise root disturbance. Seedlings were selected from within seed trays at random, except where the seedling selected was too poor to be expected to survive (either too small, lacking a terminal bud, or with some other deformity).

Each trial was protected by a fence, appropriate to site conditions. UK fences were deer proof, the fence at Dourdan was boar proof, and the fence at Monein was the least sturdy due to a lack of large herbivores in this region. A Tinytag data logger (Explorer Version 4.6, Gemini Data Loggers Ltd) was placed in a white box in the north corner of each trial to record hourly air temperature. During the first season there was heavy weed growth at all sites except Cawdor which had received a complete herbicide application prior to planting. Seedlings were swamped by weeds, so 0.5 m² black mypex mulch mats were placed around each seedling at all sites except Cawdor during the spring of 2009. Due to proximity to water courses at some sites, it was not possible to use chemical weed control.

² Nicholsons Nursery. The Park, North Aston, Bicester, Oxon OX25 6HL

	Table 3.1	Details	of the	five	trial	sites.
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Trial Site	Elevation m.a.s.l	Latitude ° N	Planted	Previous land use	Soil type	Protection	Vegetation and treatment	Site preparation and notes
Cawdor Inverness- shire	150	57.52	December 2008	pasture	sandy Ioam	deer fence, trees not protected individually	dense grass, complete herbicide application (kerb) prior to planting with minimal regrowth in year 1	surrounded by scrub, adjacent to small burn, prone to flooding in winter with water logging in north west corner
Parlington Yorkshire	110	53.81	January 2009	restock site in ash/sycamore woodland	brown earth	deer fence and mulch mat added in year 1	mixed dense vegetation, particularly grasses	mature ash adjacent to trial, resulting in much regeneration within site
Little Wittenham Oxfordshire	55	51.63	November 2008	ex-arable, lain fallow several years	silty clay Ioam	deer fence and mulch mat added in year 1	sown with clover rye grass mix April 2009	ploughed and harrowed prior to planting
Dourdan Ile de France	125	48.51	December 2008	cleared nursery area within the Forêt de Dourdan	very heavy clay loam	boar fence and mulch mat added in year 1	south half of site very thin grass, north half brambles, willow and lupins which are gradually encroaching	vegetation (brambles) cleared but site not ripped
Monein Pyrenees	138	43.33	February 2009	former oak nursery area (grown through bramble) but cleared as oak not growing well	silty clay loam	fenced and mulch mat added in year 1	cleared, but copious willow and bramble regeneration	adjacent to small river. Site flooded prior to planting resulting in much flood debris (10 – 20 cm depth) across site. Numerous molehills. Very hot and dry at time of planting.

Seedling height (to the nearest centimetre) and root collar diameter (to the nearest millimetre) were measured at time of planting with a ruler and digital callipers respectively. The 16 central trees were measured to avoid edge effects. Sixteen trees were also measured at Monein where plots were 5 x 5, starting at the same tree as at other sites, one row and one column in from the corner. Weed growth was controlled by manual weeding after budburst of planted trees (necessary to find the trees after year 1), and again in mid-summer. Survival, height and root collar diameter were assessed and measured annually for three years (2009 - 2011). Each trial was fully beaten up at the end of the first year with seedlings of the same provenance and, where possible grown in the same soil type.

3.2.6 Analyses

The German provenance was excluded from analyses as it was the only commercial provenance included in the trials, and other seedlings were much smaller at the time of planting (3.7 cm on average compared to 45.4 cm). Dead trees or trees that had reduced in height due to biotic factors (vole or mole damage) were excluded from the analyses. Trees that had died but had been beaten up were also removed from the analyses. In all plots at Little Wittenham in 2010, many trees had died from vole damage, with 100 % mortality of some plots. Annual seedling survival was calculated as the number of surviving seedlings at the end of each year, expressed as a percentage of the original total present at the start of the trial.

Statistical tests of the significance of site (soil type) and provenance effects on survival and growth were carried out using the generalised linear models (GLM) procedure in Genstat v11 (VSN international), allowing for unbalance due to mortality. Differences between means were tested by Bonferroni pairwise comparisons. Analyses of variance of height and root collar increment were carried out on plot means using the model

$$Y_{ijk} = \mu + S_i + P_j + (I)_{ij} + B_{ik} + e_{ijk}$$

where Y_{ijk} is the plot height mean of provenance *j* in block *k* at site *i*, μ is the overall experimental mean, S_i the effect attributable to the *i*th site, P_j the effect attributable to the *j*th provenance, $(I)_{ij}$ the interaction of site with provenance, B_{ik} the effect of the *k*th block within the *i*th site, and e_{ijk} the residual not accounted for by the preceding terms.

3.3 Results

3.3.1 Soil analyses

Results of soil analyses are given in Table 3.2.

3.3.2 Seedling production

Seed germination in 2007 was variable across provenances with germination of some provenances being extremely low at less than 10 %. Germination test data (chapter 5) indicated that viability was high for most seed lots (range 60.7 - 81.6 %) except for Le Tech (25.9 %, Table 3.3). When adjusted for seed viability, germination was still very low particularly for northern provenances (Table 3.3). Germination was significantly different among provenances (p<0.001), although not among soil types.

The G/V value is the percent of viable seed that would be expected to germinate with this pretreatment. The germination test data showed that this value ranged from 21.0 % for Ballindalloch to 94.6 % for Monein. However, Table 3.3 also indicates that the number of seed that could be expected to germinate was not realised for any provenance, suggesting that some factor other than pretreatment regime was inhibiting germination. For example, 21.7 % of seed from Cawdor would be expected to germinate but actual values ranged from only 3.1 % for soil from Cawdor to 8.8 % for soil from Little Wittenham.

The percentage of viable seed that germinated in 2008 was higher than in 2007 across all provenances, with a greater response in UK provenances than in French provenances (Table 3.4). However, germination of viable seed was still relatively low for the most northern provenances, with only 42.8 % of seed from Cawdor germinating (across all soil types) whereas 93.4 % of seed from Monein germinated. Both soil type and provenance were highly significant (p<0.001) for germination, with germination being lower in most seedlots in soil from Monein, Dourdan and Little Wittenham (Table 3.4). There was no soil x provenance interaction.

Table 3.2 Mean soil analysis results from nine samples at trial sites (Cawdor, Parlington, Little Wittenham, Dourdan and Monein) and fivesamples at non trial sites (Ballindalloch, Settrington, Ranmore Common, Marly and Le Tech).

Site	% organic matter	% moisture content	рН	Na mg/l	K mg/l	Ca mg/l	P mg/l	Nitrate mg/l N	Ammonia mg/l N	% N
Cawdor	5.8	22.4	4.93	0.83	1.42	6.4	9.1	0.65	0.28	0.18
Ballindalloch	6.6	29.1	3.51	1.42	3.42	0.2	0.3	0.03	0.24	0.32
Parlington	6.4	21.6	7.04	3.19	3.11	185.7	266.4	1.04	0.43	0.21
Settrington	23.6	51.3	6.23	4.10	7.77	259.6	372.4	1.55	0.84	0.55
Little Wittenham	4.8	18.9	6.58	1.01	5.30	13.6	19.5	0.01	0.14	0.10
Ranmore Common	n 14.7	31.5	5.85	3.55	2.94	100.4	144.0	0.50	0.92	0.44
Marly	4.3	21.6	4.38	0.95	2.69	5.6	8.0	0.65	0.43	0.11
Dourdan	3.6	17.5	6.21	0.47	3.33	9.4	13.5	0.21	0.22	0.06
Monein	5.4	25.0	4.38	0.80	2.93	3.9	5.7	0.24	0.56	0.12
Le Tech	3.7	7.9	6.41	0.90	3.01	13.6	19.5	0.70	0.32	0.05
Table 3.3Results of germination and viability testing and seedling production, 2007.

	Germi	nation tes	t data						
Provenance	Viability	Gmax	G/V	Cawdor	Parlington	Little Wittenham	Dourdan	Monein	Provenance Mean
Cawdor	81.6	17.7	21.7	3.1	5.0	8.8	5.0	8.0	6.0
Ballindalloch	81.6	17.1	21.0	8.4	2.3	6.9	9.2	12.2	7.8
Settrington	64.5	15.6	24.2	6.8	5.8	9.2	6.3	13.1	8.2
Parlington	80.7	20.4	25.3	10.8	13.2	10.1	9.3	23.2	13.3
Little Wittenham	60.7	20.0	32.9	13.9	13.9	13.9	10.3	17.5	13.9
Dourdan	76.4	23.7	31.0	20.4	18.0	23.3	14.7	16.0	18.5
Le Tech	25.9	13.1	50.6	24.8	14.5	9.1	7.8	39.2	19.1
Ranmore Common	71.7	50.4	70.3	31.4	32.2	32.7	24.8	27.4	29.7
Marly	68.4	34.9	51.0	32.4	31.5	37.0	26.9	32.9	31.2
Monein	64.5	61.0	94.6	53.3	51.9	47.0	40.2	32.5	45.0
Soil Mean				20.5	18.8	19.8	15.4	22.2	

Percent viability and maximum percentage germination (Gmax) after pretreatment of 10 weeks at 15 °C followed by 15 weeks at 4 °C. G/V is the percentage of viable seed that germinated in the germination test.

Percentage of seed that germinated in 2007 in soil types from different trial sites adjusted for viability, with 10 weeks at 15 °C and 16 weeks at 4 °C pretreatment. Data are ranked by provenance mean germination across soils. Means joined by lines are not significantly different from each other (Bonferroni multiple range test) at p<0.05.

	Table 3.4	Results of germination an	d viability testing and	seedling production, 2008.
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	Germir	nation test	data		Seedling production data by soil type							
Provenance	Viability	Gmax	G/V	Cawdor	Parlington	Little Wittenham	Dourdan	Monein	Provenance Mean			
Cawdor	79.1	57.5	76.1	49.4	53.5	45.8	34.6	30.8	42.8			
Parlington	88.0	67.5	76.7	72.7	56.8	36.0	61.1	45.9	54.5			
Le Tech	27.5	17.0	61.8	71.0	54.9	48.3	46.8	52.1	54.6			
Ballindalloch	74.8	68.0	91.2	56.5	73.3	58.2	52.4	55.7	59.2			
Settrington	73.0	50.0	68.5	66.5	74.8	57.4	60.5	47.7	61.4			
Dourdan	76.0	68.0	89.5	60.0	78.5	65.4	64.1	60.9	65.8			
Little Wittenham	63.0	44.5	70.6	69.4	75.7	77.7	57.5	58.9	67.9			
Ranmore Common	73.5	70.0	95.2	85.5	77.4	69.3	68.0	45.5	69.1			
Marly	67.5	59.0	87.4	87.0	84.3	78.2	69.9	87.0	81.3			
Monein	61.0	60.5	99.2	94.3	110.7	100.9	90.2	71.2	93.4			
Soil Mean				71.2	74.0	63.7	60.5	55.6				

Percent viability and maximum percentage germination (Gmax) after pretreatment of 12 weeks at 15 °C followed by 25 weeks at 4 °C. G/V is the percentage of viable seed that germinated in the germination test.

Percentage of seed that germinated in 2008 in soil from different trial sites adjusted for viability after pretreatment of 12 weeks at 15 °C and 27-28 weeks at 4 °C. Data are ranked by provenance mean germination across soils. Means joined by lines are not significantly different from each other (Bonferroni multiple range test) at p<0.05.

3.3.3 Survival

Survival was good in the first year, across sites and provenances (Table 3.5). However, while provenance was not a significant source of variation, site was (p<0.001) with survival being lowest at Monein (80.8 %). Most provenances had fewer than 40 (out of 48) individuals alive at Monein, whereas all other sites had more than 40 individuals per provenance, except for Cawdor seedlings at Cawdor and Parlington, and Parlington seedlings at Dourdan. Most mortality in 2009 was due to animal damage. At Monein, this was largely because of mole hills that completely buried some seedlings. Indeed, in 2008, between planting and measuring the trees (two days), 36 trees were buried by moles, and had to be dug out and replanted. In 2009, this number more than doubled, and half of those that died did so because of mole hills. At Cawdor and Dourdan, mortality was also largely due to moles (11.2 % and 9.6 % respectively) although some vole damage was also recorded. At Parlington, mortality (9 %) was due to voles. Survival at Little Wittenham was highest at 97.9 %, with only ten dead trees in total.

	2009		20	10	2	011
Site	n	%	n	%	n	%
Cawdor	53	88.8	189	60.6	247	48.8
Parlington	43	91.0	120	75.8	147	69.4
Little Wittenham	10	97.9	77	87.7	377	21.6
Dourdan	46	90.4	87	80.0	108	77.3
Monein	92	80.8	105	78.1	128	73.3
Mean						

Table 3.5Number of dead trees and percentage seedling survival at five trial sites over
three years.

The highest levels of mortality occurred between 2009 and 2010, being particularly high at Cawdor with an additional 28.2 % mortality from 2009 (Table 3.5) due to a number of reasons. Pheasant shooting is an important source of revenue at this estate, and the trial site had been used for this purpose and the gate left open. While a small number of trees died due to being buried by moles, the main cause of death in 2010 was trampling by people, and sheep herbivory. Also, trees at this site had not been protected by mulch mats.

The chemical vegetation control applied in 2008 held up well in 2009, but the effects were no longer apparent by 2010 resulting in the establishment of dense tall grass (largely *Dactylis glomerata*, (cocksfoot)), which also added to mortality. Mortality at Parlington, Little Wittenham and Dourdan was largely due to voles with some seedlings also buried by moles at Dourdan. However, at this site, *Lupinus* spp. were encroaching from the south to the north of the trial, and promoted vegetation growth through nitrogen fixation and additional shelter, dividing the site essentially into two treatments: good growth of *Fraxinus excelsior* but lower survival where *Lupinus* spp. were present, and higher survival but very poor growth in the absence of *Lupinus* spp.

In 2011, Cawdor again suffered high mortality of seedlings, this time due to suppression by vegetation, despite hand weeding around the trees each year. Trees that survived suffered from dieback due to smothering, resulting in less than 50 % survival in three years. At Little Wittenham, the ryegrass - clover seed mix was well established, with a resulting explosion in the vole population. Nearly 80 % of the trees were severely damaged by voles between 2010 and 2011, either through stripping of the bark or by being snipped to the base. Additional mortality at Dourdan, Monein and Parlington was relatively low, with survival remaining around 70 % (Table 3.5).

Analysis of variance showed that site was a highly significant factor in seedling survival each year (p<0.001), and the provenance effect was significant in 2010 and 2011 (p=0.002 and p=0.005 respectively). There was no provenance x site interaction.

3.3.4 Growth

Mean height at time of planting across all sites and provenances was 3.7 cm and root collar diameter was 1.8 mm. Heights and root collar diameters were significantly different among provenances (p < 0.001) and site (soil type) (p < 0.001) at the time of planting. Provenance x site was significant for height (p = 0.025) but not for root collar diameter. Site remained a highly significant source of variation in subsequent years for height and root collar diameter, although provenance was no longer a significant source of variation in root collar diameter by 2010. There were no provenance x site interactions for either trait after 2008.

At the time of planting all provenances were tallest in soil from Cawdor and shortest in soil from Monein (Table 3.6). However, within one year most provenances were tallest at Monein (except for the Ranmore Common provenance growing at Parlington and the Little Wittenham provenance growing at Dourdan) and this pattern was largely maintained in all subsequent years. Root collar diameter was greatest in soil from Cawdor in 2008, but variable across other soil types (Table 3.7). As with height growth, all provenances had the greatest root collar diameters in 2009 at Monein except for provenances from Ranmore Common, Marly and Monein, all of which were greatest at Parlington and this pattern was largely maintained in all subsequent years.

The spring of 2010 was particularly harsh with several late spring frosts occurring over several weeks. New growth was badly frost damaged twice at all sites except Monein where no frost damage was recorded. In 2010, most provenances performed best at Parlington, despite being frost damaged, except for provenances from Cawdor, Ballindalloch, Parlington and Le Tech, which all performed best at Monein. In 2011, most provenances again performed best at Monein (except for the Ranmore Common and Monein provenances which again grew best at Parlington, and the Little Wittenham provenance which again grew best at Parlington, There were no significant differences in growth among provenances at Cawdor, Little Wittenham or Monein (Table 3.8) whereas individual provenance performance was significantly different among sites (Table 3.9).

Inspection of mean incremental growth data divides the sites clearly between those showing good growth (Parlington and Monein) and those where growth was disappointing (Cawdor, Little Wittenham and Dourdan). Incremental height growth was greatest at Monein (20.9 cm) and least at Dourdan (12.0 cm) and mean diameter increment greatest at Parlington (5.4 mm) and least at Cawdor (3.2 mm) (Table 3.8).

	Cawdor							Parlin	gton		Little Wittenham				
Provenance	2008	2009	2010	2011	2008-11	2008	2009	2010	2011	2008-11	2008	2009	2010	2011	2008-11
Cawdor	4.0	5.2	8.6	18.3	14.3	3.6	5.0	7.5	11.0	7.4	3.4	4.5	7.1	14.1	10.7
Ballindalloch	4.2	5.3	10.4	14.7	10.5	4.0	6.6	11.8	18.4	14.4	3.4	4.5	7.6	13.0	9.6
Settrington	4.6	4.8	7.8	10.6	5.9	3.8	6.6	13.2	22.2	18.4	3.5	4.7	7.2	15.8	12.3
Parlington	4.3	5.7	11.8	19.4	15.1	3.6	5.8	10.9	15.9	12.3	3.4	4.8	7.8	15.5	12.1
L. Wittenham	4.0	4.9	11.4	19.6	15.7	3.6	6.4	13.9	26.8	23.2	3.6	5.4	9.3	17.4	13.8
Ranmore	4.6	5.7	10.9	16.2	11.5	3.8	8.7	18.3	31.1	27.3	3.2	4.5	7.3	15.7	12.5
Marly	5.0	6.2	13.7	21.4	16.4	3.8	8.4	18.1	24.4	20.6	3.4	5.5	9.3	17.5	14.1
Dourdan	4.2	5.9	9.8	17.1	12.9	3.6	8.3	16.6	22.2	18.6	3.7	5.6	9.7	18.4	14.7
Monein	5.1	6.8	11.6	19.5	14.4	3.9	8.4	20.6	35.4	31.5	3.5	5.8	10.1	15.9	12.4
Le Tech	4.4	4.5	6.8	12.0	7.6	3.2	5.5	10.9	14.7	11.5	3.4	4.6	7.5	12.2	8.8
Mean	4.5	5.5	10.3	16.9	12.4	3.7	7.0	14.2	22.2	18.5	3.5	5.0	8.3	15.6	12.1

Table 3.6Provenance mean heights (cm) at each site in each year and incremental height growth 2008 - 2011 (cm). The home provenance is
highlighted in yellow, the best performing provenance in green, and the worst performing provenance in red at each site.

			Dou	rdan				Monein		
Provenance	2008	2009	2010	2011	2008-11	2008	2009	2010	2011	2008-11
Cawdor	3.7	5.5	8.4	15.2	11.5	2.9	8.2	13.8	23.3	20.4
Ballindalloch	3.6	6.2	8.9	12.0	8.3	3.4	9.4	15.0	22.9	19.5
Settrington	3.9	6.5	10.8	16.1	12.2	2.8	7.5	12.9	22.4	19.5
Parlington	3.8	6.9	10.3	15.9	12.1	3.0	7.9	14.3	26.5	23.5
L. Wittenham	3.8	7.2	11.1	16.4	12.6	2.9	7.0	11.6	20.3	17.4
Ranmore	3.3	6.6	8.8	13.8	10.5	2.9	7.8	12.1	20.2	17.3
Marly	3.7	7.6	11.1	18.6	14.9	3.1	9.3	15.4	28.0	24.9
Dourdan	3.9	5.6	7.6	12.7	8.9	3.4	10.0	16.6	28.0	24.6
Monein	3.9	7.5	12.9	23.9	19.9	3.4	8.9	15.0	25.6	22.2
Le Tech	3.5	6.0	8.9	13.0	9.6	2.8	7.3	12.2	21.8	19.0
Mean	3.7	6.6	9.9	15.8	12.1	3.1	8.3	13.9	23.9	20.8

	Cawdor							Parling	ton			Little Wittenham				
Provenance	2008	2009	2010	2011	2008-11	2008	2009	2010	2011	2008-11	2008	2009	2010	2011	2008-11	
Cawdor	1.9	2.3	3.8	4.9	3.0	1.6	2.4	3.5	3.9	2.3	1.8	2.7	3.4	5.1	3.3	
Ballindalloch	1.9	2.4	4.1	5.0	3.2	1.6	2.8	4.4	5.6	4.0	1.6	2.7	3.9	5.8	4.2	
Settrington	2.1	2.4	3.7	4.1	1.9	1.7	3.4	5.8	7.0	5.3	1.8	2.9	3.7	6.9	5.1	
Parlington	2.0	2.6	4.1	5.2	3.1	1.8	2.9	5.0	5.8	3.9	1.9	3.0	4.1	6.3	4.4	
L. Wittenham	1.9	2.5	4.2	5.4	3.5	1.8	3.1	5.7	7.6	5.9	1.9	3.1	4.4	6.4	4.5	
Ranmore	1.9	2.4	4.1	4.6	2.7	1.8	3.7	7.0	8.2	6.4	1.8	3.0	4.0	5.6	3.8	
Marly	2.2	2.6	4.8	5.8	3.6	1.7	3.6	5.9	6.4	4.7	1.9	3.3	4.8	6.6	4.7	
Dourdan	1.8	2.3	3.9	5.0	3.2	1.8	3.6	6.0	6.8	5.0	2.1	3.3	4.7	6.7	4.6	
Monein	2.1	2.6	4.2	5.2	3.1	1.7	3.2	6.2	7.4	5.7	1.8	3.0	4.3	6.0	4.2	
Le Tech	1.9	2.2	3.5	4.4	2.5	1.6	2.9	5.3	5.5	3.8	2.0	3.0	3.9	6.9	4.9	
Mean	2.0	2.4	4.0	5.0	3.0	1.7	3.2	5.5	6.4	4.7	1.8	3.0	4.1	6.2	4.4	

Table 3.7	Provenance mean root collar diameters (mm) at each site in each year and incremental diameter growth 2008 - 2011 (cm).	The home
	provenance is highlighted in yellow, the best performing provenance in green, and the worst performing provenance in red at	each site.

			Dourc	lan		_			Mone	ein	
Provenance	2008	2009	2010	2011	2008-11	-	2008	2009	2010	2011	2008-11
Cawdor	1.7	2.2	3.7	5.1	3.4		1.8	3.0	4.3	6.2	4.5
Ballindalloch	1.7	2.5	3.6	4.3	2.6		1.8	3.4	4.8	6.5	4.6
Settrington	1.8	2.5	4.3	6.1	4.3		1.7	3.1	4.5	6.4	4.7
Parlington	1.7	2.5	4.0	5.2	3.5		1.7	3.2	4.6	7.0	5.2
L. Wittenham	1.9	2.5	4.1	5.3	3.4		1.8	2.8	4.1	5.6	3.9
Ranmore	1.7	2.6	3.7	5.0	3.3		1.7	3.4	4.2	5.6	3.9
Marly	1.9	2.6	4.4	5.6	3.7		1.8	3.2	4.6	6.9	5.1
Dourdan	1.9	2.4	3.3	4.8	2.9		1.9	3.7	5.4	7.7	5.8
Monein	1.9	2.7	4.7	6.8	4.9		1.8	2.9	4.4	6.3	4.5
Le Tech	1.7	2.4	3.7	5.0	3.3		1.7	3.1	4.3	6.5	4.8
Mean	1.8	2.5	4.0	5.3	3.5		1.8	3.2	4.5	6.5	4.7

Table 3.8Mean height increment (cm) from 2008 - 2011 ranked by provenance at each site. Means joined by lines are not significantly
different from each other at p<0.05 (Bonferroni multiple range test). The local provenance is highlighted in bold. Site means are
given for height increment (cm) and root collar diameter increment (mm).

				5	Site				
Provenance	Cawdor	Par	lington	Little \	Vittenham	Do	ourdan	Ν	Nonein
Settrington Le Tech Ballindalloch Ranmore Dourdan Cawdor Monein Parlington L. Wittenham Marly Site mean height increment (cm)	5.9 7.6 10.5 11.5 12.9 14.3 14.4 15.1 15.7 16.4 12.4 n.s	Cawdor Le Tech Parlington Ballindalloch Settrington Dourdan Marly L. Wittenham Ranmore Monein	7.4 11.5 12.3 14.4 18.4 18.6 20.6 23.2 27.3 31.5 18.5 ***	Le Tech Ballindalloch Cawdor Parlington Settrington Monein Ranmore L. Wittenham Marly Dourdan	8.8 9.6 10.7 12.1 12.3 12.4 12.5 13.8 14.1 14.7 12.1 n.s	Ballindalloch Dourdan Le Tech Ranmore Cawdor Parlington Settrington L. Wittenham Marly Monein	8.3 8.9 9.6 10.5 11.5 12.1 12.2 12.6 14.9 19.9 12.0 ***	Ranmore L. Wittenham Le Tech Ballindalloch Settrington Cawdor Monein Parlington Dourdan Marly	17.3 17.4 19.1 19.5 19.5 20.4 22.3 23.5 24.6 24.9 20.9 n.s
Site mean diameter increment (mm)	3.0 n.s		4.7 ***		4.4 n.s		3.5 ***		4.7 n.s

Probability >F for within site analysis: n.s. = not significant P >0.05; * significant at P \leq 0.05; ** significant at P \leq 0.01; *** significant at P \leq 0.001.

Table 3.9 Mean height increment (cm) from 2008 - 2011 of each provenance at each site. The rank of each provenance at each site is given in brackets. The site that each provenance performs best at is in bold. Provenance means across all sites are given for height (cm) and root collar diameter (mm).

					Provenance					
Trial Site	Cawdor	Ballindalloch	Settrington	Parlington	Little Wittenham	Ranmore Common	Dourdan	Marly	Monein	Le Tech
Cawdor Parlington Little Wittenham Dourdan	14.3 (5) 7.4 (10) 10.7 (8) 11.5 (6)	10.5 (8) 14.4 (7) 9.6 (9) 8.3 (10)	5.9 (10) 18.4 (6) 12.3 (6) 12.2 (4)	15.1 (3) 12.3 (8) 12.1 (7) 12.1 (5)	15.7 (2) 23.2 (3) 13.8 (3) 12.6 (3)	11.5 (7) 27.3 (2) 12.5 (4) 10.5 (7)	12.9 (6) 18.6 (5) 14.7 (1) 8.9 (9)	16.4 (1) 20.6 (4) 14.1 (2) 14.9 (2)	14.4 (4) 31.5 (1) 12.4 (5) 19.9 (1)	7.6 (9) 11.5 (9) 8.8 (10) 9.6 (8)
Marly	20.4 (5)	19.5 (7)	19.5 (6)	23.5 (3)	17.4 (9)	17.3 (10)	24.6 (2)	24.9 (1)	22.2 (4)	19.0 (8)
Provenance mean height increment (cm)	12.9 ***	12.5 ***	13.7 ***	15.0 **	16.5 n.s	15.8 ***	15.9 ***	18.2 ***	20.1 *	11.3 ***
Provenance mean diameter increment (mm)	3.4 ***	3.7 ***	4.1 ***	4.1 *	4.1 **	4.1 ***	4.7 ***	4.4 n.s	3.7 ***	3.7 *

Probability >F for within provenance analysis: n.s. = not significant P >0.05; * significant at P \leq 0.05; ** significant at P \leq 0.01; *** significant at P \leq 0.001.

Sites varied a great deal in the number of accumulated day degrees > 4 °C during the trial period with Monein receiving over twice the day degrees of Cawdor. Cawdor was the coldest site with 675 day degrees below 0 °C. However, Parlington was surprisingly mild, with the fewest day degrees below 0 °C of any site (Table 3.10).

	Trial Site										
Year	Cawdor	Parlington	L. Wittenham	Dourdan	Monein						
2009	2651	3080	3828	4429	5245						
2010	2317	2905	3729	4173	5347						
2011	2531	3280	4231	4777	5587						
Total > 4 °C	7498	9265	11788	13379	16179						
2009	160	57	70	114	53						
2010	445	270	376	386	226						
2011	71	45	106	89	128						
Total < 0 °C	675	372	552	589	409						

Table 3.10 Accumulated day degrees > 4 °C and < 0 °C from 1^{st} March 2009 to 30^{th} November 2011 at five sites.

Analysis of variance for incremental growth in height and root collar diameter showed that both site and provenance were significant sources of variation for height (Table 3.11) but site was the only significant source of variation for increment in diameter (p = 0.033). The provenance x site interaction was not significant for either trait (Table 3.11) and there is no indication that local provenances perform best.

Source of variation	d.f.	S.S	m.s	v.r	F pr
Site	4	2897.74	724.44	14.84	<.001
Provenance	9	951.86	105.76	2.17	0.032
Site x block	10	976.34	97.63	2.00	0.043
Site x provenance	36	1432.38	39.79	0.81	0.750
Residual	84	4101.86	48.83		
Total	143	10360.19	72.45		

Table 3.11Analysis of variance for height increments based on plot means of ten
provenances of *Fraxinus excelsior* from 2008 - 2011.

Provenance performance is largely consistent across sites for the best (Marly and Monein) and worst (Ballindalloch, Cawdor and Le Tech) performing provenances, while the ranking of other provenances varies among sites (Table 3.8). For example, Dourdan ranks first at Little Wittenham, but is ninth at Dourdan. Similarly, Ranmore Common is the second best performing provenance at Parlington and ranks last at Monein (Table 3.9). At both these sites, provenance is a significant source of variation.

Monein was the best performing provenance averaged across all sites, and ranked first at Parlington and Dourdan. The provenance Marly also performed well, and ranked first at Cawdor and Monein – the two sites separated by the greatest geographic distance, indicating considerable plasticity within this population (Table 3.8). At Little Wittenham the provenance from Dourdan grew best (Tables 3.6 and 3.8) in terms of height growth. In terms of root collar increment, no provenance ranked first at more than one site, although Cawdor ranked last at two sites (Table 3.7).



Figure 3.3 Mean height increment (cm) from 2008 - 2011 for ten provenances of *Fraxinus excelsior* at five trial sites, ordered from north (Cawdor) to south (Monein). The solid line indicates the site mean for all provenances at a site. Ranged around this site mean are the provenance means at each site, indicating their difference (better or worse) from the site average. The local provenance at each site (*e.g.* Little Wittenham seedlings growing at Little Wittenham) is circled.

Figure 3.3 shows clearly that the local provenance is never the best performing provenance in three-year-old *Fraxinus excelsior* seedlings, and indeed sometimes performed below the site average as can be seen at Dourdan and Parlington. Material from the south of France performed well at all sites, but the material from Scotland (Cawdor and Ballindalloch) did not do well further south, and usually performed poorly. The Le Tech provenance, which is from much higher elevation (800 m), also performed poorly at all sites.

3.4 Discussion

3.4.1 Soil analyses

According to Evans (1984), most forest stands in Britain are on reasonably fertile sites and nutrients are unlikely to limit growth. However, ash is regarded as a site demanding species, and growth is enhanced with increasing nitrogen availability. No specific data could be found to indicate adequate soil nutrient levels for ash. Seedlings grew best at Monein and Parlington and these sites had relatively higher amounts of either nitrate (at Parlington) or ammonia (at Monein) (Table 3.2). Dourdan and Little Wittenham had low levels of these compounds, and Dourdan had particularly low percentage nitrogen (0.06 %). As *Fraxinus excelsior* is known to be nitrogen demanding, this could be a factor limiting growth at these sites. Where *Lupinus* spp. were encroaching at Dourdan, growth was substantially improved resulting in a 366 % increase in height increment and 382 % increase in diameter increment between 2008 and 2011 (data not shown).

3.4.2 Seedling Production

Numbers of seedlings produced in 2007 were very low for many provenances, and this was largely attributable to insufficient chilling of seed to break dormancy, especially noticeable for more northern provenances (Table 3.3). With 10 weeks warm pretreatment followed by 16 weeks of chilling, most provenances only reached 50 % germination or less, except for those from Ranmore Common (70.3 %) and Monein (94.6 %). However, fewer seed actually germinated than expected from the results of germination testing. Germination is greatly influenced by soil moisture availability (not limited in the nursery) but also by temperature, and secondary dormancy is not uncommon where soil temperatures exceed a threshold level. In a study on direct seeding of *Fraxinus excelsior*. Piotto (1994) also investigated the effects of temperature on germination of *Fraxinus* seed, and found a temperature of 20 °C induced secondary dormancy. Seed were sown much later in 2007 (mid June) than would happen under normal nursery production conditions and were also sown in cells. High soil

temperatures were likely to have been a contributing factor to induction of secondary dormancy and reduction in germination.

In 2008, seedlots received approximately 12 weeks longer chilling than in the previous year and seed were sown earlier (early to mid May). This improved the germination capacity by over 200 % for some seedlots (Cawdor, Ballindalloch, Parlington and Dourdan), but actual germination was still lower than predicted from germination testing for some provenances, notably those from Yorkshire and Scotland (Table 3.3). It was noted at the time of sowing that both the Dourdan and Little Wittenham soils were high in clay content and hence likely to be poor for seedling production. Although both soils had grit added for seedling production in 2008, germination was lower than in soils from Cawdor and Parlington which had a higher proportion of sand, which could in turn explain low germination in these soils. Seed germination was highest in soil from Monein in 2007, but lowest in 2008, which was unexpected. Monein soil texture was good although the pH of 4.38 (Table 3.2) is close to the limit stated by Wardle (1961) of pH 4.2 for growing *Fraxinus excelsior*. However, as seedlings subsequently grew well at the Monein site, this is unlikely to be the cause of low germination, although low soil pH could impact seed germination, while not limiting later growth.

The lowest levels of germination were for Cawdor (42.8 %), Ballindalloch (59.2 %), Parlington (54.5 %) and Le Tech (54.6 %). These provenances are either from more northerly latitudes or, in the case of Le Tech, from high elevation (800 m a.s.l.), and might be expected to have higher chilling requirements (see chapter 5), which possibly were not fully met even with 28 weeks chilling.

Table 3.4 shows an anomaly for seed from Monein, with more seed germinating than were apparently viable in soil from Parlington (110.7 %) and Little Wittenham (100.9 %). Viability values were taken from the final germination test in 2008 after 12 weeks warm pretreatment followed by 24 weeks cold treatment. This pretreatment regime most closely matched the pretreatment of the seed that were sown for trial establishment and gave a viability estimate of 61 % for the Monein provenance. In the previous germination test carried out four weeks earlier after only 20 weeks cold treatment, viability of 69 % was

estimated for seed from Monein. As viability fluctuates between assessments (a sampling factor of the actual individual seed tested) a different sample may give a different, possibly higher viability, and it is unlikely that viability dropped 8 % in four weeks. Averaged across all germination tests from May 2007 to April 2008, viability was 66 %. This value would reduce the G/V value by a few percent correspondingly.

3.4.3 <u>Seedling performance</u>

While all sites were cleared of vegetation prior to planting, weed growth was substantial posing numerous problems for seedling survival. Despite hand weeding all trees four times a year, survival was poor because seedlings were swamped by competing vegetation. Due to vegetation growth, all trees were protected with mulch mats after the first year (except at Cawdor). While this aided in finding the trees, mulch mats also provided an excellent habitat for voles, and increasing damage by voles was noticeable after mulch mats were put in place. Many mats had vole nests and tunnels underneath. These influences made it impossible to assess the genotype component of survival. Mulch mats were expensive to use (mostly in terms of the time taken to place them) and while essential in this case to locate trees after the first year, would not be recommended. Delaying planting a year would have been more sensible, but was not an option in this study due to time constraints.

Most trees for forestry use are raised in nurseries under ideal conditions (tilled, fertile soil). When they are planted in to forest stands, they often exhibit a carry-over effect from the nursery and go in to check where visible growth is limited for a number of years. In this experiment, seedlings were raised in cells in the soil they were to be planted in with the expectation that this would limit nursery carry-over effects and allow adaptive differences to become visible at an earlier age than in traditional provenance trials. The nursery data showed significant differences between soil types and provenances for all variables. However, within a year, provenance ranking had substantially altered and the site with the worst soil type for growth in 2008 (Monein) was the one most provenances grew best at in 2009, suggesting that nursery carry-over effects had been eliminated within a year as opposed to the five years reported for traditional ash provenance trials (Cundall *et al.* 2003).

By 2011, provenances were only significantly different from each other at three sites, and only Marly and Monein (the best two provenances) were significantly different from other provenances. While these two provenances performed well at all sites, and some provenances (Le Tech and Ballindalloch) performed poorly at all sites, there was no consistency of ranking across sites of the other provenances. The Monein and Marly provenances are registered seed stands, and overall these provenances ranked first and second.

Interestingly, no provenance performed best at its local site (figure 3.3; tables 3.6 and 3.7), indicating a lack of local adaptation within populations, a finding also reported in the FRAXIGEN study (FRAXIGEN 2005). Most provenances grew best at Monein (overall mean increment of 20.9 cm), or, best at Parlington (overall mean increment of 18.5 cm) and second best at Monein. The site effect overrode the provenance effect, and these two sites would be predicted as the best sites for growing Fraxinus excelsior because of shelter, moisture regimes and soil structure. Looking at provenance ranks within a site, the provenance Cawdor ranked fifth, the provenance Parlington ranked third, the provenance Monein ranked fourth and the provenance Marly ranked first at both Cawdor and Monein, the two sites with the greatest geographic distance, suggesting there is plasticity within these provenances to cope with a degree of climate change. These provenances might be expected to show an increase in yield with a 3 - 5 °C increase in temperature, as indicated by Broadmeadow et al. (2005), if water does not become limiting. Le Tech always ranked in the bottom three provenances at any site which could simply be a reflection of poor quality seed and seed trees, but also a factor of altitude with this provenance being adapted to colder winters and possibly not being able to take advantage of improved growing conditions.

While many provenances performed better in more southerly locations, Monein, the most southerly provenance except Le Tech, actually performed best at Parlington, suggesting that no provenance is at its ecologically-optimal location. However, provenances do not show a consistent pattern of performance across sites. While the four northern provenances performed best at Monein (the most southerly site), suggesting that northern provenances are also not in their optimal ecological niche and endure climates that are colder than their optima, provenances from the middle of the range (Little Wittenham and Ranmore Common) actually performed better slightly further north. Dourdan, Marly and Le Tech also all performed best at Monein. As a continental site Dourdan has a high thermal sum, but also experiences cold winters. The number of day degrees below zero is much higher than for sites further north in the UK, where winter temperatures are moderated by the maritime climate. Dourdan received similar day degrees below 0 °C to Cawdor which is 9 ° latitude further north.

The meta-analysis of studies of local adaptation carried out by Leimu and Fischer (2008) reported only 45 % of populations to be locally adapted, but noted a lack of studies on trees. The results of this study would suggest that this is also the case for these populations which might not be in optimal locations. Gould (1998) points out that many populations are in their locality by chance – they are not necessarily the best population for a site; natural selection has merely selected the best that was ever there.

With a warming climate, genotypes other than those already at a location will eventually become better suited to the novel climate (Rehfeldt *et al.* 1999). Adjusting to the change occurs in the short term through physiological plasticity of individuals and in the long term through evolutionary processes of selection, migration, mutation and drift. Populations in this study showed no evidence of local adaptation (Figure 3.3) but considerable phenotypic plasticity, apparent in some populations that perform better than any other at sites that are widely separated (Tables 3.6 and 3.7) suggesting that ash has the ability of survive and grow over a large range of sites at present. Populations of *Fraxinus excelsior* presently in the UK should thus be able to cope with a degree of climate change, at least in the medium term, and may in fact show increased growth where water is not limiting.

The results presented here are from trials that are only three years old. Although the nursery carry over effect was eliminated within one year, these are still very early results, and problems with establishment are likely to be affecting results. Data from later assessments will reveal more detailed and accurate information regarding effects of site (location) and the likely impacts of a changing climate on the performance of provenances.

Whilst the view that 'local is best' may be pertinent for short-lived annual species, trees are long-lived organisms and genes travel great distances in pollen to carry novel genetic variants to other populations. When considering what to plant in UK forests for the future, it may be a good idea to use some local material, but to also include material from the near European continent. Results from this study indicate that UK populations of *Fraxinus excelsior* are not locally adapted and will be able to cope with a degree of climate change through phenotypic plasticity. Thus, maintaining a broad genetic base gives our trees the potential to adapt and is the best insurance to combat the effects of climate change.

Chapter 4 Budburst in Temperate Forest Trees

4.1 Literature Review

Perennial species such as trees are long lived organisms, and during a life cycle they encounter many unfavourable conditions. Deciduous trees growing in temperate regions, such as *Fraxinus excelsior*, have an annual cycle and enter a dormant state to avoid damage during periods of adverse conditions. They do this by shutting down photosynthesis, which is costly in terms of water use, and shedding of leaves. Active growth stops long before there is any danger of frost injury (Hänninen 1990). The factors that impose and release dormancy are complex and the mechanism by which they do so is poorly understood.

Winter chilling, spring warming and photoperiod all influence the timing of budburst, but there is no consensus as to the relative importance of these factors although it is generally recognised that short day photoperiod triggers growth cessation and bud set (Hänninen *et al.* 1990) and that temperature governs the timing of budburst in the spring (Perry 1971; Hunter and Lechowicz 1992; Bennie *et al.* 2010). Indeed, many researchers have carried out extensive experiments investigating one, two or all three of these factors and results often contradict each other (see section 4.1.3).

The timing of these natural events, including flowering, budburst (also referred to as leaf unfolding or flushing), seed set and dispersal in plants, is termed phenology. Badeck *et al.* (2004) refer to phenology as 'the science of recurring events in nature, addressing the timing of switches between recurrent developmental or behavioural phases of organisms'. Phenology is important because it relates to plant fitness (Primack 1980; Rathcke and Lacey 1985). A mistiming in phenology can result in frost injury to newly emerging leaves or flowers with a corresponding lack of growth or seed production (Hänninen 1991). Thus, phenology may contribute over time to a species distribution and abundance (Chuine and Beaubein 2001; Cleland *et al.* 2007; Chuine 2010). Phenology is also important since the duration and timing of the growing period are dependent on it, and this influences net primary production (Keeling *et al.* 1996; Chuine *et al.* 2000; Rotzer *et al.* 2004). Myneni *et al.* (1997) reported that climatic warming during the 1990s resulted in an average temperature increase of more than 1 °C in higher latitudes (and up to 4 °C in Alaska) with a corresponding increase in the active growing season of 12 ± 4 days. This accounted for an increase in net primary production of 12 % in latitudes above 45° north. The length of the growing season also has ecosystem consequences for water, nutrient and carbon cycling (White *et al.* 1997; Forrest and Miller-Rushing 2010).

Much attention has been given in the literature to the control of phenological events (Visser *et al.* 2010; Wilczek *et al.* 2010) which is known to be closely related to temperature (Rotzer and Chmielewski 2001; Doi and Katano 2008). For most species, however, it is not known: (i) which specific environmental factors are most important in determining phenology; (ii) the precise molecular and physiological processes that regulate phenological events, and (iii) whether variation in phenological timings over years or among individuals reflects genetic differences or simply plastic responses to environmental heterogeneity (Forrest and Miller - Rushing 2010).

4.1.1 Bud dormancy and its control

Trees growing in temperate regions avoid winter cold injury by entering a state of dormancy during the winter. Dormancy is a state of low metabolic activity in plants or seeds when no outwardly visible growth takes place. Many species become hardy to low temperatures in autumn, and midwinter temperatures as low as - 40 °C may do no damage. The same species may become susceptible to severe injury by frosts of only - 2 °C in spring and many are especially at risk during the early period of bud burst and extension (Savill *et al.* 1997).

Dormancy in woody perennials, particularly its induction and release, has been very well studied and yet remains poorly understood. Vegis (1964) reported a metabolic restraint on buds during dormancy, with dormancy imposing a restriction on the temperature range over which buds will grow. Lang (1987) stated that dormancy is the *'absence of visible*

growth in any plant structure containing a meristem' and described three types (Lang et al. 1987):

- ecodormancy, a period of non-active growth caused by limitations in environmental factors and lasting only while they prevail (e.g. temperature extremes, nutrient deficiency or water stress), also termed quiescence;
- *paradormancy,* where growth inhibition is caused by another part of the plant (such as apical dominance); and
- *endodormancy*, where the inhibition resides in the dormant structure itself (e.g. a chilling or photoperiodic response), also termed rest.

Rohde and Bhalerao (2007), writing about dormancy in perennial plants, reflect that Lang's definition is not a very useful one because growth within a meristem is not readily visible, and that the mere absence of growth is ambiguous because dormancy constitutes an inability to resume growth. Also, growth consists of both cell division and cell elongation; e.g. at bud burst, cell elongation of preformed leaves begins before new cell division occurs. They therefore propose an alternative definition of dormancy as 'the inability to initiate growth from meristems (and other organs and cells with the capacity to resume growth) under favourable conditions' (Rohde and Bhalerao 2007). This definition seems to fit all aspects of dormancy and is more consistent with the earlier definition of Vegis (1964) as 'a state in which growth cannot be resumed whatever the external conditions may be', the 'whatever the external conditions may be' being the key factor.

Temperate trees cease elongation and develop dormant buds long before unfavourable weather occurs or the critical daylength for the induction of dormancy is reached (Noodén and Weber 1978). Research on bud dormancy has identified many hormones and environmental signals that govern the production of and the response to these hormones. Related studies of plant growth and shoot development have identified many genes involved in meristem initiation and organ formation (Horvath *et al.* 2003). The exact mechanism that brings about dormancy is not known, although it is thought that abscisic acid plays a role (Rohde and Bhalerao 2007). In buds, levels of abscisic acid follow a seasonal cycle and reach a maximum during endodormancy. How dormancy is maintained

after it is established is unknown. Gibberellins increase in the bud after chilling and promote budburst, and auxins and cytokinins are most evident during paradormancy (Tamura *et al.* 2002).

In preparation for dormancy the tree must first stop growing. Photoperiod is well known to be the primary factor governing growth cessation in temperate trees (Wareing 1956; Nitsch 1957; Perry 1971; Hänninen *et al.* 1990). Leaves perceive photoperiod through the phytochrome red – far red photoreaction, and emit a signal to the apical meristem which stops growth and forms a dormant bud (Wareing 1956; Vegis, 1964). Once growth has stopped, the tree becomes increasingly dormant until it is impossible for cells in the meristem to respond to growth-promoting signals. The tree is now said to be in a dormant state. Buds have a maximum state of dormancy in the late autumn when cell division and growth have stopped and bud development is complete. Although temperature can affect the onset of winter dormancy, Heide (1974) working on Norway spruce (*Picea abies*) showed that dormancy occurs because of an interaction with photoperiod. More recently, Rohde *et al.* (2011) showed that temperature interacts with the critical day length (photoperiod) to modify growth cessation in identical genotypes (clones) of poplar (*Populus* sp.).

A period of chilling temperatures is now required to break dormancy. Studies have shown that it is the bud itself which must be exposed to low temperatures (Metzger 1996). However, what constitutes a chilling temperature, and for how long buds must be exposed to it is ambiguous, and perhaps chilling is an unfortunate term as temperatures as high as 10 - 12 °C have been found to break the imposed dormancy in some species of the Rosaceae family (Heide and Prestrud 2005). Perry (1971) postulates 5 °C as the optimum chilling temperature and this temperature is commonly used throughout the literature (e.g. Murray *et al.* 1989; Hunter and Lechowich 1992; von Wuehlisch *et al.* 1995). However, fluctuating temperatures (as experienced in the natural environment) appear to be more effective at breaking dormancy than constant ones (Campbell and Sugano 1975; Hänninen *et al.* 1990).

It should be recognised that that the chilling requirement of trees for budburst varies from species to species (Heide 1993a), and from genotype to genotype (Farmer 1968), with

budburst occurring later in some provenances than others (Campbell 1974; Cahalan 1981; Myking and Heide 1995). Heide (1993a) attributes this to their differences in temperature sum (also referred to as forcing units) requirements for budburst. However, it is well recognised that trees also have a chilling requirement (Vegis 1964; Perry 1971; Hänninen 1990; Heide 1993a) that ensures that budburst does not occur too early in the year with the possible consequence of injury due to spring frosts. Late spring frosts, not uncommon in a maritime country such as the UK, may cause damage to young shoots that are in the process of rehydration and thus sensitive to cold (Cottignies 1986). There is thus a trade-off between early budbreak, thereby maximising the photosynthetic potential, and the possibility of frost damage to new shoots (Hannerz 1999).

A further complication is the interaction of photoperiod and temperature during chilling of buds and also for subsequent growth of shoots. Under experimental conditions, long photoperiods (over 14 hours) have been shown to substitute for chilling (Worrall and Mergen 1967; Farmer 1968; Wareing 1969; Lavender and Hermann 1970). However, with the exception of a few species such as beech (*Fagus sylvatica*) (Wareing 1953; Heide 1993b), silver birch (*Betula pendula*) (Li *et al.* 2003), European larch (*Larix decidua*) (Wareing 1969) or poplars (*Populus* spp.) (Van der Veen 1951) which are stimulated by long photoperiods to break bud even when not chilled, long photoperiods only promote budbreak when the chilling requirement has been fully met (Lavender 1981).

Once dormancy has been released and the tree starts to grow, internal resetting needs to occur; i.e. the state in which dormancy induction is possible with a subsequent need for chilling, must be re-established. It is thought that this may occur as meristems experience an accumulated heat sum, in a form of "devernalisation", although the mechanism by which this happens is unclear (Prince and Marks 1982). However, work by Rinne *et al.* (2001) has shown that the shoot apical meristem restores symplastic organization during chilling release from dormancy, thereby enabling the tree to maintain an annual cycle of growth and dormancy.

4.1.2 <u>Budburst and its control</u>

The chilling temperature required to break dormancy restores the ability to grow, but does not promote growth (Chouard 1960). For this, a period of warmth is required, referred to as forcing temperatures and usually expressed as accumulated day degrees over a threshold temperature. Again, there is ambiguity as to what constitutes a forcing temperature. Harrington *et al.* (2010) propose a 'possibility line' above which budburst is possible. The possibility line reflects the accumulation of chilling and forcing units, and both may be accumulated at the same time. Heide and Prestrud (2005) also found that this is the case, with the same moderate temperature of 6 - 9 °C providing both chilling and forcing units in members of the *Rosaceae* family.

The timing of budburst in temperate regions varies from year to year depending on climatic conditions and results from a complex interplay between an organism's genes and environmental factors such as temperature and precipitation, and these are the predominant factors that affect budburst (Forrest and Miller-Rushing 2010).

Genes and life history

Tree species can vary widely in timing of budburst, both among and within species (Lechowicz 1984; Murray *et al.* 1989) as evidenced by provenance trials (von Wuehlisch *et al.* 1995; Robson *et al.* 2010). The timing of spring budburst is considered to be under opposing evolutionary pressures (Bennie *et al.* 2010), as earlier budburst increases the length of the growing season allowing for greater carbon capture (capacity adaptation) but delayed budburst leads to a reduced possibility of frost injury to conducting tissues once the tree has come out of winter dormancy (survival adaptation) (Bennie *et al.* 2010; Körner and Basler 2010, Kramer *et al.* 2010). However, early budburst can also make the tree more prone to insect herbivory, and thus any gain in carbon capture may be lost (Polgar and Primack 2011).

Early successional species such as *Alnus* spp, *Betula* spp and *Populus* spp tend to break bud earlier in spring than later successional species. These earlier successional species most commonly have diffuse-porous wood and produce uniformly small vessels. Wang *et al.*

(1992) found high correlation between wood type and timing of spring budburst, with ringporous species coming into leaf later than diffuse-porous species. Smaller vessels are less prone to embolism during winter (caused by bubbles in the xylem, due to freezing) which allows them to conduct water earlier in the season (Wang *et al.* 1992). Ring-porous species such as *Fraxinus excelsior* have larger vessels, which are more prone to winter freezing damage. This requires the production of new vessels in the spring, thereby delaying budburst (Lechowicz 1984; Wang *et al.* 1992). Frost kills plant tissues when ice crystals form within cells, primarily because cell membranes are damaged allowing electrolyte leakage into the apoplast (Arora and Palta 1991).

Budburst is largely genetically controlled and is thought to be under selection pressure (Doi *et al.* 2010). The amount of genetic diversity contributes to a species ability to respond phenologically to changing temperatures. Species with low genetic diversity have much less variation in timing of budburst (Doi *et al.* 2010). For many plant species, phenology is known to be a variable character with a high degree of heritability (Billington and Pelham 1991; El-Kassaby and Park 1993; Farmer 1993; Hendry and Day 2005). Differences in phenological traits between populations have been shown for some species such as *Acer rubrum* (red maple) (Perry and Wang 1960) and *Pinus strobus* (eastern white pine) (Li *et al.* 1997), but not for others e.g. *Populus balsamifera* (balsam poplar) (Farmer 1993) or *Picea glauca* (white spruce) (Li *et al.* 1993). In clonal field tests of *Populus*, Bradshaw and Stettler (1995) found that the timing of budburst was among the most highly heritable traits, with up to 98 % of total phenotypic variance of clonal means explained by genetic factors, and only 2 % by environmental factors.

When modelling genetic variation in phenological traits, Chuine *et al.* (2000) showed that differences among populations studied were weaker in warmer than in cooler climates. The models used showed that the response of budburst and flowering to climate change depended on species, latitude, the intensity of changes and each species response to the environment (its plasticity). Local adaptation for tree phenology could be a serious constraint in adapting to climate change, but this is unlikely to be the case in species with high gene flow as the timing of budburst is known to be variable with a high degree of heritability and can therefore be modified by natural selection.

Photoperiod

Long days can trigger budburst in spring despite cool temperatures, or can substitute for chilling in a few species (Campbell and Sugano 1975). However, photoperiod has been shown to be relatively unimportant in predicting the timing of budburst in the field (Worrall 1993; Hänninen 1995; Chuine *et al.* 1999; Hänninen and Hari 2002) with the notable exception of *Fagus sylvatica*. Wareing reported in 1953 that *Fagus sylvatica* had no chilling requirement and that budburst occurred in response to photoperiod alone, but subsequent work by Heide (1993b) found that although a long photoperiod was essential, the chilling requirement still had to be met first. If a tree only responded to photoperiod, it would cause mistiming of events with climate in non-normal years. Lavender (1981) concludes that, as photoperiod is a consistent factor from year to year, it probably does not control budbreak for autochthonous species in their natural environments.

Temperature: chilling and forcing requirements

Temperature affects phenology directly by influencing rates of biochemical processes. In temperate regions, the photoperiod response is modified or completely overridden by a temperature response. As a consequence, the accumulation of a certain number of day degrees (also referred to as forcing units) often predicts well the timing of budburst (Cannell and Smith 1983; Murray *et al.* 1989; Hänninen 1990) and this commonly tracks annual variation in air temperature (Fitter *et al.* 1995). However, the process is more complex than this, as there is usually an internally imposed chilling requirement for breaking winter dormancy, regardless of the accumulated day degrees received in the spring (Murray *et al.* 1989, Morin *et al.* 2009).

Many modelling studies indicate that air temperature is the most important factor regulating budburst in the majority of species (Perry 1971; Hänninen 1987; 1990; Kramer *et al.* 1996; Häkkinen 1999), first by providing (i) sufficient chilling in winter and (ii) warm spring temperatures that allow for subsequent development of buds to the point of bursting. Also, there is much evidence that the accumulated day degrees required for budburst decrease with increased chilling, down to some minimal absolute chilling

requirement, when the buds may be said to be fully dormant (Lamb, 1948; Landsberg 1974; Campbell and Sugano 1975; Cannell and Smith 1983).

(i) Breaking dormancy – chilling requirement.

Dormancy break is induced throughout the winter as individuals are exposed to chilling temperatures (Cannell and Smith 1986). Sufficient chilling is a signal to the plant that it is safe to begin development within the bud (Perry 1971). Chilling requirements vary among species (Heide 1993a; Murray *et al.* 1989; Vitasse *et al.* 2009) and among genotypes (Morin *et al.* 2009). For example, the chilling requirement of *Picea sitchensis* (Sitka spruce) is high (Cannell and Smith 1983) whereas for *Picea abies* (Norway spruce) it is low (Hänninen and Peltonen 1988). Hannerz (2003) reported that the chilling requirement of *Picea abies* is met during November and December. He also found that chilling requirement was highest in western provenances, suggesting better adaptation to maritime conditions and mild winters where a high chilling requirement would prevent early budburst and potential damage from late spring frosts. However, differences between provenances were small because Norway spruce has a low chilling requirement. Other species have a greater chilling requirement and are likely to show greater variation between provenances (Leinonen 1996).

(ii) Promoting growth – forcing requirement.

After chilling requirements are met, a certain number of forcing units (day degrees) are required for budburst and unfolding of leaves (Cannell and Smith 1986; Heide 1993a). There is assumed to be strong genetic control over this requirement, which has been subject to natural selection by past climatic conditions under which the population has evolved (Bennie *et al.* 2010). The rate of budburst progression is linearly correlated with temperature with the growing period beginning earlier in warmer years (Synder *et al.* 1999). A 1 °C rise in spring temperature was found to advance budburst in *Betula* species in Scandinavia by 7 - 9 days in maritime regions, but by less than five days in more continental areas (Karlsen *et al.* 2007).

Autumn temperatures also play an important role in tree phenology. Controlled experiments on saplings of *Betula pendula*, *Betula pubescens* and *Alnus glutinosa* revealed a highly significant positive correlation between autumn temperatures and days to budburst

in the subsequent spring (Heide 2003). Rising autumn temperatures may therefore counterbalance the earlier spring budburst that would otherwise occur in response to higher winter temperatures caused by climate warming. Heide (2003) concluded that this autumn temperature response may be important for reducing the potentially adverse effects of higher winter temperatures on dormancy stability of boreal trees during climate warming.

Because there is a close link between temperature and budburst, even a small change in temperature may affect the timing of budburst quite substantially (Myking and Heide 1995). With increasing winter air temperatures the chilling requirement of some species may not be fully met and budburst may be delayed (Zhang *et al.* 2007; Morin *et al.* 2009). Conversely, where temperatures already far exceed the biological requirement for chilling, bud burst is expected to occur earlier than at present (Murray *et al.* 1989; Heide 1993a).

Water availability

This factor is less relevant for temperate species such as those considered here, but plays a role in the tropics and in arid environments where rainfall or drought influence the timing of phenological events (Rathcke and Lacey 1985). While it is clear that water availability plays a critical role in plant processes, there is very little in the literature on how it influences the phenological response. However, Peñuelas *et al.* (2004) acknowledge that water availability can complicate the phenological response and can be a causal factor in range shifts.

4.1.3 <u>Modelling phenological responses</u>

Models that accurately predict plant phenology are important tools for predicting the response of ecosystems to climate change. Numerous phenological models have been developed to predict the timing of growth of temperate or boreal trees but they are in general, according to Chuine (2000), empirical, nonlinear and non-nested and therefore difficult to test independently and to compare. The various models use differing parameters: warming in spring, winter chilling and photoperiod which can all influence the

timing of budburst. There is no consensus in the literature as to which model is the best, although simple empirical models appear to account for more variation than more complex process-based models (Cannell and Smith 1986). Models that give the best fit for one data set may perform worst when validated against a second data set (Chuine *et al.* 1998; 1999) or may not work well for another species (Hunter and Lechowicz 1992). Also, different models can provide equally good fits to empirical data and to artificial data that are biologically incorrect (Hunter and Lechowicz 1992).

The simplest models are based solely on thermal time (day degree) requirements, and named **thermal time models** (Cannell and Smith 1983; Hunter and Lechowicz 1992; Kramer 1994a). In these models, day degrees are accumulated from an arbitrary starting point during late winter or early spring, commonly the 1st of January (e.g. von Wuehlisch *et al.* 1995; Menzel 1997; Vitasse *et al.* 2009). The model then predicts the timing of budburst on the date when accumulated day degrees reach a threshold value. However, this threshold varies greatly between species, and also between genotypes within species. The drawback of these types of models is that the date from which day degrees are accumulated, the threshold temperature and the required heat sum must all be estimated.

Most models work on thermal time and chilling requirement. There are two major hypotheses as to how chilling requirement is connected to the onset of bud development. In **sequential chilling models** (Sarvas 1972; Hänninen 1987, 1990; Hunter and Lechowicz 1992; Kramer 1994a; 1994b; Chuine and Cour 1999; Zhang *et al.* 2007) trees are assumed to have a fixed chilling requirement which must first be completely satisfied before bud development is possible. Once fulfilled, forcing units begin to accumulate, although there is no response to forcing until chilling is fully met. Thus, forcing follows sequentially from chilling. The sequential chilling model is only useful when the chilling requirement has been fully met before forcing units accumulate. Recent work by Harrington *et al.* (2010) shows that this is unlikely to be the case and that the accumulation of chilling and forcing units coincides.

An alternative to the sequential model is the **parallel chilling model** where trees are assumed to respond to increased duration of chilling with a corresponding decrease in the amount of subsequent forcing required for budburst (Landsberg 1974; Hänninen 1990; Kramer 1994b). Above a threshold temperature, the tree acquires forcing units, and below the threshold temperature, it acquires chilling units. As more chilling units accumulate, the required forcing accumulation is reduced. Much research has been carried out on parallel models (Campbell and Sugano 1975; Cannell and Smith 1983; Cannell *et al.* 1985; Murray *et al.* 1989). However, in natural conditions, such models may only be relevant where the chilling requirement is not fully met, as may be the case in milder winters (Cannell and Smith 1986; Murray *et al.* 1989) or when populations are grown beyond their natural ranges.

In all models, forcing units are accumulated for development towards budburst. In sequential models, chilling units are accumulated to break dormancy prior to the accumulation of forcing units. Thermal time models estimate the date of dormancy completion rather than simulating dormancy completion through accumulation of chilling units as in the sequential models and could perhaps be perceived as being less accurate. However, analysis of historical data sets has shown that simple thermal time models are usually most accurate at predicting budburst (Hunter and Lechowicz 1992; Häkkinen *et al.* 1998; Bailey and Harrington 2006).

4.1.4 Evidence of altered phenology and range shifts

Since the timing of budburst is highly sensitive to temperature it is a useful indicator for observing the effects of climate change (Polgar and Primack 2011). Although there can be large variations in the timing of budburst from year to year, in recent decades there has been a strong directional trend to earlier budburst, which many authors attribute to anthropogenic driven global warming (Walther *et al.* 2002; Root *et al.* 2003; Parmesan 2006; Ibanez *et al.* 2010). Global surface temperature has increased by 0.6 °C in the past three decades (Hansen J *et al.* 2006). Data sets from around the world indicate that the timing of spring budburst has advanced in many areas (Fitter and Fitter 2002; Menzel *et al.* 2006). Shifts in timing of plant activity e.g. earlier spring flowering and 'green up' of the land surface either observed directly (Sparks and Carey 1995, Fitter and Fitter 2002; Parmesan and Yohe 2003) or revealed by satellite imagery (Myneni *et al.* 1997), provide some of the

most compelling evidence that species and ecosystems are being affected and influenced by global environmental change.

Global climate change has been responsible for rapid changes in the phenology of temperate and boreal woody plants (Menzel 2000; Parmesan 2006; Primack *et al.* 2009). There are many examples in the literature of advanced spring phenology. Some examples are summarised here.

- Menzel and Fabian (1999) analysed data from 30 years of phenological observations in Europe, and reported that budburst has advanced by six days, and autumn leaf colouring has delayed by 4.8 days, thereby extending the growing period by 10.8 days since the early 1960s. They attributed these shifts to changes in air temperature.
- 2. Menzel (2000) reported that trees in northern Europe have advanced budburst on average by one week over the past 50 years.
- 3. Fitter and Fitter (2002), working on phenology in 385 species of British plants, reported that the average first flowering was 4.5 days earlier in the 1990s than in the previous four decades.
- Parmesan and Yohe (2003) reported that 62 % of 677 species studied showed advancement in spring events including budburst, flowering, and the arrival of migratory birds.
- Menzel *at al.* (2006) assessed over 125,000 observations of phenological events from 21 European countries and found that 78 % of all leafing, flowering and fruiting records were advanced, and only 3 % were delayed over the 30 year period 1971 – 2000, with an average spring advancement of 2.5 days per decade in Europe.

- At Hubbard Brook research forest in North America, *Fagus grandifolia* (American beech) broke bud 5 10 days earlier than in the last five decades (Richardson *et al.* 2006).
- In Japan, woody plants have advanced their budburst by 2 7 days with every 1 °C rise in temperature between 1953 and 2005 and autumn senescence delayed (Ibanez *et al.* 2010).

However, some 'green ups' have been delayed in certain communities. Zhang *et al.* (2007) used global satellite imagery from 1982 – 2005 to show a latitude transition zone of the start of green up in vegetation communities corresponding to warming temperatures in north America. From 40 degrees latitude northwards, the winter chilling requirement is greatly exceeded and the decrease in chilling days by warming temperatures has had no effect on the start of green up. Thus, warming temperatures have constantly advanced green up by 0.32 days per year. However, south of latitude 30 degrees, a milder winter is proving to be insufficient to meet the chilling requirement so that green up is delayed by 0.15 days per year, with a transition zone between 30 and 40 degrees north where vegetation green up onset gradually switches from an advancing trend to a delayed trend.

4.1.5 Implications of altered phenology

There are multiple implications of altered phenology in response to climate change. Some of the main consequences are highlighted below.

Mistimings between species

Ecological mismatches may occur as a result of rapid climate change. For example many tree species are breaking bud earlier, with a corresponding earlier abundance in insects. While many migratory birds arrive early, others arrive late (Miller-Rushing *et al.* 2008) and food resources may not necessarily be available. However, Buse and Good (1996) reported synchrony between budburst in oak (*Quercus robur*) and the emergence of winter moth larvae (*Operophtera brumata*). The winter moth caterpillars form an important part of the diet of blue tit (*Parus caeruleus*) chicks. Tits have evolved so that their breeding season

occurs at the time of maximum availability of caterpillars (Perrins 1991). Earlier budburst in the oak has resulted in the earlier appearance of winter moth larvae and the corresponding earlier nesting of tits. Sparks and Gill (2002) also reported synchrony between the first appearance of orange tip butterflies (*Anthocharis cardamines*) and the first flowering of garlic mustard (*Alliaria petiolata*) on which the butterflies lay their eggs.

Increased likelihood of frost damage

It has been widely observed that if trees break bud earlier, they are more likely to suffer frost damage (Cannell and Smith 1986). This was the case in North America in 2007, when an abnormally warm March triggered early budburst. Subsequent sub-zero temperatures in April killed the new foliage which led to a delay of 16 - 31 days before full leaf expansion was reached in a number of species (Augspurger 2009). A similar pattern was observed in the UK by the author in April 2010, when frosts were so severe that all foliage was killed on *Fraxinus excelsior* trees already in leaf in trials containing provenances ranging from Inverness in Scotland to the Pyrenees in the south of France. Trees just breaking bud suffered much less than those in which leaves were elongating. It is predicted that late spring frost damage will become more common as climate warming continues (Linkosalo 2000).

Increase in yield

Phenology determines the length of period over which photosynthesis can occur. Therefore, with earlier budburst, yield classes are predicted to increase for many of the UK's forest tree species under some climate change scenarios (Broadmeadow *et al.* 2005). Using the UKCIP02 climate change scenario for 2050 (Hulme *et al.* 2002), most species, with the notable exception of *Fagus sylvatica*, are likely to increase in yield under the low emissions scenario, as a result of earlier budburst and higher concentrations of carbon dioxide. Under the 2050 high emissions scenario, most species are projected to decrease in yield as water availability becomes limiting (Broadmeadow *et al.* 2005). However, because photoperiod is often the main trigger for shoot and bud growth cessation, trees may fail to exploit the extended growing season as warming continues (Saxe *et al.* 2001).

Changes in abundance and distribution

In Europe, the average growing season for many tree species has increased by 11 days (Menzel and Fabian 1999). If species with minimal photoperiod and chilling requirements continue to break bud early, they may increase their abundance and become the dominant species in their communities. However, unmet chilling and photoperiod requirements of other species may significantly slow the advance of budburst at the whole forest level (Morin *et al.* 2009).

Economic implications

Changes in the timing of budburst alter the length of the growing period, carbon sequestration, plant-animal interactions and other essential ecosystem processes which are likely to have a subsequent economic effect in crop species such as apples and pears where loss of flowers from frost can result in a lower crop for that year (Polgar and Primack 2011). There will likely be a benefit to timber growers, as least in the short term, though increased timber yields in some species due to additional carbon sequestration (Broadmeadow *et al.* 2005).

4.2 Introduction

The effect of warming temperatures on biological processes has been well documented (e.g. Rotzer and Chmielewski 2001; Parmesan and Yohe 2003; Badeck *et al.* 2004). While year to year variations in weather (e.g. warm vs. cool springs) are expected, and generally result in corresponding shifts in the timing of phenological events (Hunter and Lechowicz 1992), unidirectional phenological responses are being reported such as advanced budburst or flowering in plants (Menzel and Fabian 1999; Menzel *et al.* 2006) and migration and reproduction in animals (Sparks 1999; Sparks and Carey 1995).

The trade-off between early budburst and the possibility of frost damage is an important consideration where timber is a desired product or when considering which provenances to plant in a warming climate. For species such as *Fraxinus excelsior*, where the terminal bud is flanked by laterals, frost damage of the terminal bud usually results in a fork. As *Fraxinus excelsior* is capable of producing long straight stems that are valuable for furniture and veneer, forking as a result of frost damage can substantially reduce the value of the crop (Kerr 1995).

Provenance trials are often used to identify the best adapted seed sources for commercial use (Fletcher 1991; Konig 2005). They can also provide information regarding seed transfer guidelines for aforestation and reforestation purposes and to determine the responses of populations when transferring them to different climates (Schmidtling 1994). Generally, if provenances are moved northwards a modest distance they outperform the local provenance. However, if moved too far north, they suffer from cold damage. If moved south, they often do not perform as well as the local provenance (Wells and Wakeley 1966).

Phenological traits usually show a very high level of heritability and genetic variation (Franks *et al.* 2007; Volis 2007) and thus have the potential to evolve rapidly under strong selection pressures (Chuine 2010). While originally intended for the purposes described immediately above, provenance trials can provide valuable data for assessing the response of populations to environmental change. Provenances are assumed to have adapted to their local climate and therefore, when planted in provenance trials, show variation in time of
budburst (Chabot and Hicks 1982). Climate predications for Britain indicate a 3 - 5 °C rise in temperature by 2080 (Murphy *et al.* 2009), thereby potentially increasing the growing period, but late spring frosts are still likely to occur (Broadmeadow *et al.* 2005). Given the important influence of temperature on the phenology of trees, it is likely that climatic warming will affect the timing of the onset and cessation of growth, causing species to become less well adapted to their local environment (Kramer 1995). However, if trees are plastic in their phenotypic response to temperature, they may accommodate a temperature rise brought about by climate change.

While chilling and heat sum requirements have been studied for many species, particularly commercial conifers, the environmental control of budburst in *Fraxinus excelsior* is not understood. Several provenance trials of *Fraxinus excelsior* exist in Britain, planted by Forest Research in the early 1990s. They are used here to examine differences in the timing of budburst across several years and two sites. The effect of photoperiod is not considered in these investigations as several provenances in the trials are from the same latitude, and still display variation in budburst.

Earlier observations have shown that in the same provenance trial, some provenances break bud earlier than others. Trials at two sites were therefore assessed to determine variation in the time of budburst of various provenances across sites and years. To understand the driving forces behind these differences the chilling requirement for budburst of different provenances of *Fraxinus excelsior* was determined under controlled conditions. To understand the response of *Fraxinus excelsior* provenances to spring temperatures, the following questions were asked:

- 1. Do provenances break bud at the same or different times in a common garden experiment?
- 2. Does budburst of provenances vary between sites and between years?
- 3. What are the chilling and forcing requirements of provenances for budburst?

4.3 Methods

4.3.1 Study sites and provenances

In 1993, Forest Research established four *Fraxinus excelsior* provenance trials in England and Wales which were augmented by a further two trials planted in 1995 (Cundall *et al.* 2003). The trials comprise 22 provenances of *Fraxinus excelsior* from across its native range. Two of these trials were used in the current research to investigate the timing of budburst in *Fraxinus excelsior*: Ebberston, North Yorkshire (planted 1993), where 11 provenances are represented and Little Wittenham, Oxfordshire (planted 1995), where 15 provenances are included. Six provenances are common to both trials (Table 4.1).

The Little Wittenham site, situated in the floodplain of the River Thames, is 51 m a.s.l., with sandy clay loam soil derived from alluvial gravels. This site was formerly arable. The Ebberston site is situated in the floodplain of the River Derwent, 22 m a.s.l. The soil is described as an acid clay loam with poor fertility (National Soil Research Institute 2012). The previous land use at this site was grassland.

Maximum and minimum air temperature data were obtained from the British Atmospheric Data Centre (BADC). The closest weather station to Little Wittenham is Benson, located approximately 5 miles east (51.37 °N; 1.05 °W; 60 m a.s.l.). The closest station to Ebberston that is at a similar altitude is Topcliffe, North Yorkshire (54.10 °N; 1.23 °W; 27 m a.s.l.), approximately 38 miles west of Ebberston. A weather station on site at Little Wittenham provided incomplete records. However, correlations between available data from this station and those from Benson were very high (max temp r = 0.993; min temp r = 0.967) so it was deemed acceptable to use the Benson weather station data. No data were available to determine how well the Topcliffe data reflected the weather at Ebberston.

Table 4.1Site details of Little Wittenham and Ebberston Fraxinus excelsior provenance
trials, and provenances included at each site.

	Latitude	Longitude	Country	Little Wittenham	Ebberston
Trial Site					
Ebberston	54.22° N	0.62° W	England		
Little Wittenham	51.64° N	1.20° W	England		
Provenance					
Newton, Morayshire	57.44° N	0.84° W	Scotland	\checkmark	
Pallinsburn, Northumberland	55.38° N	2.10° W	England	\checkmark	
Settrington, Yorkshire	54.06° N	0.45° W	England	\checkmark	
Grimsthorpe, Lincolnshire	52.48° N	0.27° W	England	\checkmark	\checkmark
Bacheiddon, Powys	52.32° N	3.41° W	Wales	\checkmark	
Shropshire	52.30° N	2.40° W	England		\checkmark
Brockhampton, Worcestershire	52.11° N	2.29° W	England	\checkmark	
Glos, Hants and Surrey ¹	51.50° N	0-2.50° W	England	\checkmark	
Curdleigh, Somerset	51.00° N	3.05° W	England	\checkmark	
Friston, Sussex	50.48° N	0.10° E	England	\checkmark	
Palobe ¹	50.05° N	14.25° E	Czech Republic	\checkmark	\checkmark
Picardie	50.00° N	3.00° E	France		\checkmark
Czech Republic Unknown ¹	50.00° N	14.00° E	Czech Republic		\checkmark
Champagne	49.30° N	4.40° E	France	\checkmark	\checkmark
Upper Rhine ¹	49.00° N	8.00° E	Germany	\checkmark	\checkmark
Basse Normandie	48.30° N	0.30° E	France	\checkmark	\checkmark
Alsace	48.30° N	7.30° E	France		\checkmark
Haute Saône	47.40° N	7.00° E	France	\checkmark	\checkmark
Romania, Unknown ¹	47.00° N	23.00° E	Romania	\checkmark	
Yugoslavia, Unknown ¹	46.00° N	14.00° E	Yugoslavia		\checkmark

¹ For some provenances (Upper Rhine, Czech Republic, Yugoslavia and Romania) exact locations of seed collection were not available. Latitude and longitude have been estimated to the best of the information available. Palobe (Czech Republic) does not appear to exist but is sufficiently close in name to Polabi, which is north of Prague, and is an extensive area of *Fraxinus excelsior* woodland. The provenance Glos/Hants/Surrey was a bulk seedlot collected from across these counties.

4.3.2 Budburst in provenance trials

Both trials use a randomised complete block design with three blocks. Trees were planted at 2 x 2 m spacing and each trial is surrounded by a double guard row. The Little Wittenham trial comprises 100 tree plots (10 x 10) and the trial at Ebberston comprises 36 tree plots (6 x 6). Most detailed observations were undertaken on the trial at Little Wittenham.

At Little Wittenham, a central 25 trees in each plot were scored for budburst, and at Ebberston the central 16 trees in each plot were scored. The same trees were scored each year. Budburst was recorded at Little Wittenham annually from 2007 - 2010 and at Ebberston during 2009 and 2010 using a six-point scale from buds black and fully closed (0) to fully open with leaflets fully expanded (5) (Figure 4.1). Assessments were carried out at seven-day intervals in 2007 and 2008 and at 14-day intervals in 2009 and 2010. The progression of budburst was monitored until all trees had reached the final score of 5 in 2007 and 2008. However, only three assessments were made in 2009 and 2010 at the end of which several provenances had not completed budburst. As assessment intervals were 14 days apart, a fourth assessment was not made as all provenances would have reached a final score of 5 by then. Due to other field work commitments, it was not possible to do an intermediate assessment.

It is somewhat arbitrary as to which buds on the tree should be used to score budburst. Rarely does the apical bud come in to leaf first although in general, terminal buds open before lateral buds. In the present study, each tree was visually assessed as a whole, looking only at terminal buds. The score was attributed to the most advanced buds (usually around the midpoint of the tree), although often the apical bud would still be dormant. The intervals between budburst stages are not absolute. To minimise subjectivity, scoring was done by the same researcher in any one year.



Score 1

Score 2

Score 3

Score 4

Score 5

- Score 0 = twig is dormant and the bud is black
- Score 1 = bud is green and beginning to swell
- Score 2 = leaves begin to emerge from the bud
- Score 3 = leaves begin to open and petioles are visible
- Score 4 = leaflets open and petioles begin to extend
- Score 5 = leaves fully open and expanded

Figure 4.1 Scale used for scoring budburst in *Fraxinus excelsior*.

Data analyses

As budburst was scored on different dates at the two sites, the Julian day (JD) on which each provenance reached a particular score for budburst was determined by linearly interpolating between observation points. The mean budburst score of each provenance in each block was calculated for each assessment date and converted to the Julian day (JD) that a provenance reached budburst scores 1, 3 and 5, using the following formulae (e.g. for score 3):

1.	(4 th budburst score – 3 rd budburst score)/7	= mean budburst per day	= D
2.	(3 – 3 rd budburst score)/D	= additional days to reach score 3	= E
3.	Julian day of 3 rd budburst score + E	= Julian day to reach score 3.	

The example given is for Palobe block 1 in 2007.

Julian day	103	110	117	124	131	138
Mean budburst score	0.12	0.60	1.88	3.28	4.80	4.96

1.	(3.28-1.88)/7 = 0.2	= mean budburst per day	= D
2.	(3-1.88)/0.2 = 5.6	= additional days to reach score 3	= E
3.	117 + 5.6 = 122.6	= Julian day 122.6 to reach score 3	

The same method was used to calculate the Julian day on which plots reached budburst scores 1 and 5 to give the number of days over which budburst occurred (full data are given in Appendix 3). This enabled analysis of variance of Julian day data to determine the significance of difference between years and sites.

Figure 4.2 illustrates the validity of this approach. The data in a) show the sigmoidal progression of budburst. The data in b) are smoothed out as expected given that the calculation is based on a linear progression of budburst. The provenance performance and progression are similar using mean scores and Julian days, with the first and last provenances to break bud clearly distinct from the other provenances.



b)



Figure 4.2 a) Mean budburst score of fifteen provenances of *Fraxinus excelsior* at Little Wittenham, recorded at seven day intervals in 2007, and b) Julian day that each budburst score is reached, based on the calculated progression of budburst.

The daily mean temperature for each site was calculated as (max temp + min temp)/2 and used to calculate chilling units and temperatures sums. Accumulated day degrees were calculated using daily mean temperature above 4, 5 and 6 °C from the 1st of January and the 1st of February until the start of budburst. Chilling units were calculated as accumulated day degrees below 5 °C from the 1st November.

Statistical analyses

The calculated Julian day to reach score 3 data were used in statistical analyses as the most accurate data describing differences between provenances compared to score 1 or score 5 data through direct observation of the actual data. Analysis of variance was carried out in Genstat (11th edition, VSN International) firstly for those provenances common to both sites in 2009 and 2010, and then for each site separately. The Bonferroni multiple range test was used to test for differences between provenances and years.

Provenance performance was ranked at each site for each year, based on the Julian day on which each provenance reached a budburst score of 3. Pearson rank correlation coefficients were calculated to test the null hypothesis that any similarities in ranking of provenances in different years arose by chance.

Simple correlation coefficients were calculated for thermal time requirement (day degrees above base temperatures) as accumulated day degrees above base temperatures of 4, 5 and 6 °C from the 1st January and 1st February, chilling requirement (days below 5 °C from 1st November before the start of budburst) and mean spring temperature (daily mean from 1st January to 31st May) against the start of budburst for several provenances (Newton, Bacheiddon, Champagne and Romania) at Little Wittenham to ascertain the best model for temperature regulation of budburst. These provenances represent the broadest range of latitude.

4.3.3 Budburst of detached twigs

Twelve dormant twigs from each of 30 trees (ten in each of three blocks) were removed from each of six provenances (2160 twigs in total) in the Little Wittenham trial on 24th October 2008. Twigs were approximately 20 - 30 cm long, with at least one healthy terminal bud flanked by two laterals buds. The provenances were selected to maximise the geographic spread represented within the trial. These were (north to south): Pallinsburn, Settrington, Glos/Hants/Surrey, Basse Normandie, Haute Saône and Romania. Material removed was one to two years old. The cut ends were wrapped in moist paper towels and the twigs placed in plastic bags in a cold store at 4 °C at Alice Holt Research Station, Hampshire. The 24th October was selected as a date before which chilling temperatures would not have already started to accumulate, as few days below 4 °C occur in southern Britain before this date.

Every two weeks, 30 twigs from each provenance were removed and placed in glass jars filled with tap water in a controlled environment room at Alice Holt providing 16 hours light at 15 °C and 8 hours dark at 10 °C. Light was supplied by eight Grolux fluorescent tubes (Sylvania: F58W/GRO-T8). The glass jars were arranged in blocks as in the field, to take account of any variability in the controlled environment room. Hourly temperature was recorded with a Tinytag data logger (Explorer Version 4.6, Gemini Data Loggers Ltd). Previous work by Cannell *et al.* (1985) and Hänninen *et al.* (1990) had shown that a continuous warm temperature was not as effective in forcing buds to open as the same thermal time provided by a fluctuating temperature regime and so an alternating 10/15 °C temperature was used.

Although twigs were initially selected at random, towards the end of the experiment many were showing signs of desiccation, and so twigs were preferentially selected based on the appearance of the terminal bud and plumpness of the twig itself. The final two twigs of each provenance were not used due to poor long term survival in the cold store. The ten chilling treatments were therefore 3, 17, 31, 45, 59, 73, 87, 101, 115, and 129 days of chilling.

Twigs were assessed every two weeks for bud burst using the scoring system described above (Figure 4.1). The terminal bud was almost always the first to flush, and was the assessed bud. In a few cases, the terminal bud did not flush, and lateral buds were scored instead. Once twigs had reached a budburst score of 5 they were discarded.

Statistical analyses

Many twigs from the last chilling treatment of 129 days chilling failed to break bud even after 70 days of forcing temperatures. This was most likely due to the length of time twigs had spent in the cold store resulting in desiccation and death. This treatment was therefore removed from the analyses.

The percentage of twigs that broke bud was calculated, regardless of budburst stage. Analyses of variance were carried out to determine the effect of the number of chilling and forcing days using a generalised linear model and also repeated measures analysis in Genstat (v11 VSN International). There were no changes in the significance levels, and only results of the GLM are shown here. Regression analyses were carried out on a) all data and b) each individual provenance as the percentage budburst against accumulated chilling and forcing temperatures. Regression analyses were carried out in Minitab (version 15).

4.4 Results

4.4.1 Provenance trials

It should be noted that the earliest (Romania) and latest (Bacheiddon) provenances to break bud at Little Wittenham are absent from Ebberston, and that the earliest (Alsace) and latest (Shropshire) provenance to break bud at Ebberston are absent from Little Wittenham. This left six intermediate provenances for assessment of across site differences.

a) Common provenances to both sites

Time of budburst was significantly different between provenances (p<0.001), years (p<0.001) and sites (p<0.001) (Table 4.2). Analysis of variance indicated that there was no block effect at either site in any year. Basse Normandie in northern France was the first provenance to break bud, 5.0 days earlier than the last, Palobe from the Czech Republic (Table 4.3). Overall mean budburst in 2009 was on Julian day (JD) 125.0 (s.e 0.50) and in 2010 it was JD 127.9 (s.e 0.64), 2.9 days later than in 2009. Overall mean budburst at Little Wittenham was on JD 125.3 (s.e. 1.14), and at Ebberston it was JD 127.7 (s.e. 0.49), 2.4 days later than at Little Wittenham.

Table 4.2Results of analysis of variance of six provenances of *Fraxinus excelsior* at two sites
(Little Wittenham and Ebberston) in 2009 and 2010, using the mean Julian day on
which each provenance reached a budburst score of 3.

Source of variation	d.f	s.s.	m.s	v.r	F pr.
Provenance	5	232.281	46.456	8.15	<.001
Year	1	151.859	151.859	26.64	<.001
Site	1	93.010	93.010	16.31	<.001
Provenance x year	5	30.287	6.057	1.06	0.393
Provenance x site	5	116.822	23.364	4.10	0.004
Year x site	1	58.006	58.006	10.17	0.003
Provenance x year x site	5	11.298	2.260	0.40	0.849
Residual	48	273.662	5.701		
Total	71	967.225			

Table 4.3Mean Julian day to reach a mean budburst score of 3 of six provenances of
Fraxinus excelsior common to two sites in 2009 and 2010. Means joined by lines
are not significantly different at p<0.05 (Bonferroni multiple range test).</th>

Provenance	Latitude (°N)	Date of budburst
Basse Normandie	48.30	124.3
Haute Saône	47.40	124.5
Grimsthorpe	52.48	125.7
Champagne	49.30	127.3
Upper Rhine	49.00	127.7
Palobe	50.05	129.3

In 2009, budburst commenced four days earlier at Little Wittenham than at Ebberston (Table 4.4), but in 2010, budburst was delayed at both sites and the difference between sites was less than a day. In 2010 some provenances (Champagne, Upper Rhine and Palobe) actually broke bud later at Little Wittenham than at Ebberston. Mean budburst of all provenances was almost five days later in 2010 than in 2009 at Little Wittenham, whereas at Ebberston, budburst was only just over a day later in 2010 than in 2009.

Table 4.4 Mean Julian day and standard error to reach a mean budburst score of 3 of six provenances of *Fraxinus excelsior* at two sites and the mean number of days that provenances broke bud later at Ebberston than at Little Wittenham. Provenances are ranked south to north.

Provenance	Year	Little <i>s.e</i> Rank Ebbe Wittenham		Ebberston	s.e	Rank	Days later	
Haute Saône	2009	120.5	0.65	1	125.1	0.06	1	4.6
Basse Normandie	2009	122.1	0.72	3	125.4	0.61	2	3.3
Upper Rhine	2009	123.5	0.15	4	127.6	0.19	4	4.1
Champagne	2009	123.7	1.89	5	127.3	0.55	3	3.6
Palobe	2009	126.9	1.19	6	127.9	1.80	5	1.0
Grimsthorpe	2009	121.2	0.95	2	129.1	0.63	6	7.9
Site Mean	2009	123.0			127.1			4.1
Haute Saône	2010	124.4	0.79	2	128.1	1.96	2	3.7
Basse Normandie	2010	124.7	2.53	3	125.1	1.46	1	0.4
Upper Rhine	2010	130.5	1.19	5	129.3	0.38	5	-1.2
Champagne	2010	129.3	2.30	4	128.9	3.19	3	-0.4
Palobe	2010	133.5	3.39	6	128.9	2.96	4	-4.6
Grimsthorpe	2010	123.7	0.66	1	129.9	3.31	6	6.2
Site Mean	2010	127.7			128.4			0.7

The provenance x year interaction was not significant, indicating that the provenances were ranked in similar order in both years. However, provenance x site and year x site interactions were significant indicating that the provenances were ranked differently at the two sites and that differences between sites were not consistent across years (Table 4.4). The provenance Grimsthorpe broke bud much earlier than expected at Little Wittenham

given its latitude as the most northerly provenance. At Little Wittenham, it ranked second in 2009 and first in 2010, whereas it ranked last at Ebberston in 2009 and 2010 (Table 4.4). When Grimsthorpe was excluded from the analysis, the provenance x site interaction was no longer significant (p = 0.105). Pearson correlation for budburst of provenances between years was 0.8414 and for budburst of provenances between sites was 0.4394. When Grimsthorpe was removed from the analysis, the correlation for budburst of provenances between sites increased to 0.817 indicating that provenance ranks for budburst are similar between years, and with the removal of Grimsthorpe from the analysis, also similar between sites.

As there was a significant provenance x site interaction, and there were different numbers of provenances at the sites, the sites were further analysed independently.

b) Little Wittenham

The analysis of variance for Little Wittenham for 2007 - 2010 showed significant differences among provenances and among years, and a significant provenance x year interaction (Table 4.5). Romania was the first provenance to break bud (JD 114.2) and Bacheiddon the last (JD 133.3). Mean budburst data with standard deviations are given for all provenances at both sites for all years in Appendix 4. Grimsthorpe was again out of expected sequence, breaking bud second on JD 119.3 (Table 4.6). Mean time for completion of budburst ranged from 20.5 days for provenances from Romania and Upper Rhine to over 26 days for provenances from Haute Saône, Friston, Newton and Bacheiddon (Table 4.6).

Table 4.5 Results of analysis of variance of the Julian day on which 15 provenances of *Fraxinus excelsior* reached a budburst score of 3 at Little Wittenham in four years (2007 - 2010).

Source of variation	d.f.	<i>S.S.</i>	m.s.	v.r. F pr.
Provenance Year Provenance x year Residual Total	14 3 42 120 179	3942.642 2764.256 471.969 638.515 7817.382	281.617 921.419 11.237 5.321	52.93 <.001 173.17 <.001 2.11 <.001

Table 4.6 Mean Julian day for 15 provenances of *Fraxinus excelsior* at Little Wittenham to reach a budburst score of 3 in 2007 - 2010 and duration of budburst (from score 1 to 5). Means joined by lines are not significantly different at p<0.005 (Bonferroni multiple range test).

Provenance	Latitude (°N)	Date of budburst	Duration of budburst
Romania	47.00	114.2	20.5
Grimsthorpe	52.48	119.3	23.2
Haute Saone	47.40	119.8	26.3
Basse Normandie	48.30	120.1	23.0
Curdleigh	51.00	121.0	24.4
Champagne	49.30	122.1	23.3
Upper Rhine	49.00	123.0	20.5
Friston	50.48	124.8	26.6
Palobe	50.05	125.2	25.6
Glos/Hants/Surrey	51.50	125.6	25.7
Settrington	54.06	125.8	25.1
Brockhampton	52.11	125.9	22.0
Pallinsburn	55.38	129.5	24.3
Newton	57.44	130.0	26.0
Bacheiddon	52.32	133.3	26.4

Looking at years individually (Table 4.7), difference among provenances in the timing of budburst were highly significant (p<0.001) within a year. Romania was always the first provenance to break bud in any year (Table 4.7). This was earliest in 2007 (JD 109.1) and latest in 2010 (JD 120.3), 11 days later. Similarly, Bacheiddon was always the last provenance to break bud, with budburst occurring earliest in 2008 (JD 126.4) and latest in 2010 (JD 144.6), 18.2 days later (Table 4.7). The time that provenances took to complete budburst (the number of days to go from a budburst score of 1 to 5) were not significantly different except in 2010, with budbreak of all provenances completing in 18.8 days on average in 2008, and taking almost twice as long (30.8 days) in 2010.

Table 4.7 Mean Julian day on which each provenance reached a budburst score of 3 and the days taken for budburst to progress from stages 1 to 5 at Little Wittenham in each year from 2007 - 2010. Data are presented in rank order by Julian date for budburst in 2007. Means joined by lines are not significantly different at p<0.05 (Bonferroni multiple range test).

200	7		200	8		2009	9		201	.0	
Provenance	Days 1-5	Julian Day	Provenance	Days 1-5	Julian Day	Provenance	Days 1-5	Julian Day	Provenance	Days 1-5	Julian Day
Romania Grimsthorpe Basse Normandie Haute Saône Curdloigh	21.0 23.8 22.1 30.4 25.9	109.1 115.1 115.6 116.3	Romania Grimsthorpe Curdleigh Basse Normandie	18.4 17.8 18.3 19.2	114.4 117.8 118.2 118.4	Romania Haute Saône Grimsthorpe Basse Normandie	19.5 25.2 24.9 23.6	113.1 120.5 121.2 122.1	Romania Grimsthorpe Haute Saône Curdleigh Rasso Normandia	23.0 26.5 29.0 31.0	120.3 123.0 123.6 123.6 123.6
Champagne Upper Rhine Friston Palobe Glos/Hants/Surrey Brockhampton Settrington Pallinsburn	23.8 19.9 30.7 25.9 26.1 26.7 23.2 24.0	117.3 119.6 120.6 121.0 121.1 122.2 124.5 126.4	Haute Saône Upper Rhine Friston Palobe Glos/Hants/Surrey Brockhampton Settrington Pallinsburn	20.6 16.0 19.9 21.2 20.4 20.5 18.7 18.7	118.9 119.3 120.0 120.4 120.4 121.5 123.3 124.8	Champagne Curdleigh Palobe Glos/Hants/Surrey Settrington Friston Brockhampton Newton	23.7 22.2 22.5 22.5 18.1 23.1 22.4 22.4	123.7 125.0 126.9 127.2 127.3 128.3 128.5 131.2	Settrington Champagne Upper Rhine Friston Brockhampton Palobe Glos/Hants/Surrey Newton	27.9 27.7 26.6 32.7 30.9 32.8 34.0 37.2	128.2 127.4 129.7 130.3 131.2 132.5 133.7 135.5
Newton Bacheiddon Mean p value	26.4 23.2 24.9 0.050	127.4 128.5 120.1 <0.001	Newton Bacheiddon	17.9 16.8 18.8 0.095	126.0 126.4 120.6 <0.001	Pallinsburn Bacheiddon	21.7 24.7 22.4 0.039	131.3 133.6 125.6 <0.001	Pallinsburn Bacheiddon	32.6 41.0 30.8 <0.001	135.7 144.6 129.7 <0.001

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While there was a significant provenance by year interaction in budburst (Table 4.5), this was due to a small number of provenances, with ranking of many provenances fairly consistent across years (Table 4.7). Years were significantly different from each other. Budburst clearly occurred earlier in 2007 and 2008 with budburst occurring approximately five days later in 2009 and nine days later in 2010 (Table 4.8). Pearson correlation between years 2007 - 2010 ranged from 0.87 between 2008 and 2010 to 0.98 between 2007 and 2008 (Table 4.9) indicating that provenance ranks between years are very similar.

Table 4.8 Mean Julian day that 15 provenance of *Fraxinus excelsior* reached a budburst score of 3 at Little Wittenham in four years. Means joined by lines are not significantly different at p<0.005 (Bonferroni multiple range test).

Date of budburst					
120.1 120.6 125.6					

Table 4.9Pearson correlation coefficients of the Julian day on which 15 provenances of
Fraxinus excelsior reached a budburst score of 3 between years (2007 - 2010) at
Little Wittenham.

	2007	2008	2009
2007 2008 2009 2010	0.98 0.96 0.89	0.91 0.87	0.88

c) Ebberston

Only three observations were made at Ebberston in 2009 and 2010. Analysis of variance showed significant differences for budburst among provenances and between years although there was no provenance x year interaction (Table 4.10).

Source of variation	d.f	m.s	m.s	v.r	F pr
Provenance Year Provenance x year Residual Total	10 1 10 44 65	949.629 39.305 34.614 210.563 1234.112	94.963 39.305 3.461 4.786	19.84 8.21 0.76	<0.001 0.006 0.698

Table 4.10 Results of analysis of variance of the Julian day on which 11 provenances ofFraxinus excelsior reached a budburst score of 3 at Ebberston (2009 - 2010).

The provenances from Alsace and Picardie were the first to break bud with very small differences in timings. Alsace broke bud just before Picardie in 2009 and vice versa in 2010 (Table 4.11). Shropshire was always the last provenance to break bud by a substantial margin; 12 days later than the first provenance in 2009, and 16 days later in 2010 (Table 4.11). Grimsthorpe came into leaf as would be expected in terms of its origin. Across all provenances, budburst occurred 1.5 days later in 2010 than in 2009 at Ebberston.

Spring frosts occurred in 2010. These were moderate (lowest temperature -3.1 °C) at Little Wittenham and occurred over several nights between 4th and 13th May and although light frost damaged was observed evidenced by some browning of the leaves, terminal buds remained undamaged (Figure 4.3a). Heavy frosts (-4.7 °C) occurred at Ebberston from 11th to 13th May between the second and third assessment dates and most provenances experienced 100 % mortality of new shoots (Figure 4.3b). Calculation of the Julian day on which provenances reached a score of 3 at Ebberston in 2010 used the day on which frost damage occurred (day 131) as the progression of bud burst was halted at this point. This meant that only eight days progression between assessments was assumed, rather than the actual 14 days.



b)

a)



Figure 4.3 a) Frost damage to shoots of *Fraxinus excelsior* at Little Wittenham, picture taken on 29th May 2010, two weeks after the frost. The growing tip was not damaged.
b) Frost damage at Ebberston, picture taken on 17th May 2010. The foliage was killed, however, it was still possible to estimate the bud burst stage (this is a 5) at the time of frost.

	2009		2	2010					
Provenance	Days 1-5	Julian Day	Provenance	Days 1-5	Julian Day				
Alsace	24.7	123.0	Picardie	23.5	124.3				
Picardie	23.5	123.2	Alsace	23.2	124.4				
Yugoslavia	22.4	124.6	Yugoslavia	21.4	124.9				
Haute Saône	23.4	125.1	Basse Normandie	21.1	125.1				
Basse Normandie	24.0	125.4	Haute Saône	25.2	128.1				
Champagne	23.5	127.3	Palobe	20.2	128.9				
Upper Rhine	21.9	127.5	Champagne	25.1	128.9				
Palobe	23.6	127.9	Upper Rhine	20.5	129.2				
Czech Republic	24.3	128.5	Czech Republic	25.0	129.6				
Grimsthorpe	25.2	129.1	Grimsthorpe	22.9	129.9				
Shropshire	26.9	135.3	Shropshire	29.4	140.6				
Means	24.0	127.0		23.4	128.5				

Table 4.11 Mean Julian day on which each provenance reached a budburst score of 3 and the days taken for budburst to progress from stages 1 to 5 at Ebberston in 2009 and 2010. Means joined by lines are not significantly different at p<0.05 (Bonferroni multiple range test).

d) Correlations with environmental triggers

Budburst was highly correlated with both the degree of winter chilling (positive correlation: with greater chilling accumulation, budburst advanced) and also with mean spring temperatures (negative correlation: with lower spring temperatures, budburst was delayed) (Table 4.12). Using the Julian day that provenances reached a score of 1 as the indicator for the start of budburst, correlation coefficients of budburst with chilling units ranged from 0.34 for Romania to 0.93 for Champagne. Using the midpoint of budburst (Julian day to reach a score of 3), correlations with chilling units were much higher, ranging from 0.82 for Romania to 0.98 for Champagne. Correlations between Julian day to reach score 1 and spring average temperature were also high, -0.43 for Romania to -0.99 for Champagne, and with score 3 data, increased to -0.90 for Romania and to -0.99 for Champagne. However, correlations of these temperature cues with budburst were not as high on the UK provenances of Bacheiddon and Newton (Table 4.12).

Correlations between accumulated day degrees above a threshold temperature from 1st January and budburst were good for provenances from Romania and Champagne, and the correlation with day degrees above 4 °C was the best fit (-0.91 for Romania, score 3 and - 0.86 for Champagne, score 1). However, correlation with UK provenances Bacheiddon and Newton were poor. Calculating day degrees above 5 °C from the 1st February provided a better fit for these provenances (0.51 for Bacheiddon and 0.45 for Newton).

Day degrees accumulated above base temperatures from 1st January and 1st February until the relevant budburst score are given in Table 4.13 for years 2007 - 2010 at Little Wittenham, with annual mean spring temperature and accumulated chilling units from 1st November.

The time between the first and last provenance to reach a score of 3 ranged from 12 days in 2008 to 24.3 days in 2010 at Little Wittenham (Table 4.13). In terms of day degrees, Romania accumulated 226 day degrees over a base temperature of 5 °C in 2007 and Bacheiddon accumulated of 343 day degrees. In 2010, these provenances accumulated 129 and 203 day degrees respectively. With the large number of day degrees accumulated in 2007, budburst occurred early compared to 2010 which was much colder between January and May.

Table 4.12 Correlation coefficients of budburst for four provenances of *Fraxinus excelsior* at Little Wittenham between 2007 - 2010, with chilling (accumulated day degrees below 5 °C from 1st November) and with accumulated day degrees above 4, 5 and 6 °C, calculated from the 1st January and 1st February, until the start of budburst (score 1) and mid-way through budburst (score 3).

	Romania		Champagne		Bacheiddon		Newton	
	Score 1	Score 3	Score 1	Score 3	Score 1	Score 3	Score 1	Score 3
Chilling	0.34	0.83	0.94	0.98	0.70	0.87	0.80	0.90
Spring mean temperature From 1 st January	-0.43	-0.90	-0.99	-0.99	-0.71	-0.93	-0.75	-0.94
Day degrees above 4 °C	-0.60	-0.91	-0.86	-0.67	-0.06	-0.20	-0.20	-0.61
Day degrees above 5 °C	-0.61	-0.89	-0.83	-0.63	0.00	-0.12	-0.13	-0.50
Day degrees above 6 °C From 1 st February	-0.60	-0.82	-0.78	-0.62	-0.03	-0.08	-0.11	-0.58
Day degrees above 4 °C	-0.97	-0.37	-0.28	-0.03	0.45	0.39	0.37	-0.22
Day degrees above 5 °C	-0.97	-0.18	-0.13	-0.05	0.51	0.44	0.45	-0.22
Day degrees above 6 °C	-0.92	-0.22	-0.19	-0.04	0.47	0.39	0.39	-0.30

Table 4.13 Accumulated day degrees and Julian day of mean budburst that the first and last provenances reach budburst scores 1 and 3 at Little Wittenham, with intermediate provenances Champagne and Newton. Mean spring temperature is from 1st January to 31st May. Chilling units are accumulated below 5 °C from 1st November.

	2007	2008	2009	2010
First provenance to flush – Romania				
Julian day of score 1	102.6	107.9	103.3	107.4
Julian day of score 3	109.1	114.4	113.1	120.3
Accumulated day degrees from 1 st January	226.1	173.4	158.5	129.0
Accumulated day degrees from 1 st February	150.1	113.7	145.6	125.4
Champagne/Champagne				
Julian day of score 1	108.3	110.6	111.9	116.1
Julian day of score 3	117.3	118.5	123.7	127.4
Accumulated day degrees from 1 st January	265.9	182.6	210.5	163.8
Accumulated day degrees from 1 st February	187.1	122.9	195.6	160.3
Last provenance to flush – Bacheiddon				
Julian day of score 1	119.6	116.0	121.3	122.6
Julian day of score 3	128.5	126.4	133.6	144.6
Accumulated day degrees from 1 st January	343.3	213.7	257.1	203.3
Accumulated day degrees from 1 st February	267.3	154.0	242.2	199.7
Newton/Morayshire				
Julian day of score 1	117.3	115.5	120.0	119.8
Julian day of score 3	127.4	126.0	131.2	135.5
Accumulated day degrees from 1 st January	327.3	207.2	249.0	190.4
Accumulated day degrees from 1 st February	251.3	147.5	234.1	186.9
Days between first and last provenance to reach a score of 3	19.4	12.0	20.5	24.3
Mean temperature 1 st January – 31 st May	8.4	7.8	6.9	5.8
Chilling units below 5 °C from 1 st November	110	171	279	327

4.4.2 Detached twigs

The analysis of variance showed that provenance, days in the cold store (chilling) and subsequent days in the controlled environment room (forcing) were highly significant, as were all interactions (Table 4.14).

Source of variation	d.f.	m.s.	<i>v.r.</i>	F pr.
Provenance	5	7436.2	54.54	<.001
Chilling	6	36073.3	264.59	<.001
Forcing	4	46969.3	344.50	<.001
Provenance x chilling	30	509.6	3.74	<.001
Provenance x forcing	20	717.1	5.26	<.001
Chilling x forcing	24	5053.7	37.07	<.001
Provenance x chilling x forcing	120	245.8	1.80	<.001
Residual	420	136.3		
Total	629			

Table 4.14 Results of analysis of variance of the percentage of twigs of six provenance of *Fraxinus excelsior* that broke bud after seven chilling and five forcing treatments.

Percentage budburst increased and subsequent required forcing decreased with each successive chilling period (Table 4.15). All provenance data are given in Appendix 5. The Romanian provenance had the smallest chilling requirement of those tested, with 59 days chilling followed by 56 days forcing for 50 % of the twigs to break bud. For other provenances, the chilling requirement was much higher. Basse Normandie, Haute Saône and Pallinsburn all reached 50 % budbreak with 87 days chilling, but only when followed by a minimum of 70 days forcing. Chilling for 101 days of these provenances reduced the forcing required from 70 days to 56 days for provenances to break bud (Table 4.15). Glos/Hants/Surrey and Settrington had the highest chilling requirement of 101 days for 50 % of buds to break but then only required 56 days of forcing temperatures. By increasing the chilling to 115 days, forcing was reduced to 42 days. The minimum number of days in the cold store for any provenance to break bud was 31 days for Haute Saône but only after 70 days forcing, and then only one twig broke bud. Figure 4.4 shows the progression of budburst with increased chilling and forcing units, by provenance.

			Days forcing	5	
Days chilling	14	28	42	56	70
31	0	0	0	0	0.6
45	0	0	0	3.3	6.3
59	0	0	4.2	11.1	32.3
73	0	1.1	7.8	19.6	39.2
87	0	2.2	19.6	45.4	62.9
101	0	3.7	37.2	70.8	84.4
116	0	23.8	59.1	89.5	93.7

Table 4.15 The percentage of twigs of six provenances of *Fraxinus excelsior* that broke budafter seven chilling treatments and five forcing treatments.

Table 4.16 shows the results of regression analyses of the amount of chilling and forcing required for budburst under controlled conditions. R^2 values ranged from 54.6 % for the bulked provenance Glos/Hants/Surrey to 70.6 % for the provenance from Romania.

Table 4.16Regression equations of budburst date against days of chilling and forcing
treatments of detached twigs of six provenances of *Fraxinus excelsior*.

Provenance	equation	R ²
All data	Flush = - 62.5 + 0.642 chill + 0.862 Warm	56.9 %
Pallinsburn	Flush = - 63.4 + 0.609 chill + 0.866 warm	57.8 %
Settrington	Flush = - 49.5 + 0.514 chill + 0.613 warm	57.1 %
Glos/Hants/Surrey	Flush = - 52.2 + 0.517 chill + 0.688 warm	54.6 %
Haute Saône	Flush = - 58.9 + 0.603 chill + 0.819 warm	63.6 %
Normandie	Flush = - 67.5 + 0.697 chill + 0.919 Warm	61.8 %
Romania	Flush = - 83.3 + 0.912 chill + 1.27 warm	70.6 %

Table 4.17 details mean monthly data for both trial sites, including accumulated day degrees over a base temperature of 5 °C.







Figure 4.4a The percentage of twigs from a) Pallinsburn b) Settrington c) Glos/Hants/Surrey that break bud after seven chilling treatments (days cold) and five warm treatments (days warm).







Figure 4.4b The percentage of twigs from d) Haute Saône e) Basse Normandie f) Romania that break bud after seven chilling treatments (days cold) and five warm treatments (days warm).

Table 4.17 a) Mean monthly temperature (°C) and (b) day degrees above 5°C based on daily average temperature for Benson, Oxfordshire,2007 - 2010 and Topcliffe, North Yorkshire, 2009 - 2010.

	Benson, Oxfordshire								Topcli	ffe, Yorks	shire	
	a)				b)				a)		b)	
	2007	2008	2009	2010	2007	2008	2009	2010	2009	2010	2009	2010
Jan	6.7	6.6	2.3	0.5	78.0	59.7	14.9	3.6	2.7	0.7	7.8	0.7
Feb	5.8	4.7	3.4	3.4	54.4	25.7	26.2	10.9	3.5	1.9	36.5	0.0
Mar	6.6	6.0	6.6	5.7	61.6	55.9	60.0	60.2	6.4	5.2	60.1	45.9
Apr	10.7	7.9	9.9	8.8	170.8	97.3	148.0	115.8	9.0	8.0	120.6	93.9
May	12.1	13.5	12.3	10.6	221.2	262.4	225.0	174.5	11.2	9.8	193.7	150.5
June	15.4	14.3	14.9	15.6	311.2	280.2	297.3	317.1	14.0	14.6	270.5	284.3
July	15.6	16.4	16.7	18.2	329.0	352.9	362.5	408.1	15.2	16.2	316.1	345.8
Aug	15.5	16.5	17.0	15.8	322.6	355.0	371.0	335.9	10.8	14.6	335.7	297.7
Sept	13.6	13.3	14.1	13.3	256.5	249.5	273.6	249.6	13.5	13.5	256.2	254.0
Oct	10.1	9.0	11.4	10.0	158.9	133.6	199.6	163.9	10.4	9.4	167.7	141.0
Nov	6.4	6.8	8.8	3.2	65.1	73.6	117.8	57.2	7.4	4.2	79.3	40.1
Dec	4.6	3.3	2.9	-0.8	53.3	25.9	19.9	4.2	1.5	-2.7	8.3	0.0
Mean/Total	10.3	9.9	10.0	8.7	2082.3	1971.5	2115.5	1900.8	8.8	8.0	1852.2	1653.6

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4.5 Discussion

The calculations for converting budburst data to Julian day data assumes that budburst occurs in a linear progression. This means that the date that the budburst score reached values of 1 or 5 are less accurate than those for score 3, because trees were only assessed every seven or fourteen days and estimates of the start and end dates are less exact. Thus, the Julian day that provenances reached a budburst score of 3 was used for comparative analysis. Another reason for using score 3 data is that the differences between the provenances are greatest in the middle of the budburst progression (Figure 4.2a).

Budburst in provenance trials

Provenances clearly break bud at different times in the field trials, but also at different times on different sites. The same provenance planted at Ebberston broke bud later than at Little Wittenham and these differences also varied between years. In 2009, mean budburst commenced four days earlier at Little Wittenham than at Ebberston (Table 4.4), but in 2010, budburst was delayed at both sites and the difference between sites was less than half a day. This was because some provenances (Champagne, Upper Rhine and Palobe) actually flushed later at Little Wittenham than at Ebberston which was unexpected. These provenances are the most continental of the provenances common to both sites and would normally be expected to break bud earlier than the maritime provenances from the UK. However, these provenances had started to break bud and then got damaged by frost at Little Wittenham, whereas all provenances were badly damaged by frost at Ebberston. Other provenances at Little Wittenham commenced budburst later and therefore were not as badly damaged.

Most provenances were severely affected by frost at Ebberston in 2010, with almost 100 % mortality of leaves that had already emerged. The exception was the provenance from Shropshire which was last to break bud at Ebberston. Here, budburst had only reached an average score of 1.7 (buds just beginning to open). All foliage was killed on any tree that had reached a budburst score of 3 or above (leaflets evident). Most trees scoring 2 also suffered severely from frost damage, as did many trees only scoring 1. However, most of

the buds on trees from Shropshire with a score of 1 survived; whereas most from continental Europe didn't. This resulted in delayed completion of budburst at both sites, a delay of six days at Little Wittenham where the frost damage was slight, and 12 days at Ebberston compared to 2009.

It was unfortunate that observations were taken so far apart in 2009 and 2010. However, this was unavoidable as other trials were also being assessed. If weekly observations could have been made each year, a clearer picture might have been obtained, especially in 2010 when frost damage was so severe, particularly at Ebberston. It would have been useful to have observed the period of frost damage, and the subsequent regrowth of provenances.

At individual sites, greater differences in budburst are observed with a greater range of provenances. Romania was much the earliest provenance to break bud each year, and is the most continental in climate. Alsace and Picardie are also some of the most easterly provenances, and also came into leaf before other provenances. This is maintained from year to year, whatever the environmental signals. These provenances are adapted to a continental climate where winters are very cold. Once spring temperatures start to rise, cold spells are not common, and so forcing requirements are small as frost damage is unlikely to occur. However, provenances from the Czech Republic (Palobe at both Little Wittenham and Ebberston, and Czech (unknown) at Ebberston) that are also of eastern origin, were unexpectedly the latest continental European provenances to break bud, coming in to leaf after the more western French provenances. In an assessment of a provenance trial of *Fraxinus excelsior* containing a greater number of provenances of wider geographic distribution, also at Little Wittenham, Smith (2011) also reported eastern provenances flushing both early (e.g. Wloszczowa from Poland) and late (Osterkov, Czech Repulic). It is unfortunate that more details of the original collections were unavailable, as altitude also plays a key role in timing of budburst (Vitasse et al. 2009) which could go some way to explaining this anomaly.

Provenances originating from the UK broke bud later than provenances from continental Europe, and southern UK provenances broke bud earlier than northern UK provenances. British provenances had much higher chilling requirements than continental ones,

suggesting they are adapted to a more maritime climate where spring frosts are common, and it is of benefit to break bud later to reduce the risk of frost damage. For western continental provenances, there is not such a clear cut difference between provenances from eastern continental and western maritime regions, which is likely due to variability in local climate conditions and different elevations at which provenances were collected. This is consistent with the findings of von Wuehlisch *et al.* (1995) who also reported that provenances from eastern and south-eastern parts of the range of *Fagus sylvatica* broke bud earlier than others, and provenances from the western part of the range broke bud later.

Grimsthorpe is one of the more northerly provenances (latitude 52.48 °N) in this study, but broke bud much earlier than would be expected, responding more like provenances from latitude 49 °N which are more continental in origin e.g. Upper Rhine, Champagne. The form of the trees from Grimsthorpe was very different from others in the trial at Little Wittenham, with many trees looking more like Fraxinus angustifolia. Fraxinus angustifolia (narrow leaved ash) occurs throughout southern and eastern Europe and is not native to the UK. The buds are various shades of brown, as opposed to the black buds of Fraxinus excelsior, and the branches more twiggy. Leaflets tend to be much narrower and more variable in number than in Fraxinus excelsior (FRAXIGEN 2005). With incomplete data regarding the original collection of the seed material, it is impossible to be certain of the origin. However, it is likely that there is some parental *Fraxinus angustifolia* material in the collection from Grimsthorpe, or at least hybridisation in the progeny, at Little Wittenham. The two species are known to hybridise where there is overlap in their natural range (Fraxigen 2005; Heuertz et al. 2006). The trees from Grimsthorpe in the Ebberston trial, however, showed no such morphological differences, and this is corroborated by the timing of budburst: at Ebberston, Grimsthorpe broke bud more in line with its latitude of 52.48 °N than at Little Wittenham. When looking at five year heights, Cundall et al. (2003) also reported that Grimsthorpe's performance was highly variable across five sites. It is therefore possible that the trees in the Grimsthorpe provenance at Little Wittenham are not pure *Fraxinus excelsior*, and are either *Fraxinus angustifolia* or a hybrid of the two species. With the uncertainty of provenance collections and seedling production, it can be assumed that different seed lots were secured in the different planting years, or possibly there was a

mix up in the nursery, with the earlier collection planted at Ebberston likely to be pure *Fraxinus excelsior*.

When investigating the environmental triggers for budburst in the field, accumulated chilling units from the 1st November and mean spring temperature were both good at describing the spread of the data for all provenances. Accumulated day degrees also appeared to be a good predictor of budburst, but were more variable across provenances. Day degrees from the 1st January was better at predicting budburst in continental provenances, and day degrees accumulated from the 1st February appeared to be better at predicting budburst in UK provenances (Table 4.12). This is consistent with many other findings. Murray et al. (1989) calculated day degrees above 5 °C from 1st January and found that late flushing species had high thermal times, which decreased with increased chilling. Work by von Wuehlisch et al. (1995), also using day degrees above 5 °C from 1st January, found that eastern and south eastern provenances of beech broke bud earlier than those of more northerly and westerly origin. Early budburst provenances required 8500 degree hours, and late budburst ones 11000 degree hours in order to break bud, with a mean heat sum requirement of 9750 degree hours. Provenances from higher elevations also flushed earlier than those from lower elevations. However, the 1st of January is an arbitrary date, and results presented in this study would suggest that it is more applicable to provenances of continual origin. Cannell and Smith (1983), working on Sitka spruce (Picea sitchensis) in the UK, calculated day degrees from the 1st February. Using eight separate models to estimate heat sum and chill days to budburst, they found that 92 % of variation could be explained by a simple model of chill units from 1st November and thermal time above 5 °C from 1st February. However, Vitasse et al. 2009 reported that mean spring temperature was the best predictor of budburst in temperate tree species in France, and this would appear to be true for most provenances in this study. The rate of budburst progression is correlated with temperature and the growing season begins earlier in warmer years (Snyder et al. 1999). This was found to be true in the current study, with budburst occurring earlier in yeas with higher spring temperatures.

The difference seen between maritime and continental provenances in the field, could be because maritime provenances had not accumulated sufficient chilling units by 1st January,

and actually required less forcing temperatures once the chilling requirement was satisfied. *Fraxinus excelsior* would thus appear to have a high chilling requirement that is greater in maritime provenances than continental ones.

Budburst under controlled conditions

Budburst of provenances occurred in the same order under controlled conditions as in the field, with the exception of the bulked provenance of Glos/Hants/Surrey. In the field, this provenance broke bud before Settrington and Pallinsburn in 2007 and 2008 (years with warm springs), but in 2009 it broke bud at the same time as Settrington. In 2010 (the year with the coldest spring in this study), budburst of Glos/Hants/Surrey was delayed and in fact Settrington broke bud five days before it. In the field, the chilling requirement is exceeded for all provenances, even in the warmest years. Examining the data from the controlled environment room, for 50 % of buds to break from Pallinsburn and Glos/Hants/Surrey (the provenances with the highest chilling requirement), the chilling requirement was 101 days. In the years investigated (2007 - 2010) the warmest year was 2007, and this had 110 chilling units, and so the requirement was fully met. Where the chilling requirement is not fully met, greater forcing is required, and in these cases, budburst does not always follow a normal and complete progression, which may result in reduced photosynthetic capacity (Hänninen 1991). In the controlled environment room, in some cases, the earliest twigs to flush for several temperature regimes resulted in incomplete budbreak i.e. the twigs would break bud, but would not progress normally to complete unfolding (budburst stage 5). This was either due to mortality of individuals, or in several cases, because the buds simply did not progress to full expansion, although they remained alive. This tended to be the case with those twigs that had received little chilling. With increased chilling, budburst occurred much more rapidly, with lower forcing requirements, and budburst followed its natural course to completion.

There is no published model for the chilling and forcing requirements of bud break in *Fraxinus excelsior*, although there are many models in the literature for other species. This study has shown that, as with other species, there is a minimum chilling requirement for dormancy break, and that this varies between provenances indicating an adaptive response

under a degree of genetic control. For Romania, the most easterly provenance investigated, this minimum chilling period is only 45 days, but a long subsequent forcing period is then required. This forcing period rapidly decreased with increased chilling, as found with other species (Cannell and Smith 1983; Murray *et al.* 1989; Heide 1993a; Myking and Heide 1995). Murray *et al.* 1989 showed that after dormancy is imposed in the autumn, the requirement for forcing temperatures to break bud is high in many temperate tree species. However, as the chilling duration increases, this requirement is reduced. This reduction in forcing temperatures with increased chilling was also found in the current study for all provenances of *Fraxinus excelsior*.

With milder winters, if a certain minimum amount of cold (chilling requirement) is not reached, the required warm period will be much lengthened for bud burst to occur, and thus the growing period could be reduced. However, it is also possible that the onset of bud set in the autumn could also be delayed negating any reduction in the growing season. If the absolute minimum chilling requirement is not met (varying from 45 days for Romania to 59 days for Pallinsburn) budburst will not occur at all, regardless of the subsequent warm period. However, at present, chilling requirement of *Fraxinus excelsior* is fully met when growing in Britain, even for those provenances with the greatest chilling requirement.

There are many different models manipulating variables to explain the bud break response. While chilling units and mean spring temperature were closely correlated to budburst in all the years studied and for all provenances investigated, a single model of accumulated day degrees could not explain the response in all provenances. Various temperatures were used as base temperatures to calculate day degrees. 5° C was used as this temperature is most commonly found in the literature. However, this is an arbitrary bench mark, other than at about 5 °C cellular metabolism becomes active. Determining the precise trigger for budburst was extremely difficult. It is known to be different for various species, and results presented here indicate that it is also different for various provenances, especially between maritime and continental ones. The modelling required is beyond the scope of this study, and would benefit from additional, and more detailed observations both in the field, and under controlled conditions. While it is was evident that provenances had different chilling requirements, with more northerly and provenances requiring a greater chilling as seen in

the controlled environment room, the amount of subsequent warm is also important in the timing of bud burst. The possibility line that Harrington *et al.* (2010) propose is attractive in that both chilling and forcing units can be accumulated and this could be a factor in modelling the response in *Fraxinus excelsior*.

Cannell and Smith (1986) surmised that, in species with a large chilling requirement such as *Picea sitchensis*, milder winters might result in insufficient chilling and therefore delayed and erratic budburst in spring, while warmer spring temperatures would induce earlier budburst. Murray *et al.* (1989) predicted that tree species with a low chilling requirement would flush earlier at higher elevations in a warming climate, but not at low elevations. Kramer (1995) also hypothesised that warmer winters are likely to affect the onset and cessation of growth in many ways. However, the upper temperature limit to break dormancy has been found to be high for many species: 12 °C in *Picea abies* and *Betula* spp. (Myking and Heide 1995). Therefore, even with several degrees of warming, the natural winter chilling capacity of the climate greatly exceeds the requirement for complete dormancy breakage in temperate trees. The main effect of milder winters in temperate regions is therefore likely to be earlier bud burst, and increased risk of frost injury.

In summary, provenances of *Fraxinus excelsior* break bud at different times in a common garden experiment, and the order of budburst is more or less maintained across sites and across years, with budburst occurring earlier in years with warm springs, once the chilling requirement has been met. *Fraxinus excelsior* has a high chilling requirement which prevents budburst occurring too early in a maritime climate with mild winters and extended spring periods. Individual provenances have different chilling and forcing requirements in a common garden, which are an absolute requirement for budbreak. Phenotypic plasticity within each provenance allows *Fraxinus excelsior* to respond differently at different sites which have slightly different chilling and forcing temperatures. Continental provenances and maritime provenances from southern latitudes have lower chilling requirements. In continental climates, spring temperatures increase rapidly with less risk of damaging frosts, and the temperature sum required for budburst is lower. In such conditions it is advantageous for budburst to occur early and thus trees can benefit from a longer growing season.

Chapter 5 Chilling Requirement of Ash (*Fraxinus excelsior* L.) Seed by Population Origin

5.1 Introduction

Many temperate forest tree species produce dormant seed to prevent germination during unfavourable conditions and require periods of warm and/or cold temperatures before they can germinate. The control mechanisms which regulate seed dormancy are important adaptations of plants to their local environment, yet little is known about the control of seed dormancy within different populations of a species. A dormant seed is one that 'does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors (temperature, light/dark) that otherwise is favourable for its germination' (Baskin and Baskin 2004). Most studies have aimed at understanding seed dormancy and improving germination for nursery production and at finding artificial pretreatment regimes that maximise seedling emergence in the nursery. Germination refers to the appearance of the radicle, and seedling emergence to the production of seed leaves. Seed dormancy is complex and several categories are recognised. The most thorough classification was put forward by Nikolaeva (1977) and is today most commonly used (Baskin and Baskin 2004; Finch-Savage and Leubner-Metzger 2006). It recognises five broad categories of dormancy:

- Physiological dormancy. This is the most common type of dormancy and requires some form of temperature treatment (either warm or cold or both) to break dormancy e.g. *Acer pseudoplatanus* (Finch-Savage *et al.* 1998). Drying and photodormancy also fall into this category.
- 2. Morphological dormancy. The embryo is differentiated but small and simply needs time to grow e.g. *Annona* sp (Baskin and Baskin 2005).
- Morphophysiological dormancy. The embryo is underdeveloped, but there is also physiological dormancy. A dormancy breaking pretreatment is required in a combination of warm/cold pretreatment e.g. *Fraxinus excelsior* (Villiers and Wareing 1964).
- Physical dormancy. This is caused by water impermeable layers in the seed coat which can be overcome by chemical or mechanical scarification e.g. *Robinia pseudoaccacia* (Baskin *et al.* 2000).
- 5. Combinational dormancy. A water impermeable seed coat and physiological dormancy are both factors e.g. *Cercis canadensis* (Geneve 1991).

5.1.1 Seed dormancy in Fraxinus excelsior

Fraxinus excelsior seed is classified as having deep, simple, morphophysiological dormancy, with a long period of chilling required before germination is possible. Seed of deeply dormant species (e.g. most broadleaves) remain inactive under all conditions as opposed to shallowly dormant species (e.g. most conifers) which only remain dormant under certain environmental conditions (Gosling 2007). Simple refers to the development of the embryo. In seed with simple dormancy, the embryo develops during periods of warm (10 - 20 °C) pretreatment. Where the embryo develops during cold (5 °C) pretreatment, dormancy is referred to as complex (Nikolaeva 1977; Baskin *et al.* 2008). Seed of *Fraxinus excelsior* are naturally dispersed during autumn and winter and under natural conditions begin to germinate in the second spring after dispersal (Baskin and Baskin 1998). The seed are orthodox (they can undergo partial drying and are easy to store) and require long dormancy breaking treatments before they can germinate (Gosling 2007). The winged fruits (samaras) are approximately 4 cm long and 6 - 8 mm wide and usually contain a single seed.

Three factors appear to be involved in delaying germination in *Fraxinus excelsior* (Villiers and Wareing 1964). Firstly, at the time of dispersal the embryo, although fully differentiated, is not fully developed (morphological dormancy) and only occupies approximately half the seed cavity. The embryo normally only reaches 45 - 55 % of its final length in the first autumn, although this percentage occupancy increases at more southerly

latitudes (Suszka *et al.* 1996). A period of post-dispersal, warm, moist conditions is required to complete embryo growth (Suszka *et al.* 1996; Noweg 1998). Secondly, the pericarp is impermeable to oxygen until it has broken down, preventing maturation of the embryo (Villiers and Wareing 1964). Finally, the mature embryo requires a period under cold, moist conditions to break dormancy (Villiers and Wareing 1965; Bonner 1974, Suszka *et al.* 1996). Forest nurseries overcome this dormancy by carrying out pretreatment (stratification) so that the majority of seed germinate at more or less the same time. This is achieved through a warm phase pretreatment which allows the embryo to complete elongation, followed by a cold phase pretreatment to break dormancy (Gosling 2007). Washing the seed during pretreatment can also help break dormancy through leaching of growth inhibitors, which is thought to be abscisic acid (Kucera *et al.* 2005).

The International Seed Testing Association (ISTA 1993) pretreatment for breaking dormancy in *Fraxinus excelsior* stipulates 60 days (8.6 weeks) warm pretreatment at 20 °C followed by 210 days (30 weeks) cold pretreatment at 3 - 5 °C. However, this regime would appear to be too generalised for nursery practices: the warm period is too short and the cold pretreatment too long for many seedlots. Suszka *et al.* (1996) recommend up to 16 weeks of warm pretreatment at 15 or 20 °C depending on the origin of the seedlot, followed by a minimum of 16 weeks cold treatment at 3 °C. If embryo occupancy can be checked, the warm phase can be reduced in duration once occupancy reaches 80 - 90 % (Suszka *et al.* 1996). Other authors also recognise the importance of the duration of pretreatment, particularly emphasising the need for longer warm pretreatment than stipulated by ISTA (Nowag 1998; Doody and O'Reilly 2011). Furthermore, Doody and O'Reilly (2011) reported that additional chilling did not compensate for inadequate warm treatment.

The degree of dormancy shows variation related to the climate of seed origin and therefore may vary between seedlots of the same species (Tilki and Çiçek 2005). Nikolaeva and Vorobjera (1978) reported that embryos of *Fraxinus excelsior* are less mature in western provenances compared to eastern and southern provenances. Reciprocal transplant experiments on temperate herbaceous species have found that dormancy tends to increase with latitude (Lacey 1984) and altitude (Dorne 1981) and these factors need to be considered when pretreating seedlots. While 16 weeks chilling pretreatment may be

sufficient to break dormancy in many populations of *Fraxinus excelsior*, there is very little data looking at dormancy and chilling requirement across a range of provenances, and on the requirements for those populations at the limits of the species natural range.

5.1.2 Germination testing

It is important to know the viability of seedlots (and therefore the likely germination rate with proper pretreatment) and so germination and viability testing is commonly carried out by nurseries to determine sowing rates. Guidelines issued by ISTA (1993) state that germination testing of *Fraxinus excelsior* should be carried out over an eight week period at 30/20 °C in the dark for 8/16 hours. However, Suszka *et al.* (1996) report that that these conditions do not provide optimal temperatures for germinating *Fraxinus* species and recommend an alternating 5/15 °C (14/10 hours) which more closely mimics natural conditions and gives more rapid germination that is often complete within four weeks.

Piotto (1994) also reports that a 30/20 °C temperature for germination testing is too high. Working on three species of ash, *Fraxinus excelsior*, *Fraxinus angustifolia* and *Fraxinus ornus*, various pretreatments were applied and three separate germination temperature regimes: 30/20 °C (ISTA guidelines), 25/5 °C and constant 20 °C. The ISTA guidelines again proved suboptimal, with the 25/5 °C regime giving the most rapid and complete germination. The 30/20 °C regime not only failed to produce the highest germination rates, but also resulted in secondary dormancy in several of the seedlots tested (Piotto 1994). Gosling (unpublished data) also studied the germination requirements of *Fraxinus excelsior* seed at eight temperatures between 0 °C - 35 °C at 5 °C intervals. The greatest rates of germination occurred at 10 °C and 15 °C.

Most woodland creation in the UK occurs through planting of bareroot seedlings. Historically, woodlands in Britain have been planted (mostly with coniferous species) and intensively managed as a monoculture to maximise returns from timber production. However, with the introduction of the Broadleaves Policy in 1985, more ecologically diverse techniques have been increasingly promoted. Broadleaved planting has become more common with the adoption of different silvicultural practices including selection and

shelterwood systems. The Forestry Commission promote the use of direct seeding and natural regeneration in certain situations, where browsing and seed predation is low and where good vegetation management is possible (Willoughby and Jinks 2009). Although natural regeneration and direct seeding are potentially less expensive options than planting, they are often considered unreliable and the results can be variable, both in the level of germination and the timeframe over which germination occurs (Kozlowski 2002 and references therein). Nevertheless, reforestation by natural regeneration is increasing in popularity, partly due to the costs of tree planting, partly because it is perceived as more environmentally friendly (less use of herbicides and tree shelters), and in some cases to promote the use of local seed sources (e.g. Flora Locale 2007, Woodland Trust 2007).

With climate change predictions of an increase in temperature of 2 - 3 °C for Britain by the end of the 21st century, and the rise predicted to be greater in winter than in the summer (Murphy *et al.* 2009), the question arises, will chilling requirements be met for natural regeneration of *Fraxinus excelsior*? In relation to the adaptation of *Fraxinus excelsior* to climate change, this chapter explores whether the chilling requirement for seed of *Fraxinus excelsior* is the same for all provenances across a range of latitudes, and, if chilling requirement increases with latitude, what will the effect of higher annual mean temperatures be on natural regeneration?

5.2 Methods

Germination testing was carried out on the ten seedlots used in the RTEs (details of populations are given in section 2.3.2; details of seed collections are given in section 3.2.1) to investigate the requirements for pretreatment across a range of latitudes. Seedlots were delivered to the seed extractory at Forest Research, Alice Holt, Surrey in November 2006 where they were dried to $10\% \pm 0.5\%$ moisture content for storage.

5.2.1 Seed pretreatment

Seed pretreatment and germination testing were carried out over two years (2006 - 2007 and 2007 - 2008). The first pretreatment regime (2006 - 2007) used for seedling production in 2007 resulted in poor germination, and the study was therefore repeated in 2007 - 2008 with modified warm and cold pretreatments. In the first study approximately 1 kg of seed from each provenance was soaked in tap water for 48 hours to fully imbibe seed, and the water changed after 24 hours. After soaking, seedlots were spun by hand in a tumbler to remove excess water. Seed entered warm pretreatment on 7th December 2006. Each seedlot was placed in a separate plastic container with a loose lid to minimise moisture loss but to allow for gas exchange. No medium was used. Seed in each lot were turned every week by hand to ensure uniform moisture distribution and to prevent accumulation of moisture in the bottom of the containers and drying of seed on the top. Containers were kept in the dark in the seed extractory at 15 - 20 °C.

Every two weeks, 25 seed from each seedlot were extracted at random to determine embryo occupancy. Each seed was measured with digital callipers to the nearest millimetre. The longitudinal edges of the seed were removed with a scalpel, the seed bisected and the length of the embryo measured to the nearest millimetre. Embryo occupancy is expressed as the length of the embryo as a percentage of the entire seed length. Although seedlots had only received 10 weeks of warm pretreatment, due to time constraints and the assumed need to get seedlots into cold pretreatment ready for sowing in the spring, a mean

embryo occupancy of 72.3 % across all seedlots was deemed acceptable and all seed entered cold pretreatment at 4 °C on 15th February 2007 for 15, 23 and 31 weeks.

Moisture content of seedlots was determined by the low constant temperature oven method (ISTA 2009) of both the dried seed prior to entering warm pretreatment, and after cold pretreatment. One hundred seed from each lot were weighed and dried in an oven at 103 °C \pm 2 °C for 17 hours \pm 1 hour and then reweighed. Moisture content (as percentage of fresh weight) of dried seed prior to entering pretreatment was 10.6 % \pm 0.2 % on a weight loss basis, and 47.5 % (\pm 10.5 %) at the end of pretreatment.

Pretreatment was repeated in 2007 - 2008 with the following alterations: seed entered warm pretreatment on 7th August 2007 for 12 weeks at 15 °C. To aid removal of dormancy inhibitors and promote embryo elongation, all seedlots were washed after six weeks of warm pretreatment for 24 hours in tap water, and the seed respun to remove excess water. Seed entered cold pretreatment at 4 °C on 29th October 2007 for 13, 17, 21 and 25 weeks.

5.2.2 Germination testing

Germination testing was carried out after each cold pretreatment period (15, 23 and 31 weeks) in 2007. One hundred seed of each population were sown in a 50:50 peat - fine sand medium in separate trays. Seed that were obviously dead or empty were discarded. Seed were lightly covered to a depth of 1 - 2 cm of the same medium, and the trays inserted into loosely tied plastic bags and placed in an incubator in the dark for 56 days. Based on the findings of Gosling (unpublished data), an alternating 10/15 °C (12 hours) regime was used which more closely mimics natural conditions than a constant temperature.

Each week, germinants were removed from each tray. A seed was considered to have germinated when the radical was 5 mm long (Van de Walle 1987; ISTA 1993). After eight weeks, all ungerminated seed were recovered and assessed as chitted (the radical is visible), abnormal, dead, empty or fresh (i.e. appeared to be alive but did not germinate). Fresh seed were then tested for viability. The longitudinal edges were removed with a scalpel, and each seed individually placed in a repli-dish, covered with an aqueous solution of 1 %

2,3,5 triphenyl tetrazolium chloride and incubated in the dark at 30 °C for 18 hours. Seed were then washed in distilled water, opened and the embryo and endosperm assessed. These tissues stain a uniform deep dark red in viable seed. Where the embryonic axis or the endosperm showed white patches, seed were classified as dead (Figure 5.1). Fresh seed were classified as 'best' or 'lesser' referring to the degree of staining.

The total number of viable seed is the sum of the fresh, chitted, abnormal and germinated seed. The number of seed that germinated under a particular pretreatment regime is referred to as Gmax. The percentage of viable seed that germinated was calculated as (Gmax/viable seed)*100 and expressed as G/V. For data from 2006 - 2007, Gmax was adjusted to take into account the number of seed originally discarded.



Figure 5.1 Results from tetrazolium testing for viability. Live tissue stains dark pink. Seed are scored as best (viable), lesser (viable) or dead.

Germination testing was repeated in 2008 as in 2007 with the following alterations: one hundred seed of each provenance were selected at random (including those obviously dead or empty, in accordance with ISTA guidelines) and sown in a 50:50 peat - fine sand medium in individual trays. Two replicates of each seedlot were sown with the exception of Monein and Ranmore Common, where only one replicate was sown for the first three cold

pretreatments (13, 17 and 21 weeks) due to limited availability of seed. Two replicates of these seedlots were possible for the final cold pretreatment (25 weeks). Viability, Gmax and G/V were calculated as before.

5.2.3 Statistical analyses

Analyses of variance were carried out using Genstat (11th Edition, VSN International Ltd). To investigate the possible causes of variations in provenance responses, quadratic regressions investigating chilling temperatures (taken as mean days per year less than or equal to daily mean temperature of 4 °C, and mean days per year less than 4 °C daily mean temperature), mean annual temperature, latitude and altitude at the site of seed collection of provenances were carried out on the 2007 - 2008 germination test results. Temperature data were obtained from the nearest available weather station at similar altitude. Datasets were obtained from the Le Tech site. Here weather data were extracted by Lafont (pers comms) from the Safran system (Vidal *et al.* 2010) and averaged over three years (2006 - 2008).

5.3 Results

5.3.1 Embryo occupancy

On entering pretreatment, embryo occupancy averaged 60.1 % and was greatest in seed from Monein (69.6 %) and lowest in seed from Le Tech (51.2 %). With the exception of seed from Monein, the embryo was most advanced in northern seedlots (Inverness shire = 63.8 % \pm 2.4 %; Yorkshire = 60.6 % \pm 3.1 %), and occupancy decreased with decreasing latitude (southern England = 59.2 % \pm 3.6 %; Ile de France = 56.6 % \pm 2.1 %). At the end of warm pretreatment, embryo occupancy had increased to a mean of 72.3 % across all seedlots (range 68.1 % for Settrington to 79.7 % for Monein) (Figure 5.2). Embryos continued to elongate during cold pretreatment to give a final mean occupancy of 83.1 % (range 77.2 % for Le Tech to 95.4 % for Monein).



Figure 5.2 Embryo occupancy of ten provenances of *Fraxinus excelsior* as a percentage of the entire seed, assessed every two weeks during warm pretreatment in 2006 - 2007. A final assessment was also made at the end of the cold pretreatment (04.06.07).

5.3.2 Germination testing

The germination tests in 2008 revealed that, on average, 17.6 % of seed were empty (8.6 % at Parlington to 25.9 % at Le Tech) and 10.6 % were dead (5.6 % at Cawdor to 15.4 % at Dourdan) with the exception of seed from Le Tech where 44.9 % were dead.

Both provenance and pretreatment were highly significant sources of variation for germination in 2007, with higher levels of germination mainly in southern provenances (Table 5.1; Figure 5.3). There was no significant interaction between provenance and cold pretreatment. Cold pretreatments were at eight week intervals in 2007 which gave a rather coarse indication of the effects of chilling on germination. For example, with the first pretreatment (15 weeks cold) the provenance Monein had already reached 95 % germination. The Marly provenance doubled in germination between 15 and 23 weeks cold pretreatment, and the provenance Cawdor increased from 22 % - 78 % in the same time period. Therefore, in 2008, the first cold pretreatment was reduced by two weeks and intervals between cold pretreatments were reduced to four weeks.

Provenances also showed significant differences in 2008 for the first three cold pretreatments (13, 17, and 21 weeks) (Table 5.2; Figure 5.4). With 13 weeks cold pretreatment, only provenances from Monein and Marly were significantly different from the other provenances (Table 5.3) with most provenances having less than 20 % final germination. Cold pretreatment of 21 weeks was sufficient for all but three provenances (Settrington, Cawdor and Le Tech) to reach 50 % germination. After 25 weeks cold pretreatment, there were no significant differences between provenances, with germination ranging from 61 % in the provenance from Le Tech to almost 100 % for provenances from Monein and Ranmore Common.

Table 5.1 Results of analysis of variance of the percentage of viable seed that germinated in
ten provenances of *Fraxinus excelsior* after three cold pretreatment durations in
2007.

Source of variation	d.f.	S.S.	m.s.	<i>v.r.</i>	F pr.
Provenance	9	6622.9	735.9	5.72	<0.001
Pretreatment	2	7239.3	3619.6	28.16	<0.001
Provenance × pretreatment	18	2313.9	128.5	1.00	0.500
Total	29	16176.1			



Figure 5.3 The percentage of viable seed that germinated in 2007 in ten provenances of *Fraxinus excelsior* after 10 weeks warm pretreatment at 15 °C, followed by cold pretreatments of 15, 23 and 31 weeks at 4 °C. Provenances are displayed in order of decreasing latitude (left to right).

Table 5.2 Results of analysis of variance of the percentage of viable seed that germinated in
ten provenances of *Fraxinus excelsior* after four cold pretreatments in 2008.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Provenance	9	18802.30	2089.14	33.31	<0.001	
Pretreatment	3	43390.21	14463.40	230.64	<0.001	
Provenance × pretreatment	27	2663.95	98.66	1.57	0.105	
Residual	34	2132.14	62.71			
Total	73	61815.58				



Figure 5.4 The percentage of viable seed that germinated in 2008 in ten provenances of *Fraxinus excelsior* after 12 weeks warm pretreatment at 15 °C, followed by cold pretreatments of 13, 17, 21 and 25 weeks at 4 °C. Provenances are displayed in order of decreasing latitude (left to right).

Table 5.3 Mean percentage germination of viable seed in ten provenances of *Fraxinus excelsior* after four cold pretreatments (13, 17, 21 and 25 weeks) in 2008. Provenances joined by lines are not significantly different from each other at p < 0.05 (Bonferroni multiple range test). All seedlots received 12 weeks warm pretreatment at 15 °C.

13 weeks		17 wee	17 weeks		S	25 week	25 weeks	
Parlington	0.6	Parlington	28.4	Settrington	41.6	Le Tech	61.6	
Cawdor	1.3	Cawdor	28.4	Cawdor	47.1	Cawdor	63.9	
Ballindalloch	1.9	Settrington	32.4	Le Tech	48.9	Settrington	68.5	
Settrington	3.6	Ballindalloch	37.7	Parlington	54.1	Little Wittenham	70.8	
Little Wittenham	12.5	Le Tech	48.0	Ballindalloch	63.6	Parlington	76.6	
Le Tech	14.8	Little Wittenham	48.5	Little Wittenham	66.6	Ballindalloch	77.7	
Dourdan	15.5	Dourdan	56.6	Marly	72.8	Marly	87.2	
Ranmore	22.9	Marly	66.3	Dourdan	79.2	Dourdan	89.5	
Marly	31.0	Ranmore	69.6	Ranmore	79.7	Ranmore	95.2	
Monein	58.7	Monein	89.7	Monein	94.2	Monein	99.2	
	p = 0.00	3	p = <.001		p = 0.00	9	p = 0.14	

Regression analyses to ascertain the relationship between environmental factors and germination indicated that altitude was not associated with germination, while latitude and mean annual temperature accounted for approximately 25 % of the variation each. However, temperature at site of seed collection for provenances (as days equal to or less than 4 °C) described the most variation (Figure 5.5), accounting for up to 76.3 % of the variation in germination of viable seed.



Figure 5.5 Quadratic regressions of the percentage germination of viable seed in 2008 of ten provenances (two replicates per provenance except for Monein and Ranmore Common where there is only one replicate for the first three pretreatments) of *Fraxinus excelsior* against number of days ≤ 4 °C at the site of seed collection, with increased cold pretreatment (13, 17, 21 and 25 weeks).

Although Le Tech is a southerly latitude site it is located at 800 m a.s.l. and is an outlier in all analyses. When it is removed from the regressions, r^2 increases for the last two cold pretreatments from 38.6 % to 46.6 % for pretreatment 3 and from 43.7 % to 53.5 % for pretreatment 4 (p = 0.007 and 0.001 respectively).

Figure 5.6 illustrates individual provenance response to increased length of cold pretreatment. Even with the longest pretreatment (25 weeks – 175 days), provenances from Inverness-shire (Cawdor and Ballindalloch), Yorkshire (Settrington and Parlington) and Little Wittenham only reached about 80 % germination. However, seed from Ranmore Common, of similar latitude to Little Wittenham, reached almost 100 % germination which was more in keeping with provenances from France, and indeed, reached greater germination than the provenances from Marly and Dourdan. The provenance from Monein had the lowest chilling requirement, reaching high levels of germination (90 %) after only 17 weeks (119 days) of cold pretreatment. Le Tech is the most southerly provenance, but had one of the highest chilling requirements of all provenances, probably reflecting its altitude rather than latitude, with only 61 % of viable seed germinating after 25 weeks cold pretreatment.



Figure 5.6 Quadratic regressions of the percentage germination of viable seed of ten provenances of *Fraxinus excelsior* with increased cold pretreatments.

Provenance	r ²	Regression equation	% germ / ¹ 175 days	80 % ² germ	Days ≤ ³ 4 °C	Germination ⁴ at origin
Cawdor	87.5	-62.9 + 0.737 chilling	66.1	193.9	155	51.3
Ballindalloch	91.9	-75.2 + 0.905 chilling	83.2	171.5	175	83.2
Settrington	95.1	-60.3 + 0.728 chilling	67.1	192.7	135	38.0
Parlington	98.3	-80.6 + 0.907 chilling	78.1	177.1	118	26.4
Little Wittenham	86.4	-42.0 + 0.689 chilling	78.6	177.1	121	41.4
Ranmore	90.1	-37.2 + 0.776 chilling	98.6	151.0	95	36.5
Marly	84.7	-18.9 + 0.626 chilling	90.7	158.0	102	45.0
Dourdan	86.2	-55.9 + 0.873 chilling	96.9	155.7	111	41.0
Monein	80.7	29.2 + 0.417 chilling	102.2	121.8	88	65.9
Le Tech	73.4	-23.9 + 0.505 chilling	64.5	205.7	112	32.7

Table 5.4 Predicted percentage germination from regression equations for ten provenancesof Fraxinus excelsior with 175 days (25 weeks) cold pretreatment, and number ofdays chilling required to reach 80 % germination.

¹ Predicted germination of each provenance after 175 days (25 weeks) cold pretreatment.

² Number of days \leq 4 °C required for 80 % of seed to germinate at the site of provenances.

³ Mean number of days per year \leq 4 °C at the site of provenances between 2006 and 2009.

⁴ Predicted germination at origin with current (2006 - 2009) mean winter temperatures.

Regression equations for each provenance indicated that for 80 % of seed to germinate, the number of chilling days (\leq 4 °C) required ranged from 122 at Monein to 205 at Le Tech. Weather data extracted for each site for the years 2006 - 2009 indicate that on average Monein receives 88 days \leq 4 °C a year, and Le Tech receives 112. Ranmore Common stood out as needing rather fewer chilling days than Little Wittenham (of similar latitude) but the weather data shows that this site naturally receives a corresponding lesser number of chilling days. With current winter temperatures, only provenances from Scotland and Monein receive enough chilling for more than 50 % of seed to germinate (Table 5.4).

5.3.3 Viability

The results of the tetrazolium tests indicated that seed viability ranged from 26.1 % for the provenance Le Tech to 84.0 % for the provenance Ballindalloch in 2007. Probably due to sampling, Le Tech and Parlington actually increased in viability in 2008 (29.3 % and 84.3 % respectively) although all other provenances except Settrington decreased in viability as would be expected after a year in storage (Table 5.5). The viability of seed from Settrington increased by 8 %.

One-way analysis of variance indicated that viability of seedlots varied significantly among provenances (p<0.001). As the provenance Le Tech was an outlier the analyses were repeated without this provenance, and differences were still significant (p<0.001).

5.5 Mean percentage viability of ten provenances of *Fraxinus excelsior* over time, as determined by tetrazolium testing and the change in viability between 2007 and 2008. Provenances annual mean viability joined by lines are not significantly different from each other at p<0.05 (Bonferroni multiple range test).

Provenance	31 May 2007	30 July 2007	25 Sept 2007	Mean 2007	Provenance	28 Jan 2008	25 Feb 2008	24 Mar 2008	21 Apr 2008	Mean 2008	Change
Le Tech	24.4	27.4	26.4	26.1	Le Tech	34.0	30.0	25.5	27.5	29.3	+3.2
Monein	64.0	65.0	70.1	66.4	L. Wittenham	60.0	65.0	63.0	63.0	62.8	-3.6
L. Wittenham	55.2	66.2	77.9	66.4	Monein	63.0	68.0	69.0	61.0	65.3	-1.1
Settrington	64.1	65.0	72.9	67.3	Marly	64.5	66.5	71.5	67.5	67.5	-1.1
Marly	68.9	68.0	68.8	68.6	Ranmore	70.0	69.0	69.0	73.5	70.4	-1.2
Ranmore	76.0	67.4	71.5	71.6	l Dourdan	71.5	81.0	67.5	76.0	74.0	-2.8
Dourdan	77.1	75.7	77.7	76.8	Settrington	79.5	80.0	68.5	73.0	75.3	+8.0
Cawdor	79.6	83.6	83.3	82.2	Cawdor	80.0	81.5	75.5	79.1	79.1	-3.1
Parlington	81.5	80.0	86.2	82.6	Ballindalloch	81.5	77.0	84.5	74.8	79.5	-4.5
Ballindalloch	79.5	83.8	88.8	84.0	Parlington	86.0	87.5	75.5	88.0	84.3	+1.7

5.4 Discussion

5.4.1 Embryo occupancy

Embryo occupancy was greatest in seed from Monein, and next greatest in seed from Ranmore Common. These two provenances had high percentages of germination in both 2007 and 2008. It is possible that warm pretreatments were not sufficient for many seedlots in this study, and the embryos were not sufficiently elongated to allow high levels of germination. Doody and O'Reilly (2011) report that for populations of *Fraxinus excelsior* from Ireland, there is little embryo development between weeks 10 to 12 and that most development is completed between weeks 12 and 14. However, although Derkx (2000) reports embryo occupancy as the main indicator of dormancy progress, Doody and O'Reilly (2011) found that higher germination was achieved with an additional four to eight weeks of warm pretreatment after the embryo had filled the cavity, probably because the additional warm period contributed to further decay of the pericarp, thereby allowing for additional gas exchange.

In a report on dormancy breakage of *Fraxinus excelsior* seeds, washing of seeds was very effective at removing the inhibitory effects of the pericarp which were found to be water soluble chemical inhibitors rather than a physical effect (Jinks 1997). The study reported here showed that embryos continued to develop during cold pretreatment, which contradicts the belief that seed must have 80 - 90 % embryo occupancy before entering cold pretreatment (Suszka *et al.* 1996). Jinks (1997) also reported that embryos continued to elongate in the cold phase of pretreatment, and concluded that once the inhibition from the pericarp was removed (in this case through washing) embryos can continue to grow, and also respond to chilling temperatures.

Doody and O'Reilly (2011) found that 18 - 30 weeks warm pretreatment were optimal for Irish provenances of *Fraxinus excelsior*, which is considerably longer than the 8.6 weeks recommended by ISTA (1993), and also longer than the 20 weeks recommended by Suszka *et al.* (1996) and Piotto (1994) for continental European provenances. In any case, the relatively low level of warm pretreatment that seedlots received in this study was probably a contributing factor to low germination in some provenances. Mean embryo occupancy

was 72.1 % at the end of warm pretreatment and increased to 83.1 % during cold pretreatment, but this value is still low. Embryos may have needed to complete elongation during the germination test, which might explain low levels of germination for some seedlots. Results from this study suggest that 10 weeks warm pretreatment is too short for all provenances investigated.

The other point to note is that at time of collection, embryo occupancy was greatest in northern provenances with the exception of the provenance from Monein, and occupancy decreased in more southerly provenances, which contradicts findings of Suszka *et al.* (1996) and Nikolaeva and Vorobjera (1978).

5.4.2 Germination testing

The main finding of this study was that the chilling requirement necessary to break dormancy is higher in provenances from northern locations (Figure 5.4; Table 5.3). With 17 weeks cold pretreatment, only those provenances from France at low elevations and Ranmore Common in the south of England achieved 50 % (of viable seed) germination. Most populations from the UK had only very low germination. With 25 weeks cold pretreatment, germination levels increased, but Ranmore Common was the only UK provenance to reach 80 % germination.

The provenances that stood out as having low germination in this study are those from Le Tech, Cawdor and Settrington, which all achieved less than 70 % germination even after 25 weeks cold pretreatment. Le Tech is a high elevation site and Cawdor is the most northerly provenance in this study, and both would thus be expected to have high chilling requirements. The chilling requirement for seed from Settrington was surprisingly high, and is difficult to explain. It was noted in the cpDNA study (reported in Chapter 2) that individual trees from Settrington were not all of the same haplotype (H4). The additional haplotype found at Settrington in this study was H14, described by Sutherland *et al.* (2010) as originating from western Scotland, which could account for the higher than expected chilling requirement of seed from Settrington. The chilling requirement for the provenance from Little Wittenham was also surprisingly high given its latitude. However, the woodland

at Little Wittenham is located in a frost pocket at the bottom of hills in the floodplain of the Thames where late spring frosts are the norm, and this high chilling requirement could be an adaptation to its location. Conversely, Ranmore Common had a relatively low chilling requirement given its latitude, being similar to provenances from Marly and Dourdan in its chilling requirement for germination. The woodland at Ranmore Common is on a south facing slope, and the temperature data from this site reflect this. It received less chilling days a year than any other site except Monein, the most southerly site.

Comparing results from 2007 and 2008, the highest levels of germination were reached after 25 weeks cold pretreatment in 2008. Although the final cold pretreatment in 2007 had a longer chilling period (31 weeks) which could have helped compensate for insufficient warm pretreatment, germination was actually lower in many provenances. In this 31-week cold pretreatment, many seed had chitted (the radicle had started to emerge) and it is likely that the drop in germination was due to damage to the radicles at the time of setting up the germination test, preventing proper germination in some seed.

The results presented here largely agree with work reported by others, in that the warm pretreatment stipulated by ISTA is insufficient for many seedlots. The 210 days (30 weeks) cold pretreatment that ISTA recommends, appears to be adequate for northern provenances, but overly long for provenances from southern England and France. However, the critical factor in this study is that there was insufficient warm pretreatment for the majority of seedlots. This result supports that of Noweg (1998), who carried out germination testing with warm pretreatments ranging from 8 to 22 weeks, and found that longer warm pretreatment resulted in higher rates of germination.

5.4.3 Viability

The viability of seed from Settrington actually increased by 8 % from 2007 to 2008 which throws into question the validity of this result. In 2007, seed that appeared dead or empty were discarded prior to germination testing, and this is not the correct method to use for ascertaining seed viability (ISTA 1993). On average, 36 seed were discarded prior to each

germination test of Settrington seeds in 2007. Results from 2008 indicated that mean viability was 75.3 % in 2008 (up from 67.3 %), suggesting that more seed were discarded in 2007 than were actually dead or empty. This is likely to be the case for all seedlots to a certain degree and would also explain other provenances increase in viability (Ballindalloch and Le Tech). Thus, all viability data from 2007 should be viewed with a degree of caution.

5.4.4 Natural regeneration and climate change

The predicted days of chilling required for 80 % of seed to germinate ranged from 122 days (17.5 weeks) for the provenance Monein, to over 190 days (27 weeks) for the provenances Cawdor and Settrington (Table 5.4), with the average across all provenances being 170 days (24 weeks) chilling. However, the mean number of days per year during the period 2006 - 2009 with temperatures \leq 4 °C at Monein was only 88, and at Cawdor it was 155: each site is receiving approximately 40 days less chilling than is predicted for 80 % of seed to geminate naturally. This suggests that many populations of *Fraxinus excelsior* may not receive sufficient chilling to regenerate naturally. However, natural regeneration was evident at several sites during seed collection. Parlington and Little Wittenham had particularly high levels of natural regeneration, which was also observed at Settrington and Monein. No natural regeneration was evident at Ranmore Common, Dourdan and Marly. However, this could be a factor of deer numbers rather than a lack of germination. Other provenances were not collected from within a stand and natural regeneration was not observed in roadside margins.

Another point to consider is that mean annual chilling temperatures were averaged over only four recent years (2006 - 2009). Winter temperatures clearly fluctuate annually, and a longer historic dataset would yield different results. Even within the four years data used in this study, annual chilling temperatures varied by over 30 days at some locations. For example, Cawdor received 174 days \leq 4 °C in 2008 but only 140 days \leq 4 °C in 2007. To achieve 50 % natural regeneration at this site, 153 days \leq 4 °C are required, and this requirement was met in 2006 and 2008, but not in 2007 and 2009. However, *Fraxinus excelsior* is a prolific seeder, and masting typically occurs every two to three years (Savill 1991). In a mast year, a seed tree can easily produce 20,000 seed, and Savill (pers comms)

suggests that even 5 % natural regeneration is sufficient. Using 5 % germination as a benchmark then for successful natural regeneration, with present climatic conditions chilling requirements are fully met in all seedlots tested here even in the mildest winters. For seed from Cawdor, only 99 days chilling at \leq 4 °C would be required for 5 % germination. leading to With, annual mean temperatures are predicted to rise by 2 - 3 °C across the UK, with the rise being greater in the winter than in the summer and thus chilling requirements for natural regeneration of *Fraxinus excelsior* seed may not be met in the future. However, natural variation within a population will favour those individuals with a lower chilling requirement in years where sufficient chilling winter temperatures are marginal.

It is not clear whether accumulated chilling is carried over from one year to the next, so that the chilling requirement can be met over a two year period. Work by Stephen *et al.* (2004) on *Prunus avium* suggests that this is not the case, and that if dormancy requirements are only half met, exposure to higher temperatures induces secondary dormancy (as it does in *Fraxinus excelsior*) that negates the effects of any previous cold treatment. If this is indeed the case with *Fraxinus excelsior*, it may become necessary to consider assisted migration. Assisted migration is more commonly concerned with moving rare or endangered species outside their historic range for conservation purposes. But it can also be utilised for common species with a large range, in which climate change may make locally adapted populations less suitable for future conditions (Gray *et al.* 2011). For reforestation purposes, this then entails planting of seedlings raised in nurseries of local origin, or incorporating some seedlings from populations further south, with lower chilling requirements that are more in line with future projected climates.

In summary, populations of *Fraxinus excelsior* exhibit a range of chilling requirements, corresponding to the climate of their seed origin. High elevation and high latitude populations have a higher chilling requirement than low elevation and more southerly populations. The length of warm pretreatment is very important to allow the immature embryo to fully develop prior to cold stratification. For natural regeneration to occur, at present there is sufficient winter chilling for all provenances studied to allow for 5 % germination.

Chapter 6 Discussion

The impacts that the human population are having on both the earth's climate and its environment have become increasing apparent throughout the 20th century and today it is widely recognised that anthropogenic emissions of greenhouse gases are largely responsible for the increase in global surface air temperatures, augmented by deforestation. Global average temperature has risen by 0.8 °C since 1900 (Hansen J *et al.* 2006) and the effects of this are already apparent though altered phenological responses of species. Climate researchers agree that temperatures will increase in Europe by 2 - 3 °C in the 21st century and by 3 - 5 °C in the UK in the same timeframe (Christensen *et al.* 2007; Murphy *et al.* 2009).

Forests are particularly sensitive to changes in climate because of their long life spans which do not allow for rapid adaptation or migration (Aitken *et al.* 2008). Tree species have been exposed to long term environmental change through their evolutionary history. They have shown capacity to respond and adapt to change, and although individual populations have become extinct in the past, species extinctions appear to be very rare. Acclimation responses over the lifetime of a tree have been observed in stomatal density in *Betula pendula* in response to increasing levels of CO₂ over 50 years (Wagner *et al.* 1996) suggesting that populations have the ability to adapt to changes in climate.

The migration rates required to track ecological niches with the predicted rates of climate change are unlikely to be met by temperate tree species (Solomon 1997; Thuiller *et al.* 2008) and, if populations are unable to adapt quickly enough, assisted migration of populations better adapted to warmer climates may be an answer to maintaining productive forests (Atkins and Travis 2010; Grey *et al.* 2011). Species that have a more continuous distribution are likely to be impacted much less than those with a scattered distribution as this allows for gene flow between populations. The rate of adaptive change is likely to differ between the leading edge of the migration front and the rear end of the

distribution, with populations at the leading edge benefiting from gene flow from southern latitudes (Davis and Shaw 2001; Aiken *et al.* 2008). Local adaptation may be increased at the species level by incoming genes from populations exhibiting higher fitness than the receiving population (Lenormand 2002). With higher temperatures, populations from more southerly locations may provide pre adapted pollen to the receiving population (Lindener *et al.* 2002).

Until recently, forest policy has advocated the use of local native species in woodland creation in Britain (Herbert *et al.* 1999), but the science behind this practice is being increasingly questioned. Additionally, what actually constitutes a local seed source is not clear. Boshier (2007) argues that with the lack of extensive trials of native British trees, using only local provenance material in woodland creation schemes is potentially dangerous. Inbreeding depression (due to poor seed collection practices) and the loss of genetic diversity should be given greater consideration to allow for extensive gene flow and adaptation at a broad scale to build resilience in to populations. The emphasis that is often given to local seed sources in the UK is problematic in that the environment of a site may no longer experience the conditions under which the tree evolved.

Provenance and progeny trials can provide information regarding patterns of quantitative genetic variation and the extent of genotype x environment interactions. Evidence from provenance trials in Britain shows that British material is adapted to British conditions, whereas material from continental Europe is maladapted and often comes into leaf early and then suffers from frost damage (Cundall *et al.* 2003). Differences between UK and continental provenances are more likely to be due to geographic and altitudinal extremes within a species range e.g. extremes of north and south in Britain, and between northwest and more maritime regions (Ennos 2000). In the current study differences in latitude were not as apparent as those of altitude, with the provenance from Le Tech (the only high elevation population in this study) performing poorly at all sites. In a common garden experiment of *Fraxinus excelsior*, Boshier and Stewart (2005) reported no differences between provenances across a range of sites in England and Wales with the best trees growing best on all sites, and no evidence of a home site advantage and local adaptation. This was confirmed in the current study, with those provenances from registered seed

stands growing best at all locations. Indeed, the results indicated that no provenance was in its optimal niche, and that most would grow better in a warmer climate.

When investigating the genetic structure and mating behaviour of *Fraxinus excelsior* in a severely fragmented landscape in a deforested catchment in Scotland, Baccles *et al.* (2005) found that forest remnants maintained high levels of genetic diversity which were comparable to continuous populations in continental Europe, indicating that historical gene flow had not been limited. They reported that pollen gene flow was extensive and that pollen flow outside the restricted radius around the mother tree occurred over several kilometres. The extent of gene flow reported in their study suggests that local adaptation over a small geographic scale is unlikely unless selection forces are very strong. Thus, the scale over which species show adaptation to their local environment depends on the degree of habitat heterogeneity and gene flow. In outcrossing, wind pollinated and dispersed species such as *Fraxinus excelsior*, gene flow is extensive, and local adaptation at a small scale is unlikely.

This then leads on to adaptation to climate change at the greater scale. A lack of local adaptation in populations of *Fraxinus excelsior* can be viewed as advantageous in a changing climate. The results of the RTE in this study indicate that populations are phenotypically plastic and this allows them to grow well in a broad range of environments e.g. many provenances grew well at both Cawdor and Monein – sites that are approximately 2000 km apart in latitude and experience widely different climates. Eriksson *et al.* (1993) emphasise that the present day genetic structure of populations is transient, and it is a misconception that maximum fitness has already been obtained in nature, an observation also pointed out by Gould (1998). By conserving genetic variation, it is likely that there will be increased adaption to future evolution of species (EUFORGEN 2013). Disruptive and directional natural selection, gene flow and mutations continue to shape population structure and patterns of variation (Eriksson and Ekberg 2001). Results of half sib progeny trials also showed significant within population variation (Savill *et al.* 1999). That is to say, there appears to be ample variation in *Fraxinus excelsior* to allow for adaptation to occur.

Adapting to climate change requires adjustments in ecological, social and economic systems in response to the effects of changes in climate (Smit et al. 2000). Spittlehouse and Stewart (2003) provide a summary of adaptive actions in forestry (Box 6.1). Forest life cycles range from decades to centuries and decisions made today need to take account of likely rises in temperatures and potential impacts from drought. A high priority in adapting to climate change will be coping with and adapting to forest disturbance while maintaining the genetic diversity and resilience of forest ecosystems. Seed zone and seed transfer guidelines are essential tools in reforestation to ensure that seedlings are well adapted to the growing conditions of the planting site (Morgenstern 1996; Ying and Yanchuk 2006). In Britain, these are encompassed in the FRM regulations, and put in to practice through the Forestry Commission Practice Note 8 on Using local stock for planting native trees and shrubs (Herbert et al. 1999). Given the likely scale of (the lack of) local adaption seen in Fraxinus excelsior, the seed zones within the regions of provenance would appear to be overly restrictive, and rely on geographic distance rather than ecological distance. In terms of reforestation, this study would suggest that sourcing planting material from the local region of provenance (rather than seed zone) would be appropriate for ensuring material that is adapted to current local conditions. However, to build greater resilience into populations to aid adaptation to future climate conditions it would seem prudent to include material from more southerly location, where climate conditions are more similar to the maritime climate of the UK (e.g. material from France of more maritime origin, rather than central continental material which is better adapted to harsher winters such as are found in Romania and the Czech Republic). Indeed the Forestry Commission (Ray et al. 2010) suggest incorporating material from 2° latitude south of planting site, and even a small amount of material from 5° latitude south of planting site with the objective of incorporating some genotypes preadapted to future climates.

A warmer climate is expected to be of benefit for many tree species in temperate regions in the short term (including *Fraxinus excelsior*) through a longer growing period and greater CO_2 capture (Eggers *et al.* 2008), and this would appear to be corroborated by this study. In the longer term, populations are more likely to be negatively impacted by rising temperatures, as water and possibly nutrients becomes limited. Potential risks and opportunities in a changing climate have been reviewed for several important timber species (Hemery *et al.* 2010).

Box 6.1.	Summary of adaptive actio	ns for forestry	in a	changing climate.	Adapted fron	n
	Spittlehouse and Stewart (2)03).				

Management area	Adaptive action
Gene management Seed zone and provenance transfer guidelines need to be amended to account for the expected upwards in elevation and northwards migration of species in northern temperate regions.	 Determine the response of genotypes to climate and limits to their transferability. Provenances need to be tested at the limits of their natural range Breeding for pest and disease resistance and for a wider range of tolerances to environmental stress Re-evaluating seed orchard locations to ensure a supply of seed Planting a mixture of provenances, and possibly incorporating new species Identify drought resistant genotypes Minimise fragmentation to allow for gene flow Allow established forests to continue to allow for adaptive responses to take place.
Forest regeneration Existing forests are likely to be quite resilient to climate change but their offspring will be threatened as this is the stage when most selection pressures are evident.	 Assist migration from present to future ranges through artificial regeneration Plant provenances that grow adequately under a wide range of conditions and / or provenances from a range of locations Consider new species Control undesirable species that become more competitive in a changed climate.
Silvicultural management Productivity of temperate forest may increase, at least in the short to mid- term although nutrient availability may become limiting. Silvicultural systems will be required to manage declining and potentially stressed stands.	 Alter thinning regimes to increase vigour and lower susceptibility of insect attack. Reduce disease loses through sanitation thinning Shorten rotation lengths to decrease period of stand vulnerability to damaging insects and diseases Manage tree density and species composition to reduce vulnerability to future disturbances Underplant with other species or genotypes to augment natural regeneration.

With increased tree planting on the political agenda to offset some of the negative impacts of climate change, we must consider carefully what species and genotypes we should be planting so that populations will continue to flourish in 50 to 100 years and beyond. Tree improvements programmes have been implemented to improve the quality of many of our important broadleaved timber species in terms of form and vigour (e.g. *Fraxinus excelsior*), wood properties (e.g. *Quercus robur* and *Quercus petraea* screening for vessel size, associated with shake), and occasionally resistance to disease (e.g. *Prunus avium* resistance to bacterial canker). Such programmes ensure a broad genetic base in populations that is often greater than that found in nursery collections for reforestation as the number of parents included in such research programmes tends to be large. This is fortuitous, as, by its very nature, is builds resilience into populations against other impacts such as the effects of climate change. Factors such as budburst which are known to be under genetic control, and are highly heritable (Billington and Pelham 1991) enable researchers to select individuals within populations that break bud later and so are less likely to be damaged by frost.

The objective of this study has been to investigate various effects of climate change on populations of *Fraxinus excelsior*, with the aim of identifying provenances that may be suitable for timber production under future projected climates, and to assess levels of adaptive variation within UK populations. The climate matching analysis carried out by Broadmeadow (2004) indicates that provenances of *Fraxinus excelsior* that are matched to our projected climate under a series of emissions scenarios will come from the Loire Valley region of France in the short term and from the south of France and regions of the Adriatic at high elevations in the longer term. Populations of *Fraxinus excelsior* from these regions were tested through a series of reciprocal transplant experiments and chilling requirements of their seed were investigation through a series of germination tests. The main findings of this work are:

1. The majority of populations studied are autochthonous. New alleles were found in populations from Monein (which contained the H10 haplotype previously reported from the Czech republic; Heuertz *et al.* 2004b) and Settrington (H14 haplotype previously reported in western Scotland; Sutherland *et al.* 2010) and previously unreported alleles

were found in populations from Ballindalloch and Saint Paul, the latter of possible hybrid status.

- 2. Results from the RTEs indicate that most populations perform better in a warmer climate, and local adaptation is not evident, as would be expected in an outcrossing wind dispersed species of wide distribution. This finding supports that of other researchers (Savill *et al.* (1999), Cundall *et al.* (2003) Boshier and Stewart (2005)). Many of the provenances tested were found not to be growing in their ecological optima. The best provenances grew well on all sites, even at geographic distances of 2000 km. Most provenances performed well when moved south, benefiting from a warmer climate and longer growing season (e.g. the provenances Cawdor and Ballindalloch grew better at Monein that at Cawdor). No provenance performed best at its local site.
- 3. Provenances in a common garden break bud at different times. This appears to be closely correlated as mean spring temperatures, with provenances coming into leaf earlier in years with greater thermal sums. Provenances broke bud later at a more northerly site, which received a correspondingly lower thermal sum in the spring. Where sites received a greater thermal sum, budburst to full leaf expansion was completed more quickly. However, where forcing requirement are met early and leaves come into leaf, frost damage is common as was seen in 2010 at both sites and in nearly all provenances. Under controlled conditions, chilling and forcing requirements can be estimated for various provenances to predict the timing of budburst. Minimum chilling requirements are necessary for different provenances, with provenances from maritime regions requiring greater chilling to prevent early budburst and to avoid the associated risk of frost damage. With present winter temperatures, chilling requirements are met in all the provenances studied, even those with the greatest chilling requirements.
- 4. Provenances studied also showed variation in the chilling requirements of seed to break dormancy, again, with northern provenances requiring greater chilling than southern provenances. Germination testing with increased chilling treatments indicated that at present the chilling requirement to break dormancy is amply met in native populations.

From a management perspective for growing timber within a changing climate, from this study it would appear that populations of *Fraxinus excelsior* contain ample genetic variation which allows populations to respond to a wide array of climates. However, with uncertainty as to the degree of warming, and the effects that this will have on populations, it seems prudent to augment native populations with genotypes that are likely to be pre-adapted to future climates. It will be important to source provenances from the more maritime regions of the near continent, which have a high chilling requirement for budburst, thereby minimising risk of frost damage.

Other work was carried out within this study, but results have not been presented for a number of reasons.

- 1. Since it is being proposed to bring material from the near continent to Britain to assist populations in adapting to a warmer climate, and since theories of local adaption are endorsed to promote the use of local material, it seems prudent to ask the question, what will be the effect of introducing continental provenances on local populations? In 2006 and 2007, this question was addressed through a series of controlled pollinations in a seed orchard of British material. Pollen was collected from an adjacent provenance trial from several continental provenances with the aim of looking for outbreeding depression in the progeny. Unfortunately, although about 70 pollinations were made each year, spring frosts killed all the flowers, and no seed was set. Nevertheless, the question remains to be answered, and this work should be repeated.
- 2. To address the question of the chilling requirement of seedlots in a milder climate, seed were collected from Scottish populations of *Fraxinus excelsior* growing in a seed orchard in southern Oxfordshire and subjected to increased periods of chilling. Results were confounded by the maternal effect of the seed tree and require further analysis, beyond the scope of this study.

3. Budburst observations were made on the trees in the RTEs but results were not analysed due to time constraints and concerns that budburst was unduly influenced by the black mulch mats at four sites. Preliminary observations indicated that provenance and site differences were small, and it was hypothesised that this was due to the small size of seedlings and the influence of the mulch mats. However, datasets from a later date would yield very interesting results to address climatic differences in budburst of provenances at different sites.

Due to the time frame of a doctorate study, and also the time taken to locate populations, collect and pretreat seed and raise seedling of *Fraxinus excelsior*, only three years of data were obtained from the RTEs. Given the problems with establishing the trials, minimal information pertaining to adaptation was acquired. However, mortality is greatest in the early years of such trials, and further mortality is likely to be minimal. The trials should yield much more relevant data in future years. Assessing budburst in relatively small seedlings across a great geographic range meant that assessments were widely spaced, and data from the beginning and end of budburst were insufficiently detailed. If these assessments could be made every seven days at each site in two to three years' time, the results should prove very interesting and useful in furthering our knowledge of adaptation in populations of *Fraxinus excelsior*.

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Appendices

Appendix 1 Allele sizes of amplification products generated by primers ccmp2, 3, 4, 6, 7 and 10 and corresponding haplotype. Alleles in bold are new.

Sample	ccmp2	ccmp3	ccmp4	ccmp6	ccmp7	ccmp10	Haplotype
AH1	191	93	136	97	114	101	H4
AH2	191	93	136	97	114	101	H4
AH3	191	93	136	97	114	101	H4
AH4	191	93	136	97	114	101	H4
AH5	191	93	136	97	114	101	H4
Al1	191	93	136	97	114	101	H4
Al2	191	93	136	97	114	101	H4
Al3	191	93	136	97	114	101	H4
Al4	191	93	136	97	114	101	H4
Al5	191	93	136	97	114	101	H4
Ba1	191	93	136	97	114	101	H4
Ba2	191	93	136	97	114	101	H4
Ba3	190	93	136	97		101	NEW
Ba4	191	93	136	97	114	101	H4
Ba5	191	93	136	97	114	101	H4
Ca1	191	93	136	97	114	101	H4
Ca2	191	93	136	97	114	101	H4
Ca3	191	93	136	97	114	101	H4
Ca4	191	93	136	97	114	101	H4
Ca5	191	93	136	97	114	101	H4
Do1	191	93	136	98	113	101	H2
Do2	191	93	136	98	113	101	H2
Do3	191	93	136	98	113	101	H2
Do4	191	93	136	98	113	101	H2
Do5	191	93	136	98	113	101	H2
Le1		93	136	98	113	100	H3
Le2	191	93	136	98	113	100	H3
Le3	191	93	136	98	113	100	H3
Le4	191	93	136	98	113	100	H3
Le5	191	93	136	98	113	100	H3
LW1	191	93	136	97	114	101	H4
LW2	191	93	136	97	114	101	H4
LW3	191	93	136	97	114	101	H4
LW4	191	93	136	97	114	101	H4
LW5	191	93	136	97	114	101	H4
Ma1	191	93	136	98	113	101	H2
Ma2	191	93	136	98	113	101	H2

Sampleccmp2ccmp3ccmp4ccmp6ccmp7ccmp7ccmp7Ma31919313698113Ma4 </th <th>Cmp10 Haplo 101 H2 101 H2 101 H2 101 H4 101</th> <th>2 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4</th>	Cmp10 Haplo 101 H2 101 H2 101 H2 101 H4 101	2 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4
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Appendix 2 Site maps and trial layouts of the five reciprocal transplant experiments.

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Appendix 3 Mean Julian date for provenances to reach budburst scores of 1, 3 and 5, and the days to complete budburst (from score 1 - 5) at Little Wittenham and Ebberston by year and latitude at site of provenance.

Year	Provenance	Score 1	Score 3	Score 5	days 1-5	Latitude
2007	Romania	102.6	109.1	123.5	21.0	47.00° N
2007	Haute Saône	106.1	116.3	136.6	30.4	47.40° N
2007	Grimsthorpe	106.4	115.1	130.2	23.8	52.48° N
2007	Basse Normandie	106.8	115.6	128.9	22.1	48.30° N
2007	Curdleigh	108.1	117.3	134.0	25.9	51.00° N
2007	Champagne	108.3	117.3	132.0	23.8	49.30° N
2007	Friston	110.5	120.6	141.1	30.7	50.48° N
2007	Glos/Hants/Surrey	110.9	121.1	137.0	26.1	51.50° N
2007	Palobe	111.1	121.0	137.0	25.9	50.05° N
2007	Upper Rhine	111.8	119.6	131.7	19.9	49.00° N
2007	Brockhampton	111.9	122.2	138.6	26.7	52.11° N
2007	Settrington	115.1	124.5	138.3	23.2	54.06° N
2007	Newton	117.3	127.4	143.7	26.4	57.44° N
2007	Pallinsburn	117.4	126.4	141.4	24.0	55.38° N
2007	Bacheiddon	119.6	128.5	142.8	23.2	52.32° N

Little Wittenham 2007

Year	Provenance	Score 1	Score 3	Score 5	days 1-5	Latitude
2008	Romania	107.9	114.4	126.3	18.4	47.00° N
2008	Haute Saône	110.4	118.9	131.0	20.6	47.40° N
2008	Grimsthorpe	110.6	117.8	128.4	17.8	52.48° N
2008	Basse Normandie	110.4	118.4	129.5	19.2	48.30° N
2008	Curdleigh	110.1	118.2	128.4	18.3	51.00° N
2008	Champagne	110.6	118.5	128.7	18.1	49.30° N
2008	Friston	111.2	120.0	131.1	19.9	50.48° N
2008	Glos/Hants/Surrey	111.2	120.4	131.6	20.4	51.50° N
2008	Palobe	110.8	120.4	132.0	21.2	50.05° N
2008	Upper Rhine	111.9	119.3	127.9	16.0	49.00° N
2008	Brockhampton	111.9	121.5	132.4	20.5	52.11° N
2008	Settrington	113.2	123.2	131.8	18.7	54.06° N
2008	Pallinsburn	113.7	124.8	132.5	18.7	57.44° N
2008	Newton	115.5	126.0	133.4	17.9	55.38° N
2008	Bacheiddon	116.0	126.4	132.8	16.8	52.32° N

Little Wittenham 2009

Year	Provenance	Score 1	Score 3	Score 5	days 1-5	Latitude
2009	Romania	103.3	113.1	122.8	19.5	47.00° N
2009	Haute Saône	107.9	120.5	133.0	25.2	47.40° N
2009	Grimsthorpe	108.7	121.2	133.6	24.9	52.48° N
2009	Basse Normandie	110.3	122.1	133.9	23.6	48.30° N
2009	Curdleigh	113.9	125.0	136.0	22.2	51.00° N
2009	Champagne	111.9	123.7	135.6	23.7	49.30° N
2009	Friston	116.8	128.3	139.9	23.1	50.48° N
2009	Glos/Hants/Surrey	116.0	127.2	138.5	22.5	51.50° N
2009	Palobe	115.7	126.9	138.2	22.5	50.05° N
2009	Upper Rhine	113.7	123.5	133.3	19.6	49.00° N
2009	Brockhampton	117.3	128.5	139.7	22.4	52.11° N
2009	Settrington	118.2	127.3	136.3	18.1	54.06° N
2009	Newton	120.0	131.2	142.4	22.4	57.44° N
2009	Pallinsburn	120.4	131.3	142.1	21.7	55.38° N
2009	Bacheiddon	121.3	133.6	146.0	24.7	52.32° N

Year	Provenance	Score 1	Score 3	Score 5	days 1-5	Latitude
2010	Romania	107.4	120.3	130.4	23.0	47.00° N
2010	Haute Saône	113.6	123.6	142.6	29.0	47.40° N
2010	Grimsthorpe	113.9	123.0	140.4	26.5	52.48° N
2010	Basse Normandie	113.9	124.4	141.2	27.3	48.30° N
2010	Curdleigh	113.6	123.6	144.6	31.0	51.00° N
2010	Champagne	116.1	127.4	143.8	27.7	49.30° N
2010	Friston	118.4	130.3	151.2	32.7	50.48° N
2010	Glos/Hants/Surrey	119.1	133.7	153.1	34.0	51.50° N
2010	Palobe	119.1	132.5	151.9	32.8	50.05° N
2010	Upper Rhine	118.9	129.7	145.5	26.6	49.00° N
2010	Brockhampton	117.2	131.2	148.1	30.9	52.11° N
2010	Settrington	117.8	128.2	145.7	27.9	54.06° N
2010	Newton	119.8	135.5	156.9	37.2	57.44° N
2010	Pallinsburn	121.0	135.7	153.6	32.6	55.38° N
2010	Bacheiddon	122.6	144.6	163.6	41.0	52.32° N

Ebberston 2009

Year	Provenance	Score 1	Score 3	Score 5	Days 1-5	Latitude
2009	Alsace	110.7	123.0	135.4	24.7	48.30° N
2009	Picardie	111.5	123.2	135.0	23.5	50.00° N
2009	Haute Saône	113.3	125.1	136.8	23.4	47.40° N
2009	Basse Normandie	113.4	125.4	137.4	24.0	48.30° N
2009	Yugoslavia	113.4	124.6	135.9	22.4	46.00° N
2009	Champagne	115.5	127.3	139.1	23.5	49.30° N
2009	Palobe	116.1	127.9	139.7	23.6	50.05° N
2009	Czech Republic	116.4	128.5	140.6	24.3	50.00° N
2009	Grimsthorpe	116.5	129.1	141.7	25.2	52.48° N
2009	Upper Rhine	116.6	127.5	138.5	21.9	49.00° N
2009	Shropshire	121.8	135.3	148.8	26.9	52.30° N

Ebberston 2010

Year	Provenance	Score 1	Score 3	Score 5	Days 1-5	Latitude
2010	Alsace	109.3	124.2	132.5	23.2	48.30° N
2010	Picardie	111.4	124.3	134.6	23.2	50.00° N
2010	Basse Normandie	113.1	125.1	134.0	20.9	48.30° N
2010	Yugoslavia	113.3	125.3	134.5	21.2	46.00° N
2010	Champagne	115.2	128.9	140.1	24.9	49.30° N
2010	Haute Saône	115.4	128.1	139.8	24.4	47.40° N
2010	Palobe	115.8	128.9	137.4	21.6	50.05° N
2010	Czech Republic	115.9	129.6	140.9	25.0	50.00° N
2010	Grimsthorpe	116.6	129.9	139.9	23.3	52.48° N
2010	Upper Rhine	117.1	129.3	138.2	21.1	49.00° N
2010	Shropshire	124.8	140.9	157.0	32.2	52.30° N

Appendix 4 Mean budburst score by Julian day with standard deviation for 15 provenances of *Fraxinus excelsior* at Little Wittenham (2007 - 2010) and for 11 provenances at Ebberston (2009 - 2010).

Provenance	ID 103	ID 110	ID 117	ID 124	ID 131	ID 138
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Newton	0	0.3 ±0.6	0.9 ±1.2	2.2 ±1.5	3.8 ±1.3	4.5 ±0.9
Pallinsburn	0	0.2 ±0.5	0.9 ±1.1	2.4 ±1.3	4.1 ±1.1	4.7 ±0.7
Settrington	0	0.2 ±0.6	1.3 ±1.1	2.9 ±1.2	4.5 ±0.8	4.9 ±0.4
Grimsthorpe	0.3 ±0.7	1.7 ±1.4	3.5 ±1.4	4.4 ±0.9	4.9 ±0.3	5
Bacheiddon	0	0.1 ±0.3	0.5 ±0.9	1.9 ±1.2	3.6 ±1.2	4.4 ±0.9
Brockhampton	0.1 ±0.3	0.6 ±1.0	1.9 ±1.5	3.4 ±1.4	4.6 ±0.8	4.9 ±0.4
Glos/Hants/Surrey	0.1 ±0.4	0.8 ±1.1	2.2 ±1.4	3.6 ±1.1	4.7 ±0.6	5.0 ±0.3
Curdleigh	0.2 ±0.5	1.3 ±1.2	3.0 ±1.6	4.2 ±1.0	4.9 ±0.3	5.0 ±0.1
Friston	0.1 ±0.3	0.9 ±1.0	2.4 ±1.4	3.6 ±1.3	4.7 ±0.9	4.9 ±0.5
Palobe	0.1 ±0.4	0.8 ±1.2	2.2 ±1.6	3.6 ±1.2	4.8 ±0.5	5.0 ±0.2
Champagne	0.2 ±0.5	1.4 ±1.2	2.9 ±1.4	4.3 ±1.0	4.9 ±0.3	5
Rhine Valley	0.0 ±0.2	0.6 ±0.9	2.3 ±1.4	4.0 ±0.9	4.9 ±0.3	5
Basse Normandie	0.3 ±0.7	1.7 ±1.3	3.3 ±1.3	4.5 ±0.8	5.0 ±0.1	5
Haute Saône	0.5 ±0.8	1.7 ±1.3	3.2 ±1.5	4.3 ±1.1	4.9 ±0.5	5.0 ±0.3
Romania	1.1 ±0.9	3.3 ±1.1	4.6 ±0.7	4.9 ±0.3	5	5

Little Wittenham 2007

Provenance	JD 111	JD 118	JD 125	JD 132	JD 139
Newton	0.3 ±0.5	1.4 ±0.9	2.7 ±1.4	4.6 ±0.8	4.9 ±0.3
Pallinsburn	0.6 ±0.6	1.7 ±0.9	3.0 ±1.3	4.9 ±0.4	5
Settrington	0.6 ±0.5	1.9 ±0.8	3.4 ±1.1	4.9 ±0.4	5
Grimsthorpe	1.1 ±0.8	3.1 ±1.0	4.4 ±0.8	5	5
Bacheiddon	0.3 ±0.4	1.4 ±0.8	2.5 ±1.2	4.7 ±0.5	5.0 ±0.1
Brockhampton	0.8 ±0.7	2.3 ±0.9	3.7 ±1.1	4.9 ±0.3	5
Glos/Hants/Surrey	0.9 ±0.7	2.5 ±0.9	4.0 ±1.0	4.9 ±0.3	5
Curdleigh	1.2 ±0.8	3.0 ±1.1	4.3 ±0.9	5	5
Friston	0.9 ±0.6	2.5 ±0.9	4.2 ±1.0	4.9 ±0.2	5
Palobe	1.1 ±0.7	2.6 ±1.2	3.9 ±1.2	5.0 ±0.1	5
Champagne	1.1 ±0.9	2.9 ±1.2	4.3 ±0.9	5.0 ±0.1	5
Upper Rhine	0.7 ±0.7	2.7 ±1.0	4.3 ±0.7	5	5
Basse Normandie	1.2 ±0.7	2.9 ±0.9	4.4 ±0.8	5.0 ±0.1	5
Haute Saône	1.2 ±0.8	2.8 ±1.2	4.3 ±1.1	4.9 ±0.3	5
Romania	1.9 ±0.6	4.1 ±0.7	4.9 ±0.4	5	5

Little Wittenham 2009

Provenance	JD 103	JD 118	JD 132
Newton	0.0 ±0.0	0.6 ±0.1	3.2 ±0.6
Pallinsburn	0.0 ±0.0	0.6 ±0.1	3.1 ±0.2
Settrington	0.0 ±0.0	1.0 ±0.0	4.1 ±0.2
Grimsthorpe	0.3 ±0.1	2.5 ±0.3	4.7 ±0.1
Bacheiddon	0.0 ±0.0	0.5 ±0.2	2.8 ±0.5
Brockhampton	0.0 ±0.0	1.1 ±0.2	3.7 ±0.4
Glos/Hants/Surrey	0.1 ±0.0	1.4 ±0.3	3.9 ±0.4
Curdleigh	0.1 ±0.1	1.7 ±0.1	4.3 ±0.2
Friston	0.0 ±0.0	1.2 ±0.2	3.6 ±0.2
Palobe	0.0 ±0.0	1.4 ±0.2	3.9 ±0.4
Champagne	0.2 ±0.3	2.0 ±0.6	4.4 ±0.5
Upper Rhine	0.0 ±0.0	1.9 ±0.2	4.7 ±0.2
Basse Normandie	0.1 ±0.1	2.3 ±0.2	4.7 ±0.3
Haute Saône	0.3 ±0.2	2.6 ±0.2	4.8 ±0.1
Romania	0.9 ±0.2	4.0 ±0.3	5.0 ±0.0

Provenance	JD 113	JD 127	JD 141
Newton	0.3 ±0.3	2.1 ±0.7	3.3 ±0.7
Pallinsburn	0.0 ±0.0	1.9 ±0.1	3.5 ±0.2
Settrington	0.3 ±0.1	2.8 ±0.3	4.3 ±0.3
Grimsthorpe	0.9 ±0.3	3.7 ±0.2	4.8 ±0.1
Bacheiddon	0.1 ±0.1	1.3 ±0.3	2.8 ±0.4
Brockhampton	0.5 ±0.1	2.5 ±0.4	4.1 ±0.4
Glos/Hants/Surrey	0.3 ±0.2	2.2 ±0.4	3.6 ±0.4
Curdleigh	0.9 ±0.3	3.4 ±0.3	4.5 ±0.2
Friston	0.3 ±0.2	2.6 ±0.4	3.8 ±0.5
Palobe	0.3 ±0.2	2.4 ±0.6	3.7 ±0.5
Champagne	0.4 ±0.3	2.8 ±0.4	4.3 ±0.6
Upper Rhine	0.1 ±0.1	2.6 ±0.1	4.4 ±0.6
Basse Normandie	0.9 ±0.4	3.6 ±0.8	4.6 ±0.6
Haute Saône	0.9 ±0.4	3.5 ±0.2	4.7 ±0.3
Romania	1.7 ±0.1	4.6 ±0.1	5.0 ±0.0

Ebberston 2009

Provenance	JD 105	JD 118	JD132
Shropshire	0.0 ±0.4	0.4 ±0.0	2.5 ±0.3
Upper Rhine	0.1 ±0.0	1.3 ±0.1	3.8 ±0.2
Grimsthorpe	0.2 ±0.2	1.2 ±0.1	3.5 ±0.2
Czech Republic	0.2 ±0.3	1.3 ±0.1	3.6 ±0.2
Palobe	0.1 ±0.6	1.3 ±0.1	3.7 ±0.4
Champagne	0.2 ±0.2	1.4 ±0.2	3.8 ±0.1
Yugoslavia	0.3 ±0.1	1.8 ±0.2	4.3 ±0.3
Basse Normandie	0.2 ±0.2	1.8 ±0.2	4.1 ±0.1
Haute Saône	0.4 ±0.1	1.8 ±0.2	4.2 ±0.1
Picardie	0.5 ±0.3	2.1 ±0.1	4.5 ±0.2
Alsace	0.5 ±0.2	2.2 ±0.1	4.5 ±0.3

Ebberston 2010

Provenance	JD 109	JD 123	JD 137
Shropshire	0.0 ±0.0	0.8 ±0.3	1.9 ±0.5
Upper Rhine	0.0 ±0.0	1.6 ±0.1	3.4 ±0.1
Grimsthorpe	0.2 ±0.3	1.7 ±0.4	3.3 ±0.6
Czech Republic	0.3 ±0.1	1.8 ±0.3	3.3 ±0.1
Palobe	0.2 ±0.1	1.6 ±0.4	3.6 ±0.7
Champagne	0.3 ±0.2	2.0 ±0.5	3.4 ±0.7
Yugoslavia	0.4 ±0.1	2.5 ±0.4	4.3 ±0.4
Basse Normandie	0.4 ±0.3	2.5 ±0.4	4.3 ±0.2
Haute Saône	0.3 ±0.1	2.1 ±0.3	3.5 ±0.3
Picardie	0.7 ±0.2	2.7 ±0.2	4.4 ±0.3
Alsace	1.0 ±0.1	2.6 ±0.1	4.7 ±0.3

		Days forcing				
	Days					
	chilling	14	28	42	56	70
Pallinsburn	31	0	0	0	0	0
	45	0	0	0	0	0
	59	0	0	0	0	23.3
	73	0	0	0	10	44
	87	0	0	0	30	68.7
	101	0	0	23.4	66.7	86.7
	115	0	0	58	96.7	100
Settrington	31	0	0	0	0	0
	45	0	0	0	0	0
	59	0	0	0	3.3	7.3
	73	0	0	0	16.7	23
	87	0	0	4.2	26	38
	101	0	3.4	21	48	63.7
	115	0	10	47	81.7	87
Glos/Hants/Surrey	31	0	0	0	0	0
	45	0	0	0	0	0.3
	59	0	0	0	3.3	20
	73	0	0	0	3.3	23.3
	87	0	0	6.7	35.3	43.3
	101	0	0	13.3	63.3	76.7
	115	0	10	40	78.7	86.3
Haute Saone	31	0	0	0	0	3.3
	45	0	0	0	3.3	10
	59	0	0	0.7	10	40.8
	73	0	0	10	13.3	30.8
	87	0	3.3	13.3	30	48.7
	101	0	10.3	45.7	76	89
	115	0	23.3	48.7	83.5	89
Basse Normandie	31	0	0	0	0	0
	45	0	0	0	6.7	6.7
	59	0	0	10	3.3	23.3
	73	0	3.3	16.7	10	34.2
	87	0	0	27.7	61	85.7
	101	0	0	33.3	70.8	90.3
	115	0	24.3	68.3	96.3	100
Romania	31	0	0	0	0	0
	45	0	0	0	10	20.7
	59	0	0	14.7	46.7	79
	73	0	3.3	20	64.3	80
	87	0	10	65.7	90.3	93.3
	101	0	26.7	86.7	100	100
	115	0	75.3	92.7	100	100

Appendix 5 The percentage of twigs of six provenances of *Fraxinus excelsior* that broke bud with various chilling and forcing treatments under controlled conditions.